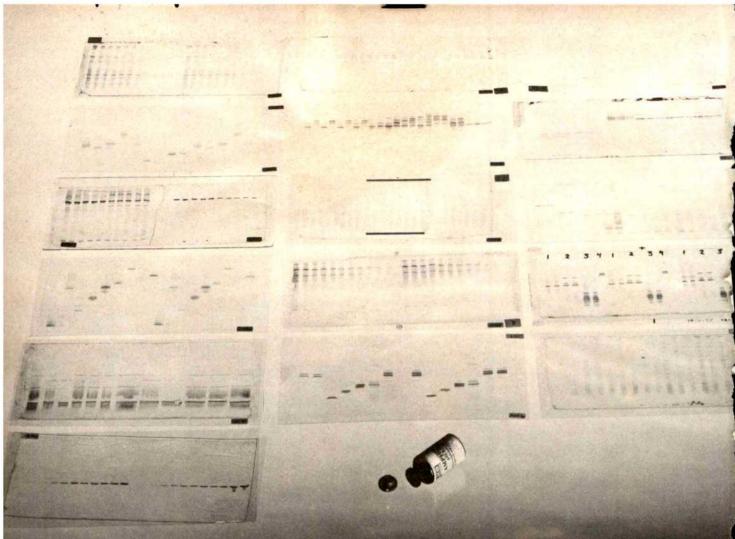
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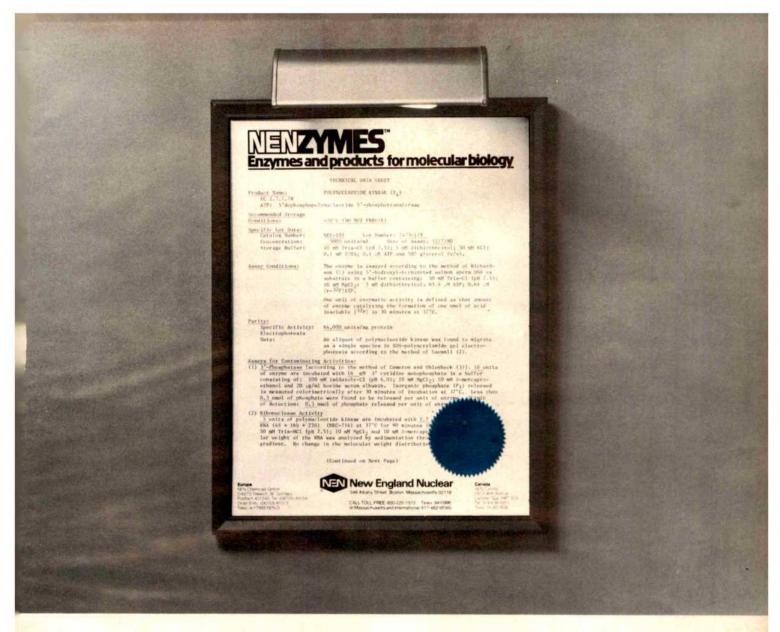


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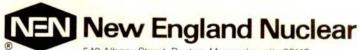
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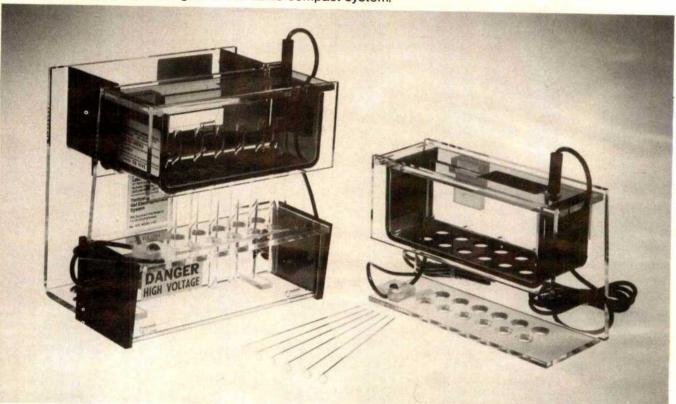


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Leech ganglion containing a Leydig cell injected intracellularly with Lucifer yellow.

Microscopy

Of special relevance to microscopy in this issue are the cover and the article which prompted it (p.17); 100 years ago (p.16), articles on pp. 9 and 93 and product Review (p.xxvii).

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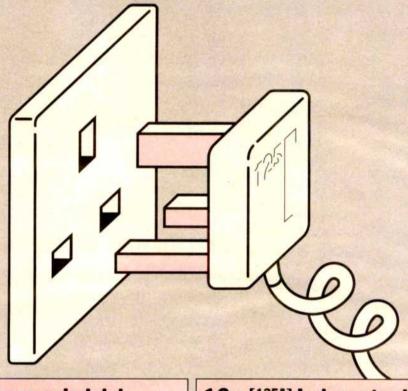
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(1) HOCHBERG, R.B. Science, **205**, pp. 1138-1140, 1979

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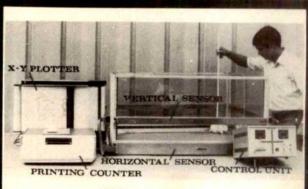
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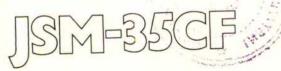
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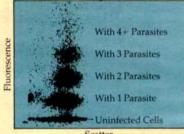
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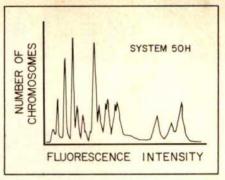


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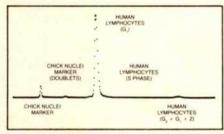
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 Histogram of CHO chromosomes stained for D.N.A. Ethidium Bromide/Chromomycin A₃.

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2. Histogram of human lymphocytes utilizing a chick nuclei marker for standardization and stained for D.N.A. with Propidium Iodide. C.V. = 1.65% (Human Lymphocytes)

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New Literature: BRL 81/82 Catalog

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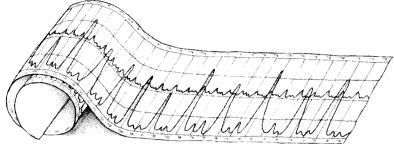
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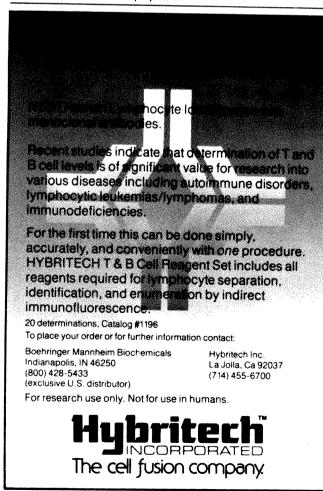
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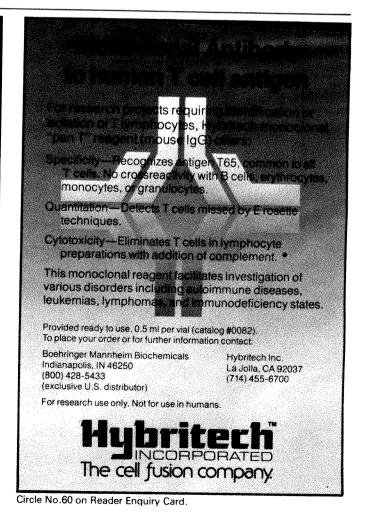
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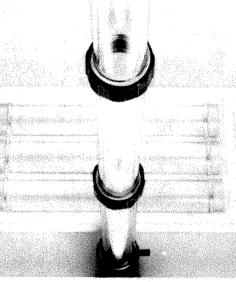
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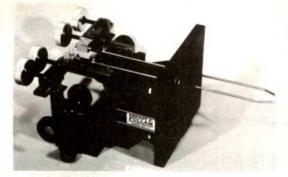
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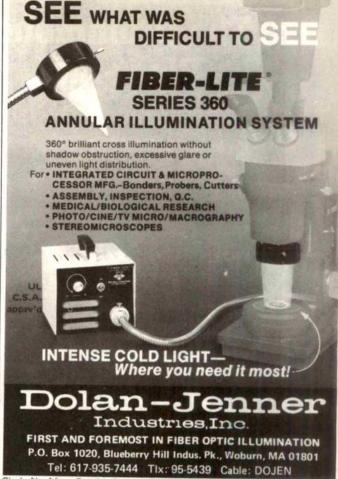
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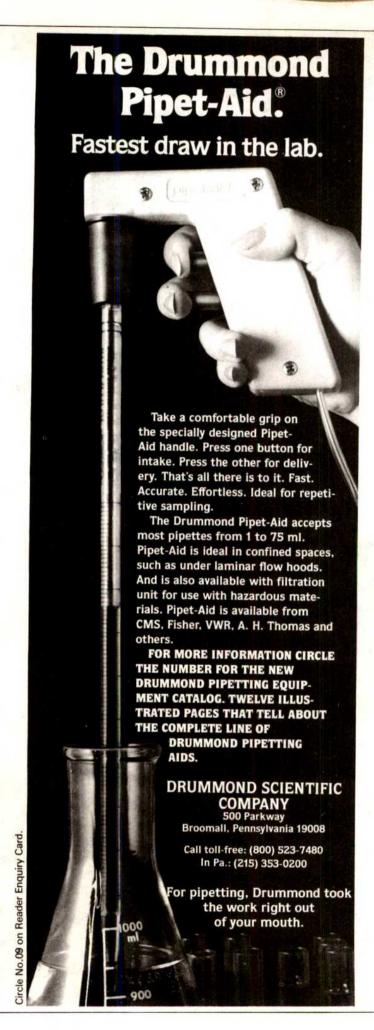
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Editor: John Maddox

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Editorial Staff

Alun Anderson Philip Campbell Sara Nash Peta Pickering Isobel Collins Konrad Guettler Judy Redfearn Miranda Robertson Robert Walgate Tim Lincoln Naomi Molson Charles Wenz

Washington News Bureau 801 National Press Building, DC 20045 Telephone: (202) 737-2355 Telex: 64280 David Dickson (Washington News Editor)

Publisher: Elizabeth Hughes Marketing Director: Ray Barker International Advertising Manager: Andy Sutherland

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New York 15 East 26 Street, New York, NY 10010 Telephone: (212) 689-5900 American Publisher: Robert Ubell American Advertising Manager: Henry Dale Marketing Manager: Sheila Kane

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nature

2 July 1981

Fighting tsetse flies and people

Opinions differ on the benefits of the transformation of the Punjab in India by the green revolution. Some hold that the transformation of traditional social life has been disastrous. Others, including most of those who live there, hold otherwise. If new strains of wheat have made it possible for India, for the first time in living memory, to be self-sufficient in wheat and thus realistically capable of turning its attention to other than agricultural development, who except those who fear competition in manufactured goods from India can count that a simple tragedy? Is it not, instead, natural to hope that similar transformations will be accomplished in other developing countries? This is the spirit in which, since the early 1970s, the World Bank has been organizing a confederation of donor governments and foundations, known as the Consultative Group for International Agricultural Research, whose frank objective has been to use the model by which the various strains of Mexipak wheat were developed by Dr Norman Borlaug (with imaginative support from the Rockefeller Foundation) to work similar revolutions elsewhere. Inevitably, not all the thirteen research institutes now under the group's wing have been equally successful. The most conspicuous cloud on the horizon is the problem of the International Laboratory for Research on Animal Diseases in Nairobi, now about to appoint its fourth director in seven vears.

The Nairobi laboratory (known as ILRAD) has been from the start the odd man out, but for good reason. Its origins lie in the accurate calculation that one of the most serious problems with which African agriculture must contend is that of the tsetse fly. The fact that the trypanosomes for which the tsetse is a vector cause African sleeping sickness among human beings is economically and socially important, but the loss of agricultural productivity because large tracts of Africa cannot be used for growing cattle is a more immediate obstacle to change. So what could be more natural than to set up (in 1974) a research institute for the battle against the tsetse fly? That was the calculation. Broadly speaking, the calculation was correct; a handful of dedicated and able scientists in Nairobi have been able to make substantial progress. There is talk of vaccination (of cattle) and of the development of resistant breeds. The trouble is that the laboratory and its management have been fighting not merely against the tsetse fly but also among themselves.

The most spectacular casualty of this squabbling is Dr A. C. Allison, whose resignation after a dispute with the board of trustees was required just a year ago. Can a laboratory that so lightly dispenses with the services of such a distinguished immunologist have serious work to do? That is what outsiders are bound to think. In practice, the board of trustees has scraped along, appointing an acting director of the laboratory first (for three months from 1 January this year) Dr C. Wells, the new chairman of its trustees, previously the head of the Canadian public veterinary service who is, by common consent, the man most likely to rescue ILRAD, and then Professor R. Zwarte from the University of Utrecht, who is also the chairman of the search committee for the new director of the laboratory. Between them, the members of the search committee have recommended to the trustees that the fourth director at ILRAD should be Dr Ross Gray from the University of Edinburgh. At their next meeting towards the end of July, the trustees will be required to decide one way or another.

Trustees of all kinds of bodies are constantly being faced with such dilemmas; which of two proposals to plump for? On this occasion, the managers of ILRAD should firmly say that they are not ready to make such a simple choice. In due course, to be sure, they may be able to enjoy such a clear-cut luxury. Before that, however, they must understand what has gone wrong at Nairobi. Why have they quarrelled so much among themselves (and why have they nevertheless allowed their numbers to grow)? How does it come about that so much good research has been done at ILRAD while such bitter fights have been carried on? Could it perhaps be that those working at the bench know as clearly as they that Africa will not feed itself until the tsetse fly is beaten? And if so, should they not invest time in putting their structure right? The cause is too good to be neglected.

Loose ends on evolution

Next week the trustees of the British Museum (Natural History) will be meeting and will probably be mulling over, as has been their custom for the past several months, the latest batch of correspondence to appear in Nature and elsewhere about the museum of which they are the custodians (and whose name they should change). Some weeks ago (see Nature 4 June, p.373), Sir Andrew Huxley was complaining at the centenary celebrations of the "obscurities and irrelevances" of much of this correspondence, but it is improbable that the trustees will feel able to skip that part of their agenda. By way of explanation, Sir Andrew also invited his listeners to reflect that in giving houseroom to these supposedly empty but entertaining letters, Nature was simply following other commercially-based journals which supply their readers with sensation. (The treatment by The Times of London of a recent sensational murder trial was mentioned.) Readers will no doubt decide for themselves whether the charge is justifiable, but that it is based on a scanty knowledge of how the press works is beyond dispute; the most immediate danger for Nature is that readers will become so bored that they will take to buying some other journal instead. This is one of the reasons why it has now been decided that the present round of correspondence must cease at the end of this month, whether or not the museum has by then chosen to reply to the issues of substance that have been raised.

Nature's role in this controversy is by no means as malevolent as some at the museum suppose. Like any other journal, Nature is to a large extent in the hands of its readers. If people write to say that this or that feature of public policy is mistaken, its first duty is to consider seriously whether what is said deserves a hearing. L. B. Halstead's original complaint that cladism has too great an influence on the museum's exhibition policy (see Nature 288, 208; 1980) is interesting and, if true, important. His further claim that cladism is a cloak for Marxism because Marxist-evolutionists tend to be cladists is illogical (and has been disowned) but is nevertheless an interesting observation even if not strong enough to stand on its own; Halstead will no doubt explain what he meant before the month is out. The most remarkable feature of the correspondence that has occupied the past several months is that it has acquired a life of its own. The original issues have been subsumed in others, while questions have arisen about other matters — the physical arrangement of the museum, for example. From time to tiem, Nature has sought to channel the argument, but not with conspicuous success. The relevance of cladistics to evolutionary studies remains unclear; its place in exhibition policy is a puzzle. The issue of punctuated (as distinct

from gradual) evolution has been raised but not resolved (and cannot be as yet). The status of the theory of evolution — metaphysical or otherwise? — is still disputed, but the museum is probably too agnostic on these questions.

The museum in its public role deserves some sympathy. Nobody should underestimate the difficulties of a public institution in mounting a defence against such a variety of charges. The most obvious difficulty is that the scientific members of this important institution differ among themselves both on the weight they give to this or that aspect of evolutionary theory and also in their opinions of the public exhibition policy. There are now suggestions that some of the criticisms that have been raised in correspondence and by Nature are concealed attacks on individual members of the museum (and Sir Andrew used the phrase "centering around the scientific thinking of members of the museum's staff"). This is not the case. The most controversial member of the staff, the head of the Public Services Division which is responsible for the exhibition policy, is acknowledged to have a flair for work of this kind, and would presumably plough some other furrow with equal skill if the trustees chose to change course. The trustees' problem is to devise a unified policy in circumstances in which the staff of the museum, distinguished as independent scientists, cannot be expected to toe some welldefined line. The museum should soon pay some attention to the problem (which afflicts all public laboratories) of how to marry the need for some coherent outward face with the inevitable diversity of opinion among its staff. The museum, which courageously has not been afraid to amend the most (and

Public but going private

needlessly) tactless part of its newest exhibition, might think of

helping other public institutions to a seemly solution of this

increasingly common problem.

Is the British government about to be still more beastly to the nationalized industries it has reluctantly inherited? There are several signs that this may be the case. Last week, the government decreed that the British Gas Corporation (which has for a year been resisting the proposal that it should cease to be a major retailer of domestic gas-burning equipment) should sell its halfshare in a small onshore oil field in Dorset, evoking the predictable chorus of anguish from the corporation, which is plainly unsure whether it is a public utility or a kind of conglomerate. A more important development has, however, gone comparatively unnoticed. For now, it seems, the British government is bent on making public agencies in the United Kingdom abandon their long-standing belief that their own definition of their own needs should properly take precedence over the needs of their customers in the marketplace. The document put out by the Department of Industry in mid-June, formally a reply to the perceptive report Research and Development for Public Purchasing put out by the Advisory Council for Applied Research and Development more than a year earlier, promises - or threatens - substantial changes in the pattern of British research and development.

The problem is uniquely British. The original report estimated that at least 40 per cent, and perhaps a half, of all applied research and development carried out in the United Kingdom is (or was, in 1975) determined by the needs of British public agencies. The public agencies (government departments, nationalized industries and the research councils) are also eager researchers in their own right; public in-house research and development was running at roughly a third of all British research and development in 1975. A rather greater proportion of defence research and development (some 60 per cent) is put out to private industry on a contract basis than in other fields of the public interest, but this appearance of virtue may be illusory in that the chief beneficiary is the aerospace industry.

The Advisory Council's perceptive complaint, in February 1980, was not that the sums of money involved were too large (which is a different argument) but that government departments

and nationalized industries have wrongly chosen the line of demarcation between themselves and private industry. For, in the most general sense, the objective of most public spending on applied research and development is to define the specification of some product — an aircraft or a repeater in a submarine telephone cable - whose manufacture will afterwards be put out to tender. Manufacturers are always willing enough to put in their bids. Who, after all, wants to turn down business, especially from a customer that can always print the money to pay for what it buys if it cannot otherwise get the funds? The snag, and the chief conclusion of the Advisory Council's report, is that in the cosy relationship that grows up between a paternalistic public sector, willing to shoulder all the cost of research and development itself, and would-be suppliers, scant regard is paid to the long-term interests of the public contractors. Too little of what they make for British government agencies will sell easily elsewhere. Too little of what they spend on research and development goes on the improvement of products that will sell in the wider marketplace.

The Advisory Council's analysis is by no means new. In the 1960s, after a long period of an over-cosy and restrictive relationship with the British Post Office, even the suppliers of telecommunications equipment came to appreciate that the benefits were illusory (yet not a lot has changed since then). The novelty of last year's report was that it advocated a few simple rules of thumb for distributing the balance of research and development between public customers and private contractors. Government and other public agencies have a proper right, even a duty, to carry out research suggesting ways in which the character of their operations may be improved or ways of improving or monitoring the conduct of their existing operations, in matters such as safety. On the other hand, if goods and services are likely primarily to sell to other public customers, the supplier should carry out the necessary research, perhaps with some public help. In intermediate cases, public and private responsibility for research and development should be shared. In general, more publicly supported research and development should be contracted out to private industry.

Rarely can a government's reply to the report of an advisory committee have been as fulsome. With a few justifiable quibbles (where the government is anxious to avoid robbing nationalized industries of their formal autonomy), all the principal recommendations are enthusiastically endorsed. Three government departments (including that for defence, the biggest spender) are said already to have decided to put out more of their research and development. Now the nationalized industries are to be asked to follow suit, but also to find ways in which their own research establishments can sell services overseas. So is a new day dawning for applied research and development in Britain?

The government's enthusiasm is accounted for by its ideology. For months (it seems like years) the government has been talking about "privatization". The Advisory Council's arguments are more objective and thus more persuasive. But everything depends on how effectively the new prescription is carried out. Here doubt persists. The government is probably right not to follow the Advisory Council in the suggestion that there should be an independent board to supervise the fashioning of a new relationship between the public and private sectors in every nationalized industry, but the result will be that final decisions will be made within government departments all too comfortable with past relationships. Conscious as many of the departments will be of the difficulties of managing a substantial shift of resources (for their own laboratories for example) the departments will draw the new lines of demarcation cautiously. This is the respect in which both the original recommendations and the British government's response are inadequate. If the balance of research and development between the public and private sectors is to be shifted substantially, there needs to be some way of anticipating the problems that will arise (such as those afflicting the 75,000 or so people on the government's own research payroll). And there needs also to be, for this purpose and others, some means of letting ministers and taxpayers know what is happening, or about to happen.

White-hot technology lives on in France

New minister plans great leap forward

Paris, June

Part of the mystique of the new Socialist government in France is its almost religious attitude to science, which has startled those on the left who are habitually critical of modern technology. Thus the left-wing daily Libération last week described the Minister of State for Science, Jean-Pierre Chevènement, as a man who embraced growth for growth's sake and sought Soviet-style state control of science. There was no room in Chevènement's policies, said Libération, for the ecological movement or for the debureaucratization, demilitarization and democratization of science.

This is in part true and part false. Chevenement has lost no time in gathering his powers but he is also calling for greater democracy in science planning going beyond the "democracy of the party faithful". He wants science to be "opened up", but chiefly towards industry. And he is not much interested in the environmentalists. On energy policy he is strongly pro-nuclear. And he will have no control of military science, which absorbs 37 per cent of a total French research and development grant of 50,000 million French francs (£5,000 million).

Chevenement sees science rather in the terms of Mr Harold Wilson's British government of 1964. It is the white heat of the technological revolution in which to forge socialism. Science has an "ethical value", he says, in that "there is no socialism without research". He includes in the term "research" most forms of new knowledge — such as social science, which may see a resurgence.

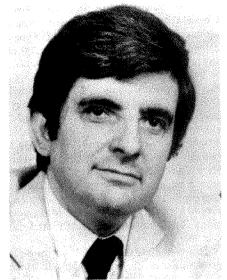
Science in the new government will be expected to help raise the French Gross National Product, to pay for the reconstruction of French society that the Socialist Party has in mind. Some think that an annual growth of 3-4 per cent of the GNP will be needed, compared with 0-1 per cent at present, whence Chevènement's interest in links between science and industry.

During the interregnum, Chevènement battled for weeks with Pierre Joxe, then Minister of Industry, for control of certain key agencies. In the end he appears to have won, although the relevant decree is not yet signed. Joxe has left the government to head the Socialist Party in the new assembly, and the promised decree should give Chevènement the powers he wants.

Specifically, he wants full control of the

Centre National de la Recherche Scientifique (CNRS), the principal body supporting basic science (including social science and humanities) in France; of the Agence Nationale pour la Valorisation de la Recherche (ANVAR), which primes the innovation pump by seeking ideas in universities and government laboratories and supporting their development in industry; of the Mission Interministerielle pour l'Information Scientifique et Technique (MIDIST), an interministerial agency which seeks to improve the national flow of information on science and technology; and of the Délégation Générale à la Recherche Scientifique et Technique (DGRST), which will perform as his administration.

Chevènement (subject to the decree) has also won power over other research agencies which will remain nominally within their present ministries. He will be in control of their budgets, which gives him effective power over their work but not their appointments — a formula he proposed to save ruffled feathers at the ministries he was plundering. These are the Commissariat à l'Energie Atomique (non-military work only), the Centre National d'Etudes Spatiales and the agricultural, medical, ocean science and overseas development research councils. In total,



Chevenement, ready to go . . .

Chevenement will control directly a budget approaching FF 20,000 million.

If the government can hold to its election promises, this budget should rise by some 10 per cent a year until 1985; and by means of low interest loans to industry for research and development projects, the FF 30,000 million industrial sector is planned to rise at the same rate.

For the moment. Chevenement -

Universities jeopardize German budget

A row in West Germany over the funding of new university buildings culminated last week in the rejection of the entire federal budget for 1981. The Bundesrat, the upper chamber of the parliament whose members are the presidents of the eleven Länder, threw out the budget approved on 6 June by the Bundestag, the lower house, on the grounds that it makes insufficient provision for new construction in universities. It was the first time since 1963 that the Bundesrat has overruled the Bundestag-approved budget.

The majority of Länder governments claim that by allocating only DM 680 million for university construction in 1981, compared with the DM 850 million originally planned, the federal government is reneging on its constitutional obligation to meet half of the costs with the Länder. The Bundesrat says that the reduced sum is not enough to pay for projects already under way, let alone for embarking on new ones. Herr Albrecht, president of the Bundesrat, is said to believe that a figure nearer DM 1,300 million is needed if the universities are to expand to meet rising demand. So great is the sense of grievance of the Länder governments that they are claiming that the Bundestag has violated a basic constitutional law - which states that the costs of construction should be shared equally between the Länder and the federal government — and they are taking their case to the constitutional court.

The matter has also been referred to an arbitration committee which is due to meet next week. An earlier attempt at compromise, which would have guaranteed the Länder reimbursement after 1984 for extra money spent in 1981, failed. But it is now hoped that the Länder will agree to accept reimbursement of DM 120 million in 1982 and the remaining DM 50 million in 1983.

Until this year, cuts in the West German federal budget were practically unheard of. But the worsening economy has forced the government to look for savings. The Länder have used the constitutional obligation on university construction funds to challenge the government's plans for savings and are said to favour cuts in agencies with large bureaucracies such as those dealing with employment and social security.

West Germany has been expanding its universities since the early 1970s and has already spent DM 25,000 million, mainly on upgrading technical colleges to university status. The plan is to invest another DM 20,000 million if the economy can stand it.

The recurrent grant to universities, which is paid by the local Länder governments, is not involved in the dispute.

Judy Redfearn

assuming the decree is forthcoming — has three targets.

First, there is an adjustment to the 1981 national budget, due in July. Here Chevenement does not expect much money, but he has promised 525 new research jobs among his agencies, 200 of them for scientists (the rest for technicians). In the agencies over which he has full control (such as CNRS) he will favour the social sciences, basic biology, medical sciences and (surprisingly) environmental science.

Second, there is the full 1982 research budget, to be fixed in August this year. Here Chevenement plans the full 10 per cent growth, in real terms, and plans to make biotechnology, microelectronics and robotics priority subjects, and to support ailing but important subjects such as chemistry.

Third, in 1982 he will seek a "loi de la programmation de la recherche", in which the National Assembly would vote a firm five-year plan and budget within which his ministry could operate without fear of political setback year to year. Such a "loi programme" has not been tried before outside defence, and it will be a supreme test of Chevènement's undoubted political powers to get such a law approved.

To that end, he is calling a national colloquium of scientists and others interested in science policy for December this year, to thrash out a "new politics" for science and rally support. Robert Walgate

US science research

Keyworth reassures

Washington

In his first public appearance since being nominated as President Reagan's Science Advisor and director of the Office of Science and Technology Policy, Dr George A. Keyworth II said last week that the new Administration "views basic research as a vital investment with a good return", that it would seek to increase industry's involvement in research and development and that greater international collaboration was essential in such capital-intensive fields as high-energy physics and space research.

On a less positive note, he warned that the United States could no longer expect to dominate research in all scientific disciplines and should accept that "our country has relinquished its pre-eminence in some scientific fields". He also stressed that support for research should be aimed primarily at those areas considered important for industrial, military or other essential technologies, or where efforts were proving particularly productive, such as computer science and genetic engineering.

Dr Keyworth's remarks were made in a speech to a meeting organized by the American Association for the Advancement of Science (AAAS) to discuss recent trends in the federal research and develop-

ment budget. Many of those present were impressed that although Dr Keyworth, a 41-year-old nuclear physicist who has spent most of his career at the Los Alamos Scientific Laboratory, has virtually no experience of Washington science policy circles, his remarks demonstrated a grasp of many of the issues facing the Administration.

Asked about attempts by the Office of Management and Budget to apply strict cost-accounting procedures to university scientists, for example, Dr Keyworth replied that he himself had never been required to punch a time-clock, but that there were arguments about public accountability that needed to be closely considered. He also expressed concern at the possible damage to international relationships that might follow budget-based decisions to withdraw from projects such as the International Solar Polar Mission, being planned jointly with the European Space Agency.

In his speech, Dr Keyworth emphasized that he saw his role as an "objective adviser" in the White House rather than an inside lobbyist for the engineering and scientific communities. "The Science Advisor in this Administration perhaps more than in any other will be an important member of a *team* of domestic and international advisers", he added.

The content of his speech made it clear that Dr Keyworth is likely to pursue many of the same goals as his predecessor, Dr Frank Press. Referring to the need to reduce the burden of "unnecessary" regulation on the private sector, he said that the Office of Science and Technology Policy would continue to work closely with others in the Administration to strengthen the scientific foundation of federal regulatory decision-making.

However, Dr Keyworth, who has been closely involved in nuclear weapons research at Los Alamos, said he believes that "our country's military might should be second to none", and has also been reported as saying that within the new Administration "science and engineering that support the development of a stronger defence have top priority."

In previous administrations such remarks might have been taken as a warning signal by the biomedical research community. But the commercial potential of genetic engineering, being a rapidly expanding field of high technology, seems to have kept such research in favour. "In general, I think we will see continued strong support for the biological and biomedical sciences", Dr Keyworth said.

Other speakers from the Administration who addressed the AAAS meeting supported the contention that basic science had little to fear from Mr Reagan, and that cuts in the research and development budget were intended to fall primarily in those areas where private industry might shoulder responsibility.

Many of those attending the conference,

however, were not totally convinced. Several questions were raised about the Administration's proposal to eliminate science education programmes at the National Science Foundation (some of which appear to have been salvaged by Congress). Others, such as Dr Ronald W. Lamont-Havers, director of research policy at the Massachusetts General Hospital, expressed concern that even though the Administration might believe in the need to support scientific excellence, budget constraints on research funding were likely to result in high-quality research not receiving adequate federal backing.

David Dickson

EEC university qualifications

Degree of agreement

Brussels

Education ministers from the European Community's ten member states, meeting in Luxembourg in June, considered the tricky problem of improving the mutual recognition of diplomas and periods of study undertaken at higher education institutes.

In theory the principle of the freedom of employment set out in the Treaty of Rome should guarantee that a qualification gained in one member state should open the same economic doors in another state, but this is far from being the case. As one example, a teacher with a degree from the University of Lille who spent her working life in Belgium was not granted her full pension rights because the Belgian state did not recognize her qualification.

No one has been foolhardy enough this time to suggest establishing a Euro-degree on the lines of the much-maligned Euroloaf or Euro-beer. The low-key approach adopted by the European Commission is to improve the availability of information. EURYDICE, the European information network on education which became operational last year, would link up with national units to provide information in five key areas - entry requirements for first degrees, transfer without loss of acquired rights from a course started in one country to a similar course in another member country, requirements for postgraduate studies or research in another member state, finding work in a member state with qualifications obtained in another, and details of career development.

The Education Council has approved these ideas and the Commission has until March 1982, when the education ministers meet again, to put forward some precise ideas on implementing the scheme.

But this leaves the crucial problem untouched. Should the Community risk an outcry from the academic world by forcing universities to accredit each others' degrees with the same value and worth? Desirable though that would be as a means of increasing the mobility of European academics, it would ride roughshod over the treasured traditions and autonomy of the institutions.

As a principle or ideal it was welcomed by the ministers and has few opponents in the academic world. The difficulty is that the Education Council realized that the edict cannot be enforced by law. Efforts over the past twenty years to encourage bilateral or multilateral agreements have not kept pace with the growth of higher education and diplomas. The Commission has now been instructed to persevere in its efforts to find ways of encouraging more agreements.

Jasper Becker

High-energy physics

CERN in a hole

The Large Electron Positron ring (LEP), the future project of the European centre for subnuclear research (CERN) near Geneva, has run foul of a small group of environmentalists formed a decade or two ago to protect the Jura Mountains, north of the CERN site, as a national park. Last week the group obtained an injunction at a Lyon tribunal which annuls CERN's licence to bore an exploration gallery for the LEP tunnel into the Jura.

The Lyon decision rests on a technicality. It stipulates that the exploration gallery is not a "temporary construction", as it must be according to the law under which CERN began drilling, but a permanent one. CERN counters that if the tunnel revealed that the construction of LEP would be impossible, then the work would indeed be temporary. However, CERN intends to use the 4 km tunnel as an entrance gallery if LEP goes ahead — so their position was not too strong.

Meanwhile, CERN has not been informed officially of the injunction. Being an international organization, its relations with states — including Switzerland and France — on whose territory it rests, are mediated through the foreign ministers of those states; and there will now be an internal wrangle in France either to get the Lyon decision overruled, or to gain approval for the gallery under another law.

The conservationists, the Association de Protection de la Nature Gessienne (that is, of the Pays de Gex where the gallery was to be drilled), are worried about potential damage to natural springs in the region, and atmospheric pollution from LEP power supplies and cooling systems.

The group timed its injunction well: it coincided with the mid-year CERN council meeting at which national positions on the LEP project were being sounded. In the event, the French delegate at the council, M. Yves Jacques, promised to appeal against the Lyon decision at the highest court — the Conseil d'Etat — and the voting was as previously expected: eight clearly in favour and four undecided. Of

the latter, Denmark expects parliamentary approval soon; after a confusing general election, the Netherlands wishes to delay a decision; Sweden is in similar position after the collapse of the Conservative government; and Norway wishes to wait until after its general election on 14 September.

A committee of the CERN council is to meet in October, and if member states are then ready, a full meeting could be convened to reach the unanimous approval that CERN seeks. (Strictly the eight-tofour majority is sufficient, as LEP will come within the normal CERN budget; but unanimity on such a big commitment more than SF 600 million (£150 million) for five years or more - is thought politic.) At that final meeting, which could be delayed to December, the central issue is likely to be not whether the states all approve of LEP, but at what budget they want it built which affects the speed of LEP construction. Argument on the budget was heated in the meeting last week, and no agreement was reached. Robert Walgate

Polish economy

Culling ministries

The Polish government's plans for economic recovery will be based on a major restructuring of the country's production ministries, Prime Minister Jaruzelski announced in the Sejm (parliament) recently. During the past few years, there has been a tendency for Polish ministries to proliferate - for example, separate ministries for the metallurgical industry, for the engineering industry, and for heavy and agricultural machinery existed at the same time. Even during the past few weeks, there have been suggestions that some of Poland's most urgent problems, environmental protection and geological surveying among others, could best be tackled by creating new ministries.

The new plan proposes replacing ten existing ministries with four larger ones. These four would cover agriculture, food procurement and production, and forestry; mining of coal and lignite and energy production; heavy industry and machine construction; and chemical processing of timber, natural and artificial fibres, and the ceramics, glass, cellulose and paper industries.

General Jaruzelski went on to remind the Sejm of some of the mistakes of past years, when separating the mining industries from power production, and machine building from foundry production caused serious losses in productivity.

The new proposals have not met with unqualified approval. The Sejm Commission for Forestry and the Timber Industry is unhappy at combining forestry with agriculture, but the corresponding commission dealing with agriculture and the food industry seems fairly happy with the union.

Vera Rich

US standards bureau

Wider horizons

Washington

The US National Bureau of Standards, traditionally a preserve of the physical and engineering sciences, is being asked by a congressional committee to broaden its horizons and take on responsibility for the burgeoning field of biotechnology. The recommendation is one of several being put to the bureau as a way of "enhancing the technological and scientific base of the nation's industrial capability".

There has been little reaction yet from the Reagan Administration to the proposals, which have been drafted by the science, research and technology subcommittee of the House Committee on Science and Technology. Secretary of Commerce Malcolm Baldridge told the committee at a hearing in Washington last week that the whole internal organization of his department, which is responsible for National Bureau of Standards, is under review. He talked of an important role for the bureau - in helping to boost US exports by providing data about foreign product standards but said it was too soon to be specific about changes.

The changes being proposed are in line with the findings of recent studies of the bureau, including reports from the General Accounting Office and the Congressional Research Service. In addition to its traditional responsibilities for weights and measures, several committee members, including chairman Doug Walgren, are convinced that the standards bureau could have a central role in federal efforts to stimulate technological innovation in the private sector.

The bureau's principal responsibility has been to help ensure the compatibility of measurement standards needed by industry, consumers, the scientific community and other organizations. In recent years Congress has given the bureau various additional functions, such as administering the Center for Fire Research and the Center for Consumer Product Technology. But often the bureau has been given a new role without adequate funding with the result that other programmes, have been cut back or eliminated.

Last year both the Senate and the House of Representatives held authorization hearings on the bureau's budget to explore possible remedies. Now the House committee is drafting a bill making the bureau's responsibilities more explicit, but also intended to enlarge its contact with the broader research community.

Many of the committee's suggestions are uncontroversial. There is general agreement that adding the biological to the physical sciences would be appropriate, in particular in the development of standards, testing procedures and measurement instrumentation needed for the advancement of biotechnology and genetic

engineering, although there is concern that the changes should not go too far for fear of conflict with work already being carried out by the National Institutes of Health.

There is widespread support for a proposed amendment that would require the National Bureau of Standards to engage in international cooperation, and that it should maintain measurement research and development as its top priority. The bureau's director, Dr Ernest Ambler, welcomed the committee's recommendation that any new new functions should be provided with adequate funds and manpower.

The committee's suggestion that the bureau be given authority to promote economic growth by "contributing to the development of the basic technologies which underlie product and process development, technological innovation and productivity" raises greater difficulties. Although the bureau does at present support research aimed at the general enhancement of industrial innovation—for example in computers or machine tools—these are undertaken only when they can be shown to be measurement-related. Members of the committee argue, however, that in practice

Support for Argentinian

Leblon, Brazil

Professor Nicolas G. Bazan, sacked in controversial circumstances as director of the Biochemical Research Institute of the University of the South in Bahia Blanca, Argentina (Nature 14 May, p.100), was greeted enthusiastically at a symposium last month organized by the Argentinian Academy of Sciences in the city of Córdoba.

Several of the foreign biologists invited to the symposium, on molecular aspects of the nervous system and memory, carried letters from their professional societies protesting against the dismissal of Bazan and the continued arbitrary attacks by the Argentinian government on scientists. Among them were letters from the American Society of Neurochemistry and from the European Society of Neurochemistry and from the European Society of Neurochemistry. In spite of the risks of reprisals, the Argentinian academy publicly endorsed these protests from the international scientific community.

Bazan has been invited to visit the United Kingdom to attend the forthcoming biannual meeting of the International Society for Neurochemistry in Nottingham next September, where a special symposium on the human rights issue in Latin America is being planned. It will deal in particular with the situation of the several hundred Argentinian scientists who are reported as "disappeared". "arrested" or "summarily dismissed".

Maurice Bazin

the bureau already seems to be stretching its definition of measurement in some of the research that it supports. If the organization feels it is limited by the terms of its existing statute, then the solution, they argue, is merely to change the wording of the legislation.

Several witnesses at last week's hearing felt the bureau could have the wider role envisaged in the proposed legislation. It would unduly limit the potential value of the bureau to the nation if its only activities were in the field of metrology.

The committee suggests that the bureau strengthen its links with the academic community by being allowed to make grants and to award fellowships in science and engineering. But Mr William Carey executive officer of the American Association for the Advancement of Science and until recently chairman of the bureau's board of visitors, warned against this on the grounds that "the granting business these days is bogged down with rules and controls for fiscal accountability" and that "adding another granting agency to the confusion would be regrettable".

How far the committee's recommendations go will depend greatly on how far they agree with the Department of Commerce's own views. Mr Baldridge has already eliminated the position of assistant secretary for science and technology, the post previously occupied by Dr Jordan Baruch, a principal author of President Carter's domestic policy review on industrial innovation. Upgrading the National Bureau of Standards would be one way of filling the resulting gap.

David Dickson

India in space

Another orbit

Lucknow

India's latest venture into space was the launch of the experimental telecommunications satellite, Apple, aboard the third Ariane test flight last month. Apple is unlikely to prove as successful as the Indian Space Research Organisation must have hoped after one of the two solar panels failed to deploy shortly after launch. But there is a chance that such a small satellite, 616 kilogrammes, can operate satisfactorily with one solar panel for long enough for the bulk of the experimental work to be done.

The Apple programme is only one of the fronts on which India is advancing into space. At the end of May, as part of the first developmental flight of its satellite launch vehicle SLV-3, India placed a 38-kilogramme satellite into a low Earth orbit. That was the second satellite to be put into orbit using the Indian rocket, the first having been launched last July (Nature 286, 324; 1980).

The latest Indian-launched satellite, designated RS-DI, carried a solid-state

sensor capable of identifying salient features on the Earth and transmitting data back to the ground stations.

The main objective of the Apple satellite launched on Ariane but built at the Space Application Centre in Ahmedabad, is to gain experience in the operation and management of communications satellites. Yet another satellite, Bhaskara II, is due for launch at the end of the year and is for the development of remote sensing technology.

Experiments planned for Apple include logging the movements of railway wagons and of railway passenger reservations in large cities, the setting up of an interlibrary information system and the development of a "national classroom" for postgraduate engineering students at the five Indian Institutes of Technology. Under the last programme, students at the five centres will be given televised lectures and be able to question the lecturers directly.

Zaka Imam

Occupational health

US industry to pay

Washington

In a decision that represents a victory for the US labour movement and a major blow to the Reagan Administration's efforts to reduce the burden of health and safety regulations on private industry, the US Supreme Court has ruled that the Department of Labor's Occupational Safety and Health Administration (OSHA) is not required to carry out rigorous costbenefit analyses of new regulations covering exposure to toxic substances before the rules are put into effect.

The drive to put occupational safety and environmental regulations on a strictly cost-benefit footing has been central to the new Administration's 'regulatory reform' strategy. In line with this, the head of OSHA, Mr Thorne Auchter, asked the Supreme Court in March to delay a decision on whether regulations introduced by the Carter Administration in 1978 to reduce workers' exposure to cotton dust were invalid since they had not been accompanied by a thorough analysis of the relative costs and benefits.

However, the court has turned a deaf ear to the agency's request. In a move that will now require both OSHA and the White House to ask for new legislation from Congress if it wants to pursue the strategy, the court ruled by five votes to three, with one abstention, that there is nothing in the law to require such analysis. Rather it has accepted the interpretation of OSHA officials under the previous administration, that the mandate requires the agency to do all it can to reduce health hazards to workers, short of bankrupting the industry concerned.

The new cotton dust regulations, which are already being implemented by most large textile manufacturers, are aimed at

lowering the incidence of byssinosis (brown lung disease) among the nation's 800,000 textile workers. In 1978 OSHA issued regulations requiring the permissible level of cotton dust to which workers could be exposed to be reduced from 1,000 to 200 microgrammes per cubic metre of air.

Even at the time the ruling was highly controversial. Whereas OSHA officials estimated that the cost of compliance to industry would be about \$650 million, industry itself put the figure at \$2,700 million.

The Supreme Court's decision is the final step in a legal challenge launched by the American Textile Manufacturers Association, asking that the rules be disregarded because the agency had not demonstrated that the expected benefits outweighed the likely costs of implementation. If the appeal had been sustained, it would have allowed the Administration to proceed relatively unimpeded with its plan to apply strict cost-benefit analysis to various regulations already in effect — for example those covering exposure to arsenic and lead — with a view to reducing their severity.

This strategy will have to be reassessed, now that the Supreme Court, in a ruling by Justice William J. Brennan, has concluded that OSHA is not required to carry out such an analysis, but merely to determine that its regulations are technically and economically feasible.

Although professing to welcome the justices' verdict, since it formally supports the government's position, OSHA officials also acknowledge that it is a set-back to their attempts to chart a new course for the agency that would be more congenial to private industry. Mr Auchter has already made clear his strong disagreement with the philosophy and policies of his predecessor, Dr Eula Bingham.

Predictably the court's verdict has been warmly welcomed by labour unions representing textile workers, but their jubilation may be premature. Already OSHA officials have revised their approach to new regulations in the light of last July's ruling by the Supreme Court, in rejecting proposed new standards for exposure to benzene (Nature 286, 97; 1980). And following the cotton dust decision, a spokesman for the textile manufacturers said that the companies "continue to believe, as does the Administration, that cost-benefit analysis is an important element in achieving rational and costeffective rule-making'

But much of the fire has disappeared from the textile industry's complaints, Many companies acknowledge that their calculations of likely costs were based on the conversion of old equipment and that new regulations have led them to buy new machines that have already produced significant gains in profitability. It is a silver lining that few would have expected when the regulations were hailed as a large black cloud over the industry

David Dickson

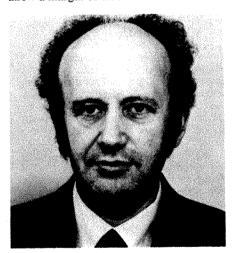
Dietary vitamin C

Bigger daily dose?

Britain's answer to Linus Pauling now argues that the government should raise the recommended daily amount of vitamin C from 30 mg to 100 mg—enough to saturate body tissues with the vitamin, but nowhere near the "megadose" levels Pauling advocates.

The new British "Mr Vitamin C" is a mild-mannered Welshman, Dr R. Elwyn Hughes of the University of Wales Institute of Science and Technology. His book on the subject has just been launched by the British Nutrition Foundation, which though supported entirely by the food industry is at pains to provide "balanced" information on nutritional matters. The foundation supports Dr Hughes's recommendation that the government should reassess the criteria by which recommended daily amounts are arrived at, but makes no commitment to the actual level of vitamin C we need. On this Dr Hughes — officially — stands alone.

We owe the British recommended amounts to a group of conscientious objectors who in 1940 were subjected to zero vitamin C diets. They soon showed the symptoms of scurvy, and their intake of vitamin C was raised slowly until the scurvy disappeared. It disappeared at 10 mg a day; so the government tripled this amount to allow a margin of error.



Britain's Pauling?

However, the past decade has seen the discovery of useful functions for vitamin C other than just preventing scurvy, says Dr Hughes, such as involvement in lipid metabolism and atherogenesis; brain metabolism; carnitine metabolism (which relates to muscle fatigue and lack of stamina); resistance to infection; and the detoxification of potentially harmful drugs, including certain tranquillizers. Although such evidence is incomplete it is sufficient to commend tissue saturation levels of the vitamin, he concludes.

According to British dieticians, the average daily intake of vitamin C in the United Kingdom is 60 mg, equivalent to the

content of two fresh oranges, But many people take much less. In a survey of old people's homes in South Wales, Dr Hughes discovered average intakes of less than 20 mg, and in some cases less than 10 mg.

Dr Hughes does not recommend megadosage, however. Its benefits are unproved, he says. Half of any excess over saturation is swept out in the faeces, and the rest rapidly in the urine; and moreover there is evidence of disadvantage, in particular that toxic metals can be forced across the placental membrane by high vitamin C fluxes.

Robert Walgate

Soviet mineral supplies

Digging deeper

Talk in the West of a "resource war" between the Soviet Union and the United States seems to have prompted a reaction from the Soviet Minister of Geology, Evgenii Kozlovskii. Talking on Radio Moscow, he said that speculation that the Soviet Union would soon become a net importer of mineral ores was mere Western propaganda.

The minister's statements may have been a response to the recent meeting of geologists and Soviet watchers at the Johns Hopkins University (see Nature 11 June, p.444), which concluded that while there was little evidence to support the view of some of President Reagan's more "hawkish" advisers that the Soviet Union was deliberately waging a resource war, there did appear to be production difficulties in the Soviet Union leading to a cutback in mineral exports and the stepping up of imports.

According to Kozlovskii, the Soviet Union can not only meet its own mineral requirements, but also has surpluses for export. Although his assertion that the Soviet Union has the world's largest known deposits of coal, gas, iron, manganese and apatite is probably true, it does not follow that sufficient supplies are available to Soviet industry.

At present, apparently, Soviet geologists are discovering new mineral deposits faster than the mining engineers can extract them. But there is still great emphasis on new methods of surveying for minerals, including satellite surveys and the drilling of deep and "super-deep" boreholes.

Deep boreholes, in fact, are to play a big part in Soviet geophysical research in the 1980s. The first such hole, on the Kola peninsula, reached a depth of 10,800 metres and a new hole to be drilled in Western Siberia is thought to be of particular significance because of hopes of finding oil deposits in Pre-Jurassic strata at depths of between 5,000 and 7,000 metres. Even deeper drillings are being planned, to depths of greater than 13,000 metres, where high ambient temperatures (up to 350°C) and high pressures will require new drilling techniques and monitoring Vera Rich instruments.

CORRESPONDENCE

Defence for Chelsea

SIR — Your correspondent, in summarizing the recommendations of the Committee on Academic Organisation of London University, has failed to comment on an alarming feature of the discussion document. The committee state that their judgements on academic standards in institutions are based on "general reputation" and their own personal opinions. It is on this basis that it is implied that the majority of departments at Chelsea College are not of the standard of the university at all. How else can those who survive the proposed "peer review" be accommodated on a "single site", given the present size of the college?

The University of London is a great, but complex, institution. It faces brutal cuts in funding over an alarmingly short period of time and it was thus wise to have set up the Committee on Academic Organisation under Sir Peter Swinnerton-Dyer to assess the implications of the expected cuts and advise on their implementation with minimal damage to academic standards. But the committee has yielded to the temptation of extrapolating objective financial analysis into arbitrary recommendations based on subjective and highly damaging prejudgements. Instead of setting the scene for cooperative rationalization and contraction, the document has generated anger and dissention.

The committee had already clearly established that for years the sub-allocation of the UGC block grant to London by the Court of the University has favoured certain colleges at the expense of others. Inevitably, the high unit-cost colleges, with their favourable facilities and staff ratios, have tended to attract more research grant monies than have the low unit-cost colleges. Nevertheless, and in spite of the conditions under which they have been forced to operate, the under-provided schools have not only become better integrated and more innovative than the giants, but contain many departments of true distinction.

Yet the committee's conclusion is that it is "unthinkable" that the necessary savings should be made where the high costs have been identified. Instead, the worst of the burden is to fall on colleges that, having been starved from the centre, are now judged, a priori, not to be academically excellent — as if, in any case, excellence can ever be ascribed to a college rather than to individual departments.

Chelsea College has survived a succession of externally-engineered crises, and in the face of them has grown from strength to strength. Operating at exceptionally low unit-costs we still have built up departments that are among the biggest and best of their kind in the university, and some of which have a major international reputation. Why then have we been singled out for gratuitous attack? Is it simply and cynically that we are a sitting target, fully-stretched financially and spatially, and big enough if destroyed to spare others their share of the agony?

A simple way to destroy a college is to malign its reputation, so that potential students and benefactors and the various grant-giving bodies shy away. If this is not what the committee consciously set out to do, then the publication of the discussion document represents a sad error of judgment.

The attack on academic standards at Chelsea College not only offends us, it insults boards of studies of the university and its external examiners, jointly charged with maintaining standards in their subjects. They are, by implication, accused of incompetence.

The committee should now retract their scenarios and their gratuitous insults to Chelsea and to the other colleges judged to be "weak", and leave the schools themselves voluntarily to suggest rationalization within objectively defined guidelines. Otherwise, the university should forthrightly reject as incompetent the latter part of the report.

H. BAUM

Department of Biochemistry, Chelsea College, London SW3, UK

Postdocs are OK

SIR — I object strongly to your comments regarding the future for postdoctoral fellows in the United States (*Nature* 11 June, p.441). The 50 per cent increase in postdoctoral grants since 1972 represents an expansion of the equality of opportunity for recent graduates, and the federal policy-makers responsible ought to be applauded, rather than attacked with words like "demeaning employment", "exploited", and "scandal". You confuse an equality of *opportunity* with an equality of *outcome*. Sadly, this is a common confusion that muddies the minds of the liberal thinkers of both our peoples, and breeds frustration amongst the unfortunate victims that subscribe to their thought.

The value of postdoctoral study and its "prize" of an academic post remain intact ("88 per cent of the most recently appointed assistant professors in chemistry had done a postdoctoral stint" etc., p.443). For many, this study is preceded by an overkill of classroom exercises and examinations, and followed by endless faculty committee meetings and governmental paperwork, leaving only those precious postdoctoral years where one can devote full time to research, and attempt to achieve the scholastic maturity necessary for undertaking truly independent investigations. (Incidentally, independence is the mark of a "professional", not the social status, health insurance, or salary implied by your comments and the juvenile anecdotes of the disappointed. Anything less denotes hired help, regardless of training, qualification, or special ability.) The statistics of the Grodzins Committee fail to take into account those students of highest ability and fortune who achieve this maturity while earning their degree (all are supposed to) and therefore do not need a postdoctoral period of additional opportunity. For the remaining majority, it stands to reason that an increase in the numbers electing to enter this race for a fixed number of prizes will be followed by an increase in the numbers who do not win. Is that so surprising as to deserve front page coverage? Is it morally offensive? Is it indicative of someone in authority having failed in their responsibility, as you state? I think not. You propose that those who do not win "should be paid a bounty, a kind of retrospective recompense for deprivations" Now that I do find morally offensive, lying somewhere between race-fixing and squandering public money on a losing proposition, or to be blunt, on losers.

Dexter B. Northrop University of Wisconsin-Madison, USA

Cox sure?

SIR — Barry Cox's comments on the British Museum (Natural History) exhibit on Origin of Species (*Nature* 4 June, p.373) contain an incredible statement which must not pass without challenge. Otherwise, the creationists' claim that evolutionary science is really dogma will have received the *imprimatur* of your journal. The statement is:

"We [biologists] don't even think that it [the evidence] could support a dramatically different scientific (sic) theory, in the way that earlier observations of the heavens were transformed from being compatible with an Earth-centred Universe to demonstrating a Sun-centred Solar System."

As a practising biologist, I wish to register my dissent. Surely, Dr Cox got carried away. Does he understand the implications of novel findings in genetics so completely that he can make such a statement with serenity? Is it certain that new data on genome organization and variation will not lead to fundamentally new ideas about the mechanisms of speciation? Have we fully assimilated the lessons of overlapping and interrupted coding sequences, mobile genetic elements, and somatic differentiation by chromosome rearrangements? With all due respect to Dr Cox and the many scientists who believe that the problem of evolution is solved "in principle", let me state my conviction that there is a great deal of aptness in the analogy between Ptolemaic astronomy and our current understanding of evolution.

JAMES A. SHAPIRO Department of Microbiology, University of Chicago, Illinois, USA

Fit for what?

Sir - In his article (Nature 4 June, p.373) on the new Origin of Species exhibition in the British Museum (Natural History), Dr Cox quotes the sound track of a film loop "The Survival of the Fittest is an empty phrase, it is a play on words". This appears to refer to the widespread belief that "survival of the fittest" is a tautology, because our only measure of the fitness of an organism is its ability to survive. This is not so; in the long run survival is a problem for palaeontology, because fossils are our only evidence of what has failed to survive. And in palaeontology, there is a technique which can be used to estimate fitness (in the ordinary dictionary sense). This uses the "paradigm" method developed by Rudwick¹ for testing functions inferred from structures in fossils. The structure concerned is compared with a paradigm which is the ideal for the performance of that function (due allowance being made for the nature of animal materials). Paul² has taken this farther, and compared with the appropriate paradigms a series of structures which perform the same functions in different primitive echinoderms. He concluded that, for the functions of protection, feeding and respiration, the echinoderms of the Cambrian and Ordovician were less efficient than their successors. Moreover, this was compatible with the elimination of the unfit by competition (which is "survival of the fittest" in reverse).

Continued on p. 95

NEWS AND VIEWS

Modern light microscopy

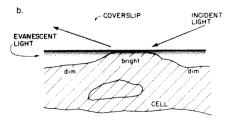
from Michael Spencer

New techniques in electron microscopy have over the years been matched by innovations in light microscopy, which retains the unique advantage of being applicable to living cells. Two recent papers illustrate this well, and also emphasize that in several respects the conventional formula for the resolving power of a light microscope may be said to underestimate the potential of the instrument.

The first, by Daniel Axelrod (J. Cell Biol. 89; 141, 1981), is about a new application to microscopy of total internal reflection fluorescence (TIRF), a branch of spectroscopy dating from the sixties. It complements another existing technique (also introduced about twenty years ago) called interference reflection microscopy, which can show up cell-substrate contacts as darker regions against a background of light reflected from other areas of cell surface. In the case of TIRF, however, the opposite situation applies: only the contact areas give rise to light collected by the objective.

Light from a laser (Fig. 1) enters a glass or fused quartz cube at such an angle that it undergoes total internal reflection at the interface between a glass coverslip and a film of medium containing cultured cells. The cell membranes have previously been 'doped' with a fluorescent label. Although geometrical optics predicts no transmission of light across the interface, electro-

COVERSLIP



magnetic theory shows that a disturbance which Axelrod calls an evanescent wave (a term taken from diffraction theory) will penetrate into the liquid medium. This wave propagates parallel to the surface with an intensity that decays exponentially with perpendicular distance from the interface; although in the ideal theoretical case no energy crosses the boundary, many observers (from Newton onwards) have shown that this is not true in practice. Within a thin layer adjacent to the interface, fluorescent molecules can be excited by the evanescent wave.

The depth d at which the wave's intensity falls to 1/e of the incident value depends on the angle of incidence; the larger this angle, the smaller the value of d. It is possible to arrange that most of the fluorescence is confined to a layer only about 100 nm thick, which is considerably less than the wavelength of the light; even smaller values would be feasible with the use of highrefractive index components. The technique allows membrane-bound receptors, or elements of cytoskeletal structure, to be selectively labelled and shown up without interference from fluorescence arising elsewhere in the specimen. Furthermore, by varying the angle of incidence (and hence the depth of excitation) one can map the topography of the membrane facing the substrate. Figure 1(c) illustrates the kind of picture that is obtained.

The second paper concerns the application of polarizing microscopy to biological systems. Shinya Inoué is the doyen of workers in this field, and has for many years been engaged in pushing the technique to the limits set by current technology. Visitors to the inner sanctum where he conducted some of his experi-

Fig.1 TIRF microscope apparatus for viewing cells in culture. (a) The optical system on the microscope stage; (b) magnified diagram of the evanescent wave at the coverslip/solution interface, exciting fluorescence of those portions of a cell in close contact with the coverslip. (c) Human skin fibroblasts labelled with a fluorescent marker and illuminated by TIRF. Angle of incidence 74.3°, d = 105 nm. Bar = 30 nm. From Axelrod, D. J. Cell Biol. 89, 141 (1981).

ments were required to exchange their shoes for carpet slippers, lest particles of New England dust should contaminate the optical surfaces and give rise to unwanted scattering. His latest report (*J. Cell Biol.* 89; 346, 1981) concerns the improvements now possible with the use of video image processing.

The most serious problem in observing weakly birefringent objects in a polarizing microscope is that the method is a darkfield one; very little light is collected, and even if the dark-adapted observer sees something clearly, it can require a long photographic exposure to record the image. Furthermore, the background level is raised (and contrast reduced) by polarization effects arising at lens surfaces. Inoué tackled this problem more than twenty years ago and showed that it could he ameliorated by the use of 'rectified optics' incorporating additional elements. However, this still does not enable one to use the highest-quality objectives that are essential for achieving maximum resolution, because these incorporate fluorite lenses that contain crystalline inhomogeneities whose effects cannot be compensated for.

With video recording and reproduction, however, it is simple to subtract an unwanted background level electronically, and thus enhance the contrast. With a highly sensitive video camera one can also



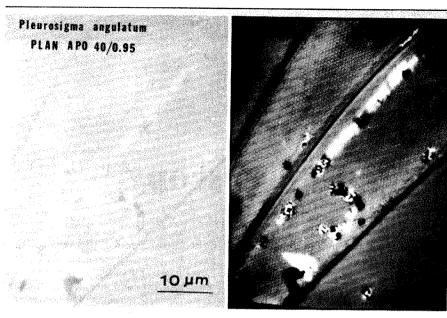


Fig.2 Appearance of a diatom in video-polarized light microscopy, using a plan apochromatic objective. The left scene was adjusted to match that seen by eye through the ocular, while the right scene was electronically adjusted for optimum contrast. The diatom was embedded in a medium of nearly matching refractive index. From Inoué, S. J. Cell Biol. 89, 346 (1981).

reduce the exposure time for photography, so that rapidly moving objects can be followed. Just how striking the improvement can be is illustrated by Fig.2, which compares a diatom as seen by eye through

the microscope with the image photographed from a video system adjusted for optimum contrast. Comparable improvements are also posible in the field of interference contrast microscopy.

Inoué's paper includes pictures, taken by the new technique, of beating cilia, acrosomal processes in the act of extending and rotating bacterial flagella. All of these have diameters much smaller than a wavelength of light. Dark-field photography of sub-microscopic objects is not, of course, new, but the use of polarized light adds new information about the orientation of macromolecules within the structures; in particular, birefringent structures show up without interference from the non-birefringent background. Inoué illustrates this point with a high-resolution picture of the ridges on the surface of an epithelial cell.

He adds a caveat that to get the best results one must take account of the nonideal performance of both optical and video components, and exercise great care in optimizing the illumination. The light levels are so low that by maladjustment one can easily lose the image in a background of instrumental noise. Such problems, and the rival merits of different commercial systems, are the subject of hot debate among the cognoscenti; there is no doubt, however, that future advances in video technology will give further boosts to the older branches of microscopy.

Michael Spencer is a Research Fellow in the Department of Biophysics at King's College, London.

Seabirds and oil: the worst winter

from Chris Mead and Stephen Baillie

LAST winter more oiled seabirds bearing rings were reported to the British ringing office than ever before: a total of 103 birds of ten species from December to March. Many were found in areas where exceptional numbers of dead and dving oiled birds had been counted on beaches (see the figure). A single incident in the Skagerrak accounted for 22 ringed birds and resulted in a total of 30,000 oiled seabirds being found on beaches. Other areas where recoveries of ringed birds were concentrated suffered oil pollution from a variety of sources rather than the acute pollution from a single source experienced on the Skagerrak. The aggregate of beach counts of oiled birds in Western Europe last winter probably approached 60,000 (ref. 1).

The lack of comparative figures from earlier years for counts of beached birds over all the affected areas precludes the use of such counts for annual comparisons. However, details of ringing recoveries for many years are available and allow the scale of the oiling mortality of British populations to be assessed. The worst affected species, the two large auks guillemot (Uria aalge) and razorbill (Alca

Chris Mead and Stephen Baillie are research workers at the British Trust for Ornithology,

torda), spend a large part of their time swimming and are therefore likely to encounter floating oil^{2,3}. These two species accounted for 84 per cent of the oiled ringed birds. Information on the numbers of ringed birds at risk and numbers recovered from last winter and 1967-68 to 1978-79 is given in the table. This shows that guillemots experienced four times as much oiling as usual and razorbills about three times as much. For guillemot, but not for razorbill, more birds were also 'found dead' than usual. These figures conceal some age-related differences between the two species. For guillemot the youngest birds were worst affected (4.5-fold mortality increase) with immatures suffering a 2.3-fold increase but adults only 1.4 times normal mortality. For razorbills only adults were badly affected (2.5-fold increase in mortality) with younger birds surviving as well as or better than normal. These results reflect the species' different migration patterns4-6: most sub-adult razorbills winter well south of the areas of oiling and adult guillemots mostly winter close to the breeding areas which were unaffected by oil.

Although several thousands of each species are marked each year, the ringing effort is not uniformly distributed throughout the birds' breeding range in

Britain and Ireland. There are four areas where it is possible to speculate about the effect of the 1980-81 mortality of guillemots: Orkney and Shetland, Grampian region, north-west Scotland and Saltee, Co. Wexford. If there is no compensatory increase in the survival of the remaining birds and the age of first breeding does not change, any excess mortality during a single winter might affect breeding numbers in three ways: immediately, by the loss of adults from the breeding population; annually for four years, by the loss of immature birds in second to fifth winters; and after four years by the loss of first year birds now entering the breeding population for the first time.

When some allowance has been made for the variation in ringing effort, it seems likely that the guillemots from eastern areas have suffered worst. The Orkney and

Mead, C. BTO News 112, 1 (1981).

Andrews, J.H. & Standring, K.T. Marine Oil Pollution and Birds (RSPB, 1979).
Mead, C.J. & O'Connor, R.J. Seabird Ringing Recoveries

and Oil Pollution. (Evidence to Royal Commission on Environmental Pollution - BTO, 1980).

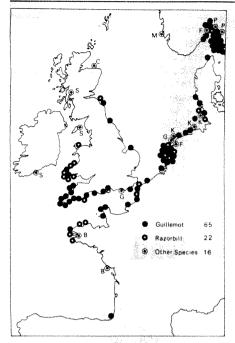
Mead, C.J. Bird Study 21, 45 (1974)

Lloyd, C.S. Bird Study 21, 102 (1974).

Birkhead, T.R. Bird Study 21, 241 (1974). Birkhead, T.R. & Hudson, P.J. Ornis Scand. 8, 145

eabird Group Seabird Counting Manual: Auks (1980).

^{9.} Stowe, T.J. Ibis (in the press).



Finding localities of oiled seabirds ringed in Britain and Ireland and reported from December 1980 to March 1981. Black circle—guillemot (*Uria aalge:* 65 records). White star — razorbill (*Alca torda:* 22 records). Black star — other species: 16 records keyed as follows: (F) Fulmar (*Fulmarus glacialis:* 2); (G) Gannet (*Sula bassana:* 2); (C) Cormorant (*Phalacrocorax carbo:* 1); (S) Shag (*Phalacrocorax aristotelis:* 3); (M) Red-breasted merganser (*Mergus serrator:* 1); (B) Great skua (*Stercoraius skua:* 2); (K) Kittiwake (*Rissa tridactyla:* 3); (P) Puffin (*Fratercula arctica:* 2).

Winter mortality, December to February, for 1980-81 compared with 1967-79 for British and Irish ringed guillemots and razorbills

	Guillemot (Iria aalge)	Razorbill (A	lca torda)
Ringed birds at risk+	1980–81	1967-79	1980-81	1967-79
	18,000	83,000	15,500	122,500
Recoveries reported: Oiled 'Found dead'++ Total	61	68	20	55
	20	56	4	84
	81	124	24	139
Recoveries/1,000 at risk: Oiled 'Found dead' Total	1.1	* 0.8 0.7 * 1.5	1.3 * 0.3 1.5	0.4 0.7 1.1

* indicates significantly higher rate than for the other period with probability < 0.001

+The figure for birds at risk has been calculated from the ringing totals reported over the last 25 years (1980 figures provisional) with allowance made for first year mortality (50 per cent), annual immature mortality of 16 per cent and adult mortalities of 6 per cent for guillemot and 9 per cent for razorbill⁴⁻⁷.

++'Found dead' recoveries include only beached birds not reported as oiled. Birds known to have been shot, snared or caught during fishing operations have been excluded throughout.

Shetland guillemot populations will be reduced by 2 per cent immediately, followed by 1 per cent per annum for three more years and a further 4-5 per cent in 1985 when the 1980 cohort should start to breed. It is possible that the guillemots from Grampian colonies may have suffered as badly. However, the adults and immatures from north-west Scottish colonies seem to have survived better but the increased mortality of first year birds will affect breeding numbers in four years. In this area 5 per cent of the breeding razorbills may have been lost. Saltee results indicate that neither species suffered particularly high mortality last winter.

Even the largest predicted annual

decreases in populations (about 5 per cent) are probably too small to be detected by current census techniques8. Recent breeding population studies indicate that population changes on this scale (or slightly greater) have affected many auk colonies in Britain and Ireland over the last decade^{7,9}. However, this oiling mortality, if repeated over several winters, would result in a steady decline of guillemot and razorbill populations over much of northern Britain. Together with the threat of summer pollution at colonies posed by inshore oil exploitation, all those responsible for the conservation of seabirds, particularly in Scotland, have a very real cause for concern.

Moonquake meanings

from Peter J. Smith

As the Earth has earthquakes, so the Moon has moonquakes; and insofar as events of both types have definite foci, generate seismic waves and radiate energy, they are clearly recognizable as belonging to the same class of phenomena. Thereafter the resemblance weakens, for the distributions of lunar and terrestrial shocks in both space and time are quite different. Whereas most earthquake epicentres lie along narrow bands coincident with major crustal features, moonquake epicentres exhibit no such correlation. Whereas earthquake foci lie at depths of 0-700 km, their counterparts in the Moon mostly occur in the depth range 700-1,100 km (deep moonquakes), although there are also a few at less well determined depths of rather less than 200 km (shallow moonquakes). And whereas earthquakes taken together demonstrate no clear temporal patterns,

Peter J. Smith is in the Department of Earth Sciences, Open University, UK.

deep moonquakes have a strong periodicity of 27 days. Lunar quakes also differ from the terrestrial variety in that they occur repeatedly at a limited number of foci.

The reason for all these differences is evidently the influence of different tectonic styles, the importance of which is reemphasized by new calculations of lunar seismic energy and moonquake source parameters carried out by Goins et al. (J. geophys. Res. 86; 378, 1981). This is not the first time that an attempt has been made to determine the seismic energy released by moonquakes, although Goins and his colleagues claim, certainly correctly, that previous estimates were unsatisfactory in that they failed to take sufficient account of such factors as instrument frequency response and the effects of intense scattering near the lunar surface. On the other hand, it is not entirely clear that many of the conclusions drawn by Goins et al. could not equally have been drawn from the earlier calculations, notwithstanding that they apparently underestimated seismic energy by a factor of about 100 and moonquake magnitudes by a factor of about 2. But that is no argument against having the correct, or at least more 'realistic', figures at last; and in any case Goins and his co-workers have also made the first determinations of additional source parameters such as seismic moment and stress drop.

According to the new figures, then, the 500 or so deep moonquakes with body wave magnitudes (m_b) greater than 1.6 (maximum $m_b = 3.0$) recorded annually by the Apollo Passive Seismic Net release energy of 8×10^{13} ergs a year. So where does this energy come from? Or to put it another way, what is the basic mechanism for the generation of deep moonquakes? Comparison with the Earth is no help here, for deep moonquakes have no terrestrial equivalents. What is relevant, however, is the fact that the marked periodicity of 27 days in the event occurrence frequency at each deep moonquake focus exactly matches the dominant 27-day period of the

tide induced in the Moon by the Earth. Moreover, the number and size of deep moonquakes in any given month also correlates with the 206-day variation in the lunar tide resulting from the perturbation of the Moon's orbit by the Sun. The occurrence times of deep moonquakes are thus evidently controlled by tidal forces; and since the tidal energy dissipated in the Moon is $10^{21}-10^{22}$ ergs a year, this source is obviously more than sufficient.

But the tidal energy dissipated in the Earth is, at 10²⁷ ergs a year, even greater than that dissipated in the Moon. Why, then, are there apparently no tidally induced earthquakes? For this interesting conundrum Goins and his colleagues offer three explanations, not necessarily mutually exclusive. One is that terrestrial lithospheric stresses due to lunar forces are about an order of magnitude smaller than the lunar stresses due to Earth forces at moonquake depths. The second is that there may indeed be some tidal earthquakes but that most, if not all, of them are masked by tectonic earthquakes and microseismal noise. And third, the 'dynamic character of the Earth's interior' generally precludes the development of long-term strain buildup at terrestrial depths of greater than about 700 km.

The latter two explanations thus bring us directly to tectonic style, which seems to be much more important than the relative magnitudes of possible driving forces when it comes to a planet's seismic characteristics. For example, the thermal energy available in the Moon $(2 \times 10^{26} \text{ ergs a})$ year) is not much less than that in the Earth $(9 \times 10^{27} \text{ ergs a year})$, but the different sizes of the two bodies lead to different distributions of temperature with depth and hence to different tectonic regimes. The result for the Earth is a thin lithosphere, lithospheric breakup, plate motion and a dominant near-surface seismic distribution generated tectonically. On the Moon, by contrast, the lithosphere is much too thick to fracture; and the temperature-depth distribution, and hence the shear modulus-depth variation, is such as to concentrate the tidal stress into a particular depth range. Thus whereas heat is the chief fundamental influence on terrestrial seismicity, the corresponding lunar influence is tidal.

Not that things can be quite as simple as that. Although tidal stresses vary smoothly over the lunar surface, moonquake epicentres do not. Most of them lie on the Moon's nearside, although that could merely reflect the difficulty of detecting farside events with nearside seismometers. But of greater significance, perhaps, is the highly uneven distribution of epicentres on the nearside, and in particular, a marked disinclination of epicentres to lie in the south-east quadrant of the face. This could be simply a matter of chance; but it might also suggest that, notwithstanding the dominance of tidal forces, the precise locations of foci within the 700-1,100 km

layer are governed by pre-existing inhomogeneities or zones of weakness.

Then there are those shallow moonquakes, hitherto mentioned but otherwise ignored. Although the passive seismic net has only been recording about five of these a year with m_b greater than 2.2, their maximum magnitude (4.8) is much greater than that of deep moonquakes (3.0) and the total seismic energy released by them annually is 2,500 times greater (2×10^{17} ergs a year). Yet they demonstrate no obvious periodicities in their times of occurrence, are not related to any obvious surface features, and do not occur repeatedly at the same foci. Moreover, although they release far less energy than even intraplate earthquakes (about 3 per cent of terrestrial seismic activity), their stress drops are comparable with, and sometimes even higher than, those of most terrestrial events.

approach by A. Salin (University of

Bordeaux), who finds an interference dip

in this secondary peak. However, H.

Knudsen (Aarhus, Denmark) claimed that

her group had not seen this interference

experimentally. L. Dubé and J. S. Briggs

(Freiburg, Germany) have shown that in

capture from one excited state to another,

the second Born contribution is dominant

over the first Born at high impact

velocities, and capture occurs into final

Europe regains the lead in atomic physics?

from M.R.C. McDowell

THE First European Conference* on Atomic Physics (including Molecular Physics) shattered the belief that the United States necessarily leads the world in any branch of physics. This resurgence in Europe is not confined to a few countries—important new advances were reported from Poland and Spain, for example.

The charge exchange reaction

 $(X^{q+} + e) + Y^{p+} \rightarrow X^{q+} + (Y^{p+} + e)$ in which an electron is transferred from one ion (or atom) to another, is attracting much theoretical and experimental interest. One reason is that it is now clear that charge exchange-assisted recombination can move the ionization equilibrium in thermonuclear plasmas several stages of ionization away from the 'coronal equilibrium' values. At impact velocities Vgreater than the orbital velocity of the captured electron, energy and momentum are transferred with the electron, and the corresponding momentum transfer factors in the expression for the cross-section can greatly reduce the rate of the process. The process leads to highly specific final states.

A. Macias and A. Riera (Universidad Autonoma de Madrid) have clarified this momentum transfer problem by showing that the translation factors introduce a new class of coupling which may be more important than dynamical coupling in determining the final state. Further advances in our understanding of charge exchange came from J. H. McGuire (Hans-Meitner Institute, Berlin) who, with his student P. R. Simony (Kansas State University), has shown that the v^{-11} dependence of the proton-hydrogen charge exchange cross-section is manifested in a secondary maximum in the differential cross-section, and this was confirmed in a different theoretical

states with maximal overlap with the initial state. It became clear in discussion that no one knows the exact velocity dependence, at high velocity, of any charge exchange process.

Theoretical advances also dominated the discussion on the behaviour of atoms in very intense electromagnetic fields, the latter being of great interest in the astrophysics of White Dwarfs and Neutron Stars, as well as in the ablation of inertial fusion targets. Fields of interest range up to 10^{13} G, although currently available laboratory fields are restricted to about 4×10^{5} G. P. Cavaliere, C. Leone and R. Zangara (University of Palermo, Italy) have shown that the normal double-loop

Zangara (University of Palermo, Italy) have shown that the normal double-loop pattern observed in electron impact triple differential ionization measurements splits into a quadrupole pattern when a laser beam, at right angles to the electron beam, also interacts with the target. In the field-free case the transition is $s \rightarrow p$, but with the field on, it may be $s \rightarrow s$ or $s \rightarrow d$, the electron emitting or absorbing one photon. The quadrupole pattern appears to be associated with absorptions $[s \rightarrow (p+1)]$. They also show that the process of laser-assisted electron impact ionization of atoms can lead to appreciable laser gain. F.

M.R.C. McDowell is Professor of Applied Mathematics at Royal Holloway College, University of London, and was assisted by both D.N. Stacey, Clarendon Laboratory, Oxford and G.V. Marr, University of Reading in preparing this report.

^{*}The Conference, sponsored by the Atomic Physics Division of the European Physical Society, was held at the University of Heidelberg (April 6-10 1981), and was organized by Professor G. zu Putlitz. The two-volume Book of Abstracts (eds. J. Kowalski, G'zu Putlitz, H. G. Weber) is published in Europhysics Conference Abstracts.

M. Faisal (University of Bielefeld, Germany) with A. Larni and N. K. Rahman (University of Pisa) have demonstrated theoretically that laser gain should also be exhibited in laser-assisted recombination. Detailed calculations for recombination to He (51P) suggest that high gain can be obtained at low laser intensity if the electrons are highly monochromatic. Photoionization of atomic hydrogen in intense magnetic fields has been calculated by S. M. Kara (British Nuclear Fuels), who finds intense resonances at the Coulomb-modified Landau levels. An exact scaling theorem for the isoelectronic sequence has subsequently been given by G. Wunner, H. Ruder, W. Schmitt, H. Herold (University of Erlangen, Germany) and M. McDowell (Royal Holloway College, London University), which reduces to a simple scaling in the dipole approximation. This should be experimentally testable, using a synchrotron as a light source.

For field-free photoionization, U. Heinzmann (University of Munster, Germany) followed up his earlier work on production of spin-polarized photoelectrons by reporting absolute measurements of the eigen-quantum defects in Ar, Xe and Kr from threshold to 40 eV, which join on smoothly through threshold with the bound-bound transition values of n³ times the oscillator strengths f_{nn} from level n to level n'. This is very direct experimental evidence for the validity of Seaton's multichannel quantum defect theory, which is of considerable astrophysical importance. (For Xe, see U. Heinzmann Appl. Opt. 19; 4087, 1980.)

Dramatic developments were reported in the study of nuclei by optical spectroscopy. Two experimental advances, the tuneable laser and on-line mass separation, have allowed the charge radii, spins and moments of long sequences of radioactive nuclei to be measured. In particular, the variations in charge radius (giving rise to so-called 'isotope shifts' in atomic spectral lines) have given a great deal of nuclear structure information over the years, but until quite recently very little work had been done on light elements or unstable nuclei. Suddenly, vast quantities of data are appearing, largely due to the efforts of two groups (based at Mainz and Orsay) working at the ISOLDE mass separator at CERN. Rubidium, for example, has two naturally occurring isotopes; results for over 20 isotopes are now available, including several isomers. They show, remarkably, that the nuclear size actually decreases as the neutron number rises from 40 to the shell closure at 50; there is then a sharp increase, much more rapid than is predicted by the usual nuclear models.

Other elements show equally interesting features, the most striking so far being the shape isomerism found in neutron-deficient mercury isotopes.

The results are a valuable testing ground for nuclear theory. The variations in

nuclear size show broad overall features which should lead to a better insight into the systematics of nuclear behaviour, while the finer details, for example isomer shifts, depend sensitively on the structure of the individual nuclei concerned.

The work of V. S. Letokhov (Academy of Sciences, Moscow) on the resonance interaction of laser radiation with single atoms now allows the simultaneous availability of high spectral resolution with the ultimate in sensitivity and detection selectivity in that he claims to be able to detect and do physics on one atom in 1012. High Rydberg atomic states have now been demonstrated to be very attractive for high-resolution spectroscopy and metrological work (H. Walther, Munich). Exciting a single Rydberg state by laser opens the possibility of higher-resolution measurements of the fundamental constants. Beam foil spectroscopy continues to be used and recently has allowed the observation of radiative transitions between bound states of the negative ion of lithium (I. Martinson, Lund).

The study of molecular systems is entering a new and exciting phase. New avenues of experimental study are being opened up by the multichannel quantum defect theory (M. J. Seaton, London; Ch. Jungen, Orsay), typified by work reported by S. Krummacher (Freiburg) using the Orsay synchrotron light source to study the inner shell photoionization of CO. It is important to note that Heinzmann's direct verification of this theory allows absolute calibration. High-resolution spectroscopy of Ch reported by J. Le Calvé (Saclay), threshold electron spectroscopy (M. Kurepa, Belgrade) and resonance fluorescence (T. Möller, Hamburg) on the same molecule show that there is much which can be obtained by application of both new and traditional techniques to the analysis of individual molecular systems.

A gradient of membrane protein in the retina

from Colin J. Barnstable

THE MECHANISM by which cells sense and signal their position within an organ or organism remains one of the least understood problems in biology. Many experimental systems such as chick limb development, hydra regeneration and insect cuticle formation have indicated that gradients of positional information exist and can be sensed and acted upon by the relevant cells. Both the nature of these gradients and the sensing mechanisms are unknown. In the nervous system, the position of an individual neurone has important consequences for the nature of its synaptic input and the target it innervates. For example, motor neurones in the developing spinal cord migrate to particular positions and the resulting clusters of cells can be shown to be committed to innervate a particular muscle1. Innervation is only achieved after the growing axons reach this correct target some distance away. In this system the mechanism that specifies which motor neurones will innervate which muscles, and the mechanisms by which the growing axon reaches its target, remain unknown.

This general problem of neural specificity has also been intensively studied in the visual system and particularly in the topographical projection of retinal ganglion cell axons to the optic tectum. One current debate centres around the mechanism by which this ordered

Colin J. Barnstable is Instructor in Neurobiology, Harvard Medical School. projection is formed. In some fishes it may be the result of a maintained topography in the optic nerve². In the adult cat³, on the other hand, the ganglion cell axons are mixed up in the optic nerve and sort out at their targets. This implies that some form of 'chemoaffinity' between axon and target cell exists. Models for this have varied from a strict 'lock-and-key' relationship between each ganglion cell axon and its tectal target neurone to a quantitative gradient or gradients of interaction molecules arranged according to particular retinal axes⁴.

These interaction molecules have usually been studied in terms of selective cell adhesion using methods developed for studying the aggregation and sorting of a variety of embryonic tissues (see ref. 5 for a review). In the retino-tectal system it has been shown that in general cells from a given portion of retina preferentially adhere to cells from the tectal area that includes their normal synaptic target. From studies such as these it has been concluded that there are several interacting gradients that can be used to specify cell position and subsequent interaction. None of these studies, however, has successfully identified the molecular nature of these gradients.

A recent paper by Trisler, Schneider and Nirenberg⁶ has demonstrated that a gradient of concentration of a particular molecule does exist across the avian retina. To demonstrate this Trisler et al. looked

for, and found, a monoclonal antibody that bound more strongly to dorsal retina than ventral retina. When 14-day chick embryo retinas were cut into eight segments along a dorsal-ventral axis and assayed for antibody binding, the most dorsal segment bound 35-fold more antibody than the most ventral. Presumably the extremes of the gradient show a much greater difference. The antigen detected by the monoclonal antibody has been named TOP, after 'toponymic' which the authors define as 'a marker of position'. It appears to be a cell surface protein since it can be assayed on living cells, is heat labile and is trypsin sensitive. Immunocytochemical localization of antigen in 14-day chick embryo retina showed that it was present in all layers and, therefore, possibly in all cell types. The gradient is thought to represent differences in the amount of antigen per cell rather than regional differences in cell or membrane concentration since mechanically dissociated cells gave different intensities of immunofluorescence according to the part of the retina chosen. Essentially all the features of the gradient were found at every age tested from 4-day old embryo to adult.

Whatever the mechanism for generating the gradient, simple 'organizing' or 'controlling' areas outside the retina seem not to be involved. The evidence for this comes from an unusual embryo found by Trisler et al. that had a third eye in the middle of the forehead facing in a dorsoanterior direction. Even though the eye was in an abnormal orientation, the gradient of TOP antigen showed a normal orientation with respect to its own dorsalventral axis defined by the choroid fissure.

The importance of this paper for biology in general is that it has demonstrated a molecular gradient in a particular organ. Its importance in aiding our understanding of neural specificity will depend upon whether the cells within the retina or in the optic tectum use the information contained in the gradient for their physiological interactions.

Landmesser, L. J. Physiol., Lond. 284, 391-414 (1978).

- Sperry, R.W. Proc. natn. Acad. Sci. U.S.A. 50, 703 (1963). Moscona, A.A. in *Neural Recognition* (ed. Barondes, J.S.) 205 (Plenum, New York, 1976).
- Trisler, G.D., Schneider, M.D. & Nirenberg, M. Proc. natn. Acad. Sci. U.S.A. 78, 2145 (1981).

Galactic nuclei

from R.A.E. Fosbury

SINCE the upsurge of interest in Seyfert galaxies and the discovery of quasars in the early 1960s, galactic nuclei have been prime targets for the application of new observational techniques. The more active galactic nuclei have not disappointed observers as their high luminosity extends over the whole electromagnetic spectrum. The current status of research on galactic nuclei, both active and 'normal', was examined by the participants at a recent conference* at the Royal Greenwich Observatory.

In the spiral Seyfert galaxies, the nucleus has a profound influence over the physical conditions in a region of typical diameter one kiloparsec $(3 \times 10^{21} \, \text{cm})$. The optical observers call this the narrow line region (NLR) since it is the source of the strong forbidden emission lines with widths of order 300 km s-1 characteristic of this class of object. The region is also now recognised to be a source of radio radiation with a relatively steep spectrum. Andrew Wilson (University of Maryland) reviewed new radio observations, primarily from the Very Large Array (VLA) in New Mexico, which showed the Seyferts to be intermediate between normal and radio

galaxies. Indeed, some show a double or triple structure on a scale of a few arc seconds, reminiscent of a classical double radio source but prevented from bursting out of the immediate nuclear environment by a spiral of dense interstellar medium. Wilson noted an interesting lack of correlation between radio and optical axes in the Seyferts which may suggest that the nucleus is not influenced very much by what the rest of the galaxy is doing. Spencer, Booler, Steward and Lonsdale (Jodrell Bank) complemented the VLA work by showing similarly high-resolution radio maps made at lower frequencies with the new multi-telescope radio-linked interferometer which spans a significant fraction of the western side of Britain. Because of the steep spectra, this instrument is particularly suited to studies of the nuclear surroundings. The small angular size subtended by the nuclei makes it difficult to reconstruct the geometric and kinematic state of the various components. It has long been realised that the emission line profiles are a rich source of such information, although difficult to interpret. Mark Whittle (Institute of Astronomy, Cambridge) reported an extensive study of the profiles originating in the NLR and at last offered some hope of being able to distinguish

between inflowing and outflowing material from careful comparisons of the asymmetries seen in lines from different ionization states. Although it is generally agreed that most of the emission line radiation emitted by active nuclei is the result of photoionization processes, there is still considerable uncertainty whether the origin of the ionizing continuum is nonthermal with a power-law spectrum or a combination of this with thermal radiation. Shock processes have been advocated for some nuclei which do not show a strong continuum. Even for these. Daniel Péquignot (University of Meudon) illustrated the versatility of photoionization models to explain some of the lowionization spectra. His models produce the strong auroral line with regions of high density and reproduce the optical spectra quite satisfactorily using a two-component ionizing spectrum; the approach was, however, running into some difficulties with the UV emission line observations.

Bernard Pagel (Royal Observatory, Herstmonceux) considered the elemental abundances of both normal and active nuclei. With the caveat that the abundance determination methods used for nuclei are somewhat unreliable, it is perhaps surprising that activity seems to have little effect on chemical composition. In fact, all the observed galactic nuclei seem to have very similar abundances and, in particular, there is no evidence for secondary processing in nuclear HII regions as the nitrogen to oxygen ratio is consistent with that of oxygen to hydrogen. How normal are 'normal' galactic nuclei anyway? Robert Sanders (University of Groningen) sketched a picture of recurrent activity in which gas clouds within the inner few hundred parsecs lost a small fraction of their mass to a black hole on each collision and produced one event of about 1056 ergs every 107 years. He supported the thesis with reference to our own Galaxy by pointing out the expanding ring seen in molecular emission lines which should oscillate with about that period. New X-ray observations of our own nucleus were presented by Brin Cooke (University of Leicester). The orbiting Einstein Observatory has shown a number of hardspectrum point sources together with a diffuse component extended along the galactic plane. One of the (non-variable) point sources is coincident with the radio source Sagittarius A west and he pointed out that the radio/X-ray flux ratio is about the same as that in the radio galaxy Centaurus A. Willem Wamsteker (VILSPA) described some optical observations of the nucleus of the nearby spiral galaxy M83 which showed noncircular motions very similar to those seen in our Galaxy.

The second half of the conference collected together important observations

R.A.E. Fosbury is at the Royal Greenwich Observatory, Herstmonceux Castle, UK.

Scholes, J.H. Nature 278, 620-624 (1979).
 Horton, J.C., Greenwood, M.M. & Hubel, D.H. Nature 282, 720-722 (1979).

^{*}The 25th Herstmonceux Conference was held at the Royal Greenwich Observatory in April, 1981.

ARTICLES

Lucifer dyes—highly fluorescent dyes for biological tracing

Walter W. Stewart

National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205, USA

Lucifer dyes are intensely fluorescent 4-aminonaphthalimides which are readily visible in living cells at concentrations and levels of illumination at which they are nontoxic. Because of their low molecular weight they frequently pass from one cell to another; this widespread phenomenon, termed dye-coupling, is thought to reveal functional relationships between cells. Lucifer dyes can also be used for ultrastructural tracing by comparison of electron micrographs with light micrographs of the same thin section. In addition, they show promise for backfilling neurones through cut nerves, for visualizing the results of retrograde axonal transport and for the covalent labelling of macromolecules.

SINCE their introduction in 1978 Lucifer yellow CH and Lucifer yellow VS have been used with considerable success as intracellular markers in a wide variety of biological systems. Both dyes contain the same sulphonated 4-aminonaphthalimide moiety, but they differ in the substituent on the imide nitrogen (Fig. 1). Their syntheses, to be reported elsewhere, are based on the commercial wool dye brilliant sulphoflavine (CI 56205). The dyes have similar spectral properties: absorption maxima at 280 nm and 430 nm, corrected emission maxima near 540 nm, and quantum yields of about 0.25. The high quantum yields make possible the detection of the dyes at low concentration, and the wide separation between the absorption and emission maxima facilitates excitation at one wavelength and observation of fluorescence at another.

The dyes differ in their chemical properties, but both can be bound to tissue. Lucifer yellow VS is a vinyl sulphone that reacts rapidly with amino and sulphydryl groups but is extremely stable in water; even in weak base (pH 9.0) its half life is about one week at room temperature. The dye combines rapidly and covalently with proteins and presumably with other tissue constituents containing sulphydryl or amino groups. Lucifer yellow CH, on the other hand, has a free hydrazido group and reacts with aliphatic aldehydes at room temperature. Aldehydecontaining fixatives bind the CH dye to tissue, presumably through the hydrazido group. Aqueous solutions of this dye appear to be chemically stable for at least several months at room temperature.

So far the CH dye has been used more often than the VS dye, perhaps because the latter is not yet commercially available;

Fig. 1 Structural formula of Lucifer dyes. Both dyes are 3,6-disulphonated 4-aminonaphthalimides. For the lithium salt of Lucifer yellow CH (often referred to simply as Lucifer yellow CH), R is -NH-CO-NH-NH₂; for the lithium salt of Lucifer yellow VS, R is m-phenyl-SO₂-CH=CH₂. About ×24,000,000.

which one is best suited to a particular application can only be determined empirically.

The most frequent application of Lucifer yellow CH has been to reveal the shape of individual neurones 1-5. It has been used to determine the branching pattern and course of regenerating neurones 6-7. Because of the low toxicity of the dye, direct in vivo observations on the regeneration of dye-filled neurones may be possible. Lucifer yellow CH has been used in the study of developing systems: in the development of molluscs 8, for example, and in the exciting work by Goodman and Spitzer on the development of the grasshopper nervous system 9. I describe here other applications of these fluorescent tracers in the hope that their many potential uses can be rap-dly exploited.

Dye-coupling revealed by intracellular injection of Lucifer yellow

Neurones and other cells can be marked with Lucifer yellow CH by injecting the dye through a micropipette using pressure or iontophoresis. The dye spreads quickly throughout the body and processes of the injected cell, but does not cross the cell membrane¹. The low molecular weight (457.2) and intense fluorescence of the dye offer several advantages over other tracers. (1) A cell can be stained in its entirety with a relatively small amount of dye. Illumination sufficient for photomicrography of live cells does not noticeably alter their physiology. (2) In preparations which are sufficiently transparent one can determine whether the distribution of dye found after fixation is the same as that seen in the living state. (3) Most importantly, the dye frequently spreads in vivo from the injected cell to certain nearby cells. This movement of dye from cell to cell has been termed dye-coupling¹. In certain cases, dye-coupled cells are known to be electrically coupled to each other. Two examples are horizontal cells in the turtle retinal and the septate axon of the crayfish (K. Futamachi and W.S., unpublished). In the embryo of the mollusc Patella, however, dye-coupling between macromeres was detected without knowing whether the cells were electrically coupled and was used to study the development of the embryo⁸. Presumably in many (but not necessarily all) instances dye-coupling and electrical coupling have a common basis. In any case, dye-coupling offers a method of recognizing certain functional connections between cells by morphological means.

A pair of neurones called Leydig cells occurs in each segmental ganglion of the leech *Hirudo medicinalis*. These cells are known to be electrically coupled³⁰, and Fig. 2 shows that they are dye-coupled as well. Two neurones in the ganglion contain dye: the Leydig cell that was injected and the contralateral Leydig cell into which dye spread. The transfer of dye presumably occurred at the sites of contact between the arborizations of the two neurones. Unexpectedly, dye also spread from the injected neurone to processes in the ipsilateral axon bundles





Fig. 2 Leech ganglion containing a Leydig cell injected intracellularly with the lithium salt of Lucifer yellow CH. a, The lower cell was pressure-injected with a 5% solution of dye. The tissue was allowed to stand for 90 min at room temperature and was then fixed at 4 °C for 12 h in 4% formaldehyde in 0.15 M sodium phosphate buffer, pH 7.4. The ganglion was embedded in glycol methacrylate as previously described 1 . The fainter cell body (top) is the contralateral Leydig cell, which is dye-coupled to the injected cell. $\times 107$. b, Detail from a. The ipsilateral nerve contains two fluorescent processes—one from the injected neurone, the other from a dye-coupled neurone in the adjacent ganglion (see text). $\times 180$.

leaving the ganglion (Fig. 2b). These are probably axons from Leydig cells in adjacent ganglia, as Kent Keyser has independently demonstrated in greater detail (personal communication). As both adjacent ganglia had been removed before injection, the sites of coupling to the two dye-coupled processes must have been in the remaining ganglion or in the short lengths of attached nerve. Here and in similar situations Lucifer yellow CH is useful for gross localization of the sites of intercellular communication.

The sensitivity of Lucifer yellow CH was compared semiquantitatively with that of two other tracers, Procion yellow and horseradish peroxidase. Horseradish peroxidase is often used for intracellular staining^{18,19} and is detectable by both light and electron microscopy. When 5% solutions of Lucifer yellow CH and horseradish peroxidase were injected by pressure into leech neurones, the two tracers appeared roughly equal in their ability to reveal morphological detail as judged visually with the light microscope (W.S. and K. Muller, unpublished). Horseradish peroxidase, however, cannot be seen in living tissue and is thought not to pass between dye-coupled cells²⁰. Procion yellow, a fluorescent tracer previously used for intracellular injection²¹, has about 1% of the fluorescence intensity of Lucifer yellow and in comparably marked cells reveals much less morphological detail¹.

Backfilling with Lucifer yellow CH

Neurones can also be filled with dye through their processes rather than by injection of dye into their somata. Backfilling, introduced by Iles and Mulloney²², is carried out by simply immersing the cut end of a nerve in a pool of tracer. The tracer moves up the axons of the nerve, either by diffusion or in response to an externally applied electric field, and fills the cell bodies of the neurones with processes in that nerve. This technique has been used with Procion yellow²², with cobalt chloride^{23,24}, and with horseradish peroxidase²⁵. Lucifer yellow CH is at least as suitable for backfilling as these tracers. Moreover, since neither the dye nor the process of backfilling appears to alter physiological properties noticeably, and since the dye can usually be seen *in vivo*, one can first identify a backfilled cell by its fluorescence, then impale it with a micropipette and investigate its electrophysiology.

Figure 3a is a fluorescence photomicrograph of the buccal ganglia of the freshwater planorbid snail Biomphalaria glabrata. The preparation was backfilled with the lithium salt of Lucifer yellow CH through the nerve innervating the left salivary gland. The two largest cells containing dye can usually be identified by other criteria such as size, position, and branching pattern. These cells appear to be salivary effector neurones and may be homologous to the cells described by Kater in Helisoma26 and Benjamin in Lymnaea²⁷. The fluorescent cells were impaled after they had been backfilled, and their physiology appeared normal. In particular, action potentials in the left cell produced one-for-one depolarizing potentials in the salivary cells (Fig. 3b, c), and depolarizing and hyperpolarizing current passed from either cell to the other, suggesting that they were electrically coupled (Fig. 3d, e). These results are similar to those obtained from preparations which had not been backfilled. After fluorescence photomicrography of the ganglia (Fig. 3a), the physiology of both cells was still normal, and they remained electrically coupled (Fig. 3f, g). In this system, therefore, the amount of light needed for fluorescence photomicrography is much less than that which causes photosensitized damage or killing of cells that contain dye. In the method of cell killing devised by Miller and Selverston, the illumination level which killed Lucifer-filled cells in 5 min was 1,000 times the level required for visualization of processes in the neuropil²⁸.

Lucifer dyes as tracers in electron microscopy

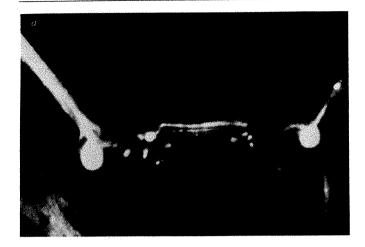
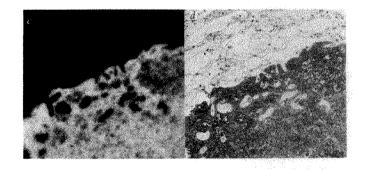
Since the Lucifer dyes described here do not contain a heavy atom, they have no useful electron density. Their intense 

Fig. 3 Live neurones stained by backfilling with the lithium salt of Lucifer yellow CH, and electrical responses recorded after staining. The cut end of the left salivary nerve of a snail (B. glabrata) was drawn into a small drop of a 3% solution of dye on a square of polyethylene about 1 mm on each side. For 1 h a potential was imposed so that a positive, constant current of 30 nA flowed from the bath to the drop of dye. The buccal ganglia were rinsed in a Ringer's solution, and the stained cells were identified with fluorescence optics. The large dye-filled cell in the left buccal ganglion was impaled with a microelectrode (b and c, lower trace). A salivary cell was impaled with a second electrode (b and c, upper trace). The microelectrode was then withdrawn from the salivary cell and used to impale the large cell on the lateral margin of the right buccal ganglion (d and e, records for the right cell are on top). The current traces show when current (about 0.25 nA) was injected, using an active bridge circuit (not accurately balanced in all records), first into one cell and then the other. The live prepartion, a, was then photographed using epifluorescence with a Zeiss Ultraphot. The conditions of photography were: 6.3X Neofluar lens (0.20 numerical aperture), HBO 200 W mercury arc lamp, heat filter and BG-12 excitation filter, FL-500 dichroic splitter, 500 nm barrier filter, 30 s exposure. ×66. After photography, both lateral cells were impaled again, producing the records shown in f and g. Vertical calibration indicates 50 mV for all records; horizontal calibration indicates 1s for c and 2s for all other records.

fluorescence, however, allows them to be used as tracers for electron microscopy by an indirect procedure. An ultrathin section mounted on a grid is examined with a fluorescence microscope to identify cells or processes that contain dye, and then the same section is examined with the electron microscope. Figure 4 shows light and electron micrographs of the same areas of a neurone in the right parietal ganglion of the snail B. glabrata; this neurone can often be identified by its size and position. To make this type of comparison, tissue is conventionally processed for electron microscopy except that osmication is omitted, since even brief exposure to osmium tetroxide greatly reduces Lucifer fluorescence.





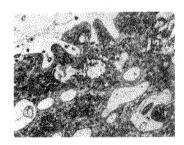


Fig. 4 A cell in the right parietal ganglion of the freshwater snail Biomphalaria glabrata was injected with Lucifer yellow CH, fixed for 4 h at 23 °C in a pH 7.4 12.5 mM Na°/H° HEPES buffer containing 2% glutaraldehyde, 1% formaidehyde, and 25 mosM balanced salts, dehydrated in graded alcohols, and transferred to anhydrous acetone at 4 °C. The ganglion was stained for 1 h at 4 °C in 1% hafnium tetrachloride in acetone, then rinsed in acetone and embedded in Spurr's resin 42. (The fixative and rinse solutions also contained 1% HfCl₄ in this experiment, but not in most.) a, Silver section on a hexagonal grid (Polaron) was illuminated through a Zeiss 63X OD lens (0.90 numerical apertume) with an Osram 50 W HBO lamp and photographed with a 15 s exposure on Ektachrome 400 film pushed to ASA 800. ×640. b, The same section shown in a, photographed with the electron microscope after uranium and lead staining. ×640. c, Detail from a. ×1,500. d and e, Detail from b. ×1,500 and ×3,700. Photographed at 80 kV.

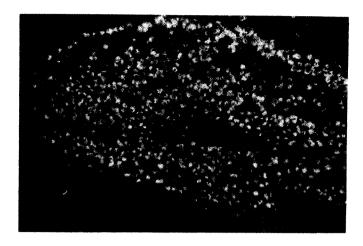


Fig. 5 Isthmo-optic nucleus of chick labelled by retrograde axonal transport of Lucifer yellow VS. The vitreous humour of the contralateral eye was injected with 25 μl of 5% Lucifer yellow VS; the injection damaged a portion of the retina. After 21 h the chick was anaesthetized and fixed by perfusion with buffered 4% formaldehyde. The other eye was not injected, and its contralateral isthmo-optic nucleus showed almost no fluorescence. $\times 200$.

Post-fixation staining can be omitted entirely, but tissue processed in this way generally lacks sufficient contrast and electron density to give useful images. Adequate contrast is generated by staining tissue en bloc in 1% hafnium tetrachloride in anhydrous acetone at 4 °C, a procedure introduced by Morris Karnovsky (personal communication). The tissue is then rinsed in acetone and embedded in Spurr's resin. When a thin section (grey or silver) on an ordinary electron microscope grid is illuminated using epifluorescence with an intense arc lamp, the fluorescence of the marked cell is bright enough to be photographed on a fast colour film with a 15-s exposure. The same thin section is then conventionally stained with uranium and lead; the areas that had been found fluorescent are located and photographed with the electron microscope. Surprisingly small areas can be identified with confidence when the light and electron micrographs are compared. In Fig. 4c, d, for example, there are dye-free areas only 500 nm in diameter that are easily recognized in both the light and electron micrographs. The areas from which dye is excluded lie within the perimeter of the cytoplasm of the injected cell and may be penetrating processes of other cells²⁹ Dye-filled processes 500 nm in diameter can usually be found with ease, while 200 nm processes are locatable with greater difficulty.

The necessity of avoiding osmication to preserve fluorescence entails some disadvantages. Membranes either cannot be seen or, more often, appear as thin grey lines (Fig. 4e) instead of the dark black bilayer commonly seen with conventional fixation. The poor visualization of membranes makes the method described here unsuitable for some applications. For example, synapses in the leech central nervous system are difficult to recognize because membranes and associated synaptic specializations lack the contrast they normally have with osmium post-fixation (K. Muller, personal communication).

Since Lucifer yellow CH has no significant electron density of its own, it was possible to test whether either the dye or the process of injection had an effect on the ultrastructure of an injected neurone. The paired Retzius cells of the leech³⁰ were convenient test objects. In each of three segmental ganglia, one Retzius cell was injected with dye and one was not. The tissue was immersed in a cacodylate-buffered fixative²⁰, then processed as described in the legend to Fig. 4. When thin sections

were examined with the electron microscope, both cells were recognizable by their size and position. (The injected cell had previously been identified by its fluorescence.) The ultrastructural morphology of the two cells was virtually the same, suggesting that neither the dye nor the process of injection had caused significant morphological changes (K. Keyser and W.S., unpublished). This experiment illustrates an advantage of an ultrastructural tracer that does not rely on electron density for its detection: the appearance of the marked tissue is not necessarily changed.

Retrograde axonal transport

A useful technique for studies of vertebrate neuroanatomy relies on the phenomenon of retrograde axonal transport, which causes certain exogenous proteins to be carried from axon terminals to the cell body³¹. Kristensson, Olsson, and Sjöstrand

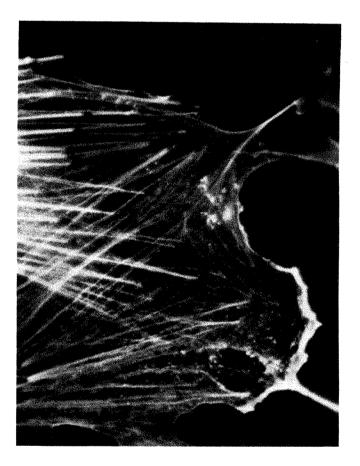


Fig. 6 Indirect immunofluorescence staining of actin filaments with Lucifer-labelled antibodies. Cultured human lung cells, W138, were fixed and treated with rabbit anti-actin antibody, then with Lucifer-labelled goat IgG anti-(rabbit IgG); the procedures were those described by Lazarides⁴³. The Lucifer-labelled antibody was prepared as follows. To 50 mg of Miles-Yeda goat IgG anti-(rabbit IgG) dissolved in 1.8 ml 0.5 M pH 9.0 carbonate buffer was added 0.52 mg Lucifer yellow VS in 1.0 ml carbonate buffer. These weights correspond to a nominal 3 mol of Lucifer per mol of protein, but since Lucifer yellow VS is hygroscopic, less dye was actually present. The mixture was allowed to stand for 3 h at room temperature, then unreacted dye was removed by chromatography with phosphate-buffered saline on Sephadex G-25. The optical densities at 280 nm and 430 nm indicated a dye to protein molar labelling ratio of approximately 2.3. About ×500.

in changes conformational monitoring pH-induced macromolecules.

showed in 1971 that horseradish peroxidase appeared in the cell bodies of neurones near whose terminals a solution of horseradish peroxidase had been introduced³². This marker has since been used both to trace pathways in the central nervous system and to study the mechanism of transport itself. The LaVails and others have shown that peroxidase moves within axons at a rate of about 100 mm per day (ref. 33). Some exogenous proteins seem not to be subject to retrograde axonal transport³⁴, but the reasons for this are not understood. Kuypers and his colleagues have recently shown that certain other fluorescent dyes are also subject to retrograde movement 35.36, so it is not surprising that Lucifer yellow VS serves as an effective marker for retrograde axonal transport from the chick eye to the cell bodies of neurones which have their axon terminals within the retina³³ (Fig. 5). Perhaps the dye is transported as a protein conjugate formed in situ. It is not known whether Lucifer yellow VS will prove useful for tracing connections in other parts of the nervous system.

Recently wheat germ agglutinin labelled covalently with Lucifer yellow VS (see below) has been found to provide good staining of motoneurones in the spinal cord of chick embryo after a small amount (0.5 µl of 0.5% agglutinin) was injected into leg muscles (M. McPheeters and L. M. Okun, personal communication). After retrograde transport had occurred, it was possible to dissociate the spinal cord and isolate the labelled neurones by means of a fluorescence-activated cell sorter37.

Covalent labelling of macromolecules with Lucifer yellow VS

Lucifer yellow VS labels proteins rapidly and covalently under mild conditions. In 0.1 M NaHCO₃ the reaction is complete in 2 h at room temperature. The conjugates generally have quantum yields between 0.1 and 0.2 (R. Chen and W.S., in preparation), so Lucifer-conjugated antibodies should be suitable for immunofluorescence staining. Figure 6 shows actin filaments of cultured fibroblasts stained with Lucifer-labelled antibodies. Lucifer yellow VS is similar to fluorescein isothiocyanate in its ease of use and fluorescence intensity and has the advantage that its emission peak at 540 nm is considerably to the red of fluorescein's at 515 nm, so that its fluorescence contrasts more strongly with tissue autofluorescence. But since Lucifer has no other clear-cut advantage and since less is known about the chemistry and the stability of the bond that is formed, it is likely that fluorescein isothiocyanate will remain the dye of choice for most immunofluorescence applications. When fluorescence is needed at pHs below 6, however, Lucifer yellow VS may be useful. The fluorescence intensity of Lucifer VS is unchanged from pH 2 to pH 10 (ref. 1), an unusual property for a watersoluble dye. This pH-independence makes the dye suitable for

Other uses of Lucifer yellow dyes

Among published examples of the possible uses of Lucifer yellow dyes it is worth noting that intracellular staining with Lucifer has been followed by immunocytochemical staining with rhodamine-labelled antibodies38; the goal of this approach is to characterize the immune reactivity as well as the electrophysiology of the same cell. This dye also has been used to follow changes in junctional permeability caused by moderate transjunctional polarization³⁹. Another interesting application is suggested by experiments in which an individual dye-filled neurone or even a part of a neurone was selectively damaged by means of an intense, focused spot of light²⁸. This phenomenon is sometimes referred to as "cell killing," but as the originators of the technique have pointed out⁴⁰, it is not known whether the affected cells are killed or simply inactivated temporarily. Also, it is not yet known what margin of safety there is for cells that do not contain dye. Finally, there is an interesting recent report that when turtle retina is bathed in a calcium-free Ringer's solution containing Lucifer, the bipolar cells, but not other cell types, take up dye from the medium⁴¹. Uptake is not influenced by light, but is blocked by metabolic inhibitors. It will be of interest to see whether similar phenoma occur in other neuronal

Dye-coupled systems of cells have been discovered through the use of Lucifer yellow CH^{10,11}. It has been used to monitor dye-coupling in regenerating neurones¹²⁻¹⁴, and it greatly facilitated the discovery that an electrical connection between two leech neurones was mediated by a pair of small interneurones¹⁵ and was not direct, as had previously been thought. The dye has been used to obtain information on the position of the micro-electrode tip, either in neurones¹⁶ or, in one case, in giant mitochondria¹⁷. But because of its rapid spread, presumably by diffusion, this dye generally gives only limited information on tip position, and so it may not be the best marker for this purpose.

In addition to the two sulphonated 4-aminonaphthalimides that have been used until now, more than 40 compounds in this group have recently been prepared by a convenient one-step synthesis44. These compounds have a wide range of substituents on the imide nitrogen, and most are water-soluble and have an intense yellow-green fluorescence. Because of the ease with which functional groups can be incorporated into sulphonated 4-aminonaphthalimides, these compounds will probably find new applications as biological tracers.

The experiment shown in Fig. 2 was performed at the 1978 Leech Course at Cold Spring Harbor Laboratory. I thank Elias Lazarides for carrying out the immunofluorescence staining and Kent Keyser, Enid Applegate, and Jennifer LaVail for collaboration in performing the experiments described in the legends to Figs 3, 4 and 5 respectively.

- Stewart, W. W. Cell 14, 741-759 (1978).
 Peacock, J. H., Rush, D. F. & Mathers, L. H. Brain Res. 169, 231-246 (1979).

- Peacock, J. H., Rush, D. F. & Mathers, L. H. Brain Res. 169, 231-246 (19/9).
 Shafer, M. R. & Calabrese, R. L. Cell Tissue Res. 214, 137-153 (1981).
 Crawford, A. C. & Fettiplace, R. J. Physiol., Lond. 306, 79-125 (1980).
 Takato, M. & Goldring, S. J. comp. Neurol. 186, 173-188 (1979).
 Murphy, A. D. & Kater, S. B. Brain Res. 186, 251-272 (1980).
 Bulloch, A. G. M. & Kater, S. B. Science 212, 79-81 (1981).
 Laat, S. W. de, Tertoolen, L. G. J., Dorresteijn, A. W. C. & Biggelaar, J. A. M. van den Nature 287, 546-548 (1980).
- Goodman, C. S. & Spitzer, N. C. Nature 280, 208-214 (1979)
- Gutnick, M. J. & Prince, D. A. Science 211, 67-70 (1981)
- Spencer, A. N. & Satterlie, R. A. J. Neurobiol. 11, 13-19 (1980). Muller, K. J. & Scott, S. A. Science 206, 87-89 (1979).
- Muller, K. J. & Scott, S. A. Nature 283, 89-90 (1980). Scott, S. A. & Muller, K. J. Devl Biol. 80, 345-363 (1980).

- Scott, S. A. & Winler, K. J. & Scott, S. A. J. Physiol., Lond. 311, 565-583 (1981).
 Fettiplace, R. & Crawford, A. C. Proc. R. Soc. B203, 209-218 (1978).
 Bowman, C. & Tedeschi, H. Science 209, 1251-1252 (1980).
 Snow, P. J., Rose, P. K. & Brown, A. G. Science 191, 312-313 (1976).
 Muller, K. J. & McMahan, U. J. Proc. R. Soc. Lond. B194, 481-499 (1976).
- Muller, K. J. & Carbonetto, S. J. comp. Neurol. 185, 485-516 (1979)
 Stretton, A. O. W. & Kravitz, E. A. Science 162, 132-134 (1968).
- Iles, J. F. & Mulloney, B. Brain Res. 30, 397-400 (1971
- 23. Pitman, R. M., Tweedle, C. D. & Cohen, M. J. Science 176, 412-414 (1972).

- Tyrer, N. M. & Bell, E. M. Brain Res. 73, 151-155 (1974).
 Zipser, B. Brain Res. 182, 441-445 (1980).
- Kater, S. B., Murphy, A. D. & Rued, J. R. J. exp. Biol. 72, 91-106 (1978).
 Benjamin, P. R., Rose, R. M., Slade, C. T. & Lacy, M. G. J. exp. Biol. 80, 119-135
- Miller, J. P. & Selverston, A. I. Science 206, 702-704 (1979)
- 29. Coggeshall, R. E. & Fawcett, D. W. J. Neurophysiol. 27, 229-289 (1964).
- Nicholls, J. G. & Baylor, D. A. J. Neurophysiol. 31, 740-756 (1968).
- Kristensson, K. Acta neuropath. (Berl.) 16, 293-300 (1970).
 Kristensson, K., Olsson, Y. & Sjöstrand, J. Brain Res. 32, 399-406 (1971).
 LaVail, J. H. in Methods in Physiological Psychology, Vol. 2 (ed. Thompson, R. F.) 355-384 (Academic, New York, 1978).

 34. Hendry, I. A., Stach, R. & Herrup, K. Brain Res. 82, 117-128 (1974).
- 35. Kuypers, H. G. J. M., Catsman-Berrevoets, C. E. & Padt, R. E. Neurosci. Lett. 6, 127-135
- 36. Kooy, D. van der, Kuypers, H. G. J. M. & Catsman-Berrevoets, C. E. Brain Res. 158, 189-196 (1978).
- McPheeters, M. & Okun, L. M. Soc. Neurosci. Abstr. 6, Abstr. 247.19 (1980). Reaves, T. A. Jr & Hayward, J. N. Proc. natn. Acad. Sci. U.S.A. 76, 6009-6011 (1979). Spray, D. C., Harris, A. L. & Bennett, M. V. L. Science 204, 432-434 (1979).
- Selverston, A. I. & Miller, J. P. J. Neurophysiol. 44, 1102-1121 (1980). Detwiler, P. B. & Sarthy, P. V. Neurosci. Lett. 22, 227-232 (1981).
- 42. Spurr, A. R. J. Ultrastruct. Res. 26, 31-43 (1969)
- Lazarides, E. J. Histochem. Cytochem. 23, 507-528 (1975).

R5481 19418 Stewart, W. W. J. Am. chem. Soc. (in the press).

Active folding in the Algerian earthquake of 10 October 1980

G. C. P. King

Bullard Laboratories, Department of Earth Sciences, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, UK

C. Vita-Finzi

Department of Geography, University College London, Gower Street, London WC1E 6BT, UK

A fold controlling the form of the local topography near El Asnam, Algeria, increased in amplitude at the time of the earthquake of 10 October 1980. The deformation occurred rapidly in an elastic-brittle manner and not in the gradual ductile fashion in which folds are normally thought to develop.

AN earthquake of magnitude 7.3 (M_s) occurred near El Asnam, in north Algeria, at 12.25 GMT on 10 October 1980. Hypocentral locations provided by the US Geological Survey (1°25′E, 36°9′N) and the International Seismological Centre (1°26′E, 36°11′N) are broadly consistent with the observed damage distribution¹ but their accuracy cannot be assumed to be better than ± 10 km. Aftershocks recorded by French² and British³ groups define a broader region 50-km long aligned broadly NE–SW with shocks having depths of up to 15 km. Extensive surface faulting occurred within this zone (Fig. 1a). A magnitude of 7.3 suggests a geometric moment⁴.5 of 2×10^9 m³, consistent with a slip area 30-km long and 12-km deep with a mean displacement of 6 m. Further work will constrain the seismic parameters more closely but is unlikely to affect the present arguments.

We examine here some of the more striking features of the surface deformation associated with the earthquake and relate them to subsurface processes. Archaeological and stratigraphical evidence is used to demonstrate that some 30 similar earthquakes could account for the local topography and geological structure.

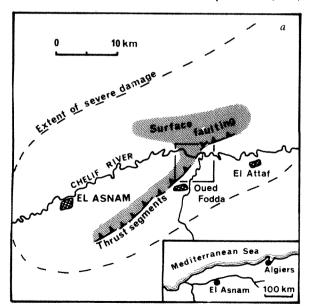
The central section of a detailed fault map of the area is shown in Fig. 1b; the complete map, which was compiled in conjunction with an aftershock study, will be published elsewhere³. A restricted area is chosen for discussion here for three reasons. First, it displays a well developed thrusting surface break which furnished a reliable measure of horizontal slip direction; and, as

this direction is consistent with that determined in preliminary fault plane solutions, the surface break probably bears a simple relationship to the seismogenic fault at depth. Second, the zone of deformation behind the fault is typical of that observed elsewhere in the area. Third, the channel and fill terraces of the Chelif river cross the section and yield information on recent and late Quaternary deformation.

Compressional faulting

In the region shown in Fig. 1b the thrust forms a clear scarp (Fig. 2) crossing fields and orchards which were level before the earthquake. Figure 3 shows the scarp cutting across one of several lines used for laying irrigation pipes in a potato field. The lines in this field show no lateral offset (excluding some disturbance close to the fault scarp) and must therefore be parallel to the horizontal component of the slip vector. The azimuth of N 140° E derived from this site was confirmed wherever the fault crossed other linear features such as irrigation channels and embankments. The form of the scarp suggests that its dip lies between 30° and 60° , a value consistent with the preliminary fault plane solution^{2,3}.

Slickensides observed near the southern end of the faulting give a spurious slip direction. This is not surprising as rock mechanics does not predict the formation of slickensides at the surface except in unusual conditions⁶. Brittle failure near the surface should always be tensile (as usually observed) and shear motion near the surface will occur only if it can exploit a very



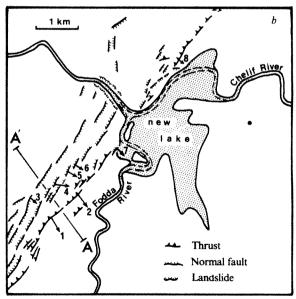


Fig. 1 a, Epicentral region showing approximate extent of severe damage, the principal segments of thrust surface breaks and the region of extensive surface faulting, most of which was tensional. b, Surface breaks near the Chelif river. Slip vector measurements: 1, 1.8 m (vertical component only), azimuth 140° E. 2, 3.2 m (vertical component only), azimuth 135° E. 3, 1.4 m (vertical component only), azimuth 130° E. 4, 1.0 m (total), azimuth 105° E, dip 50°. 5, 0.7 m (total), azimuth 125° E, dip 60°. 6, 0.7 m (total), azimuth 125° E, dip 60°. 7, 1.4 m (vertical component only), azimuth 130° E. 8, 1.2 m (vertical component only), azimuth 130°. The slip vector measurements used in Fig. 2 from section A-A' are not repeated here.

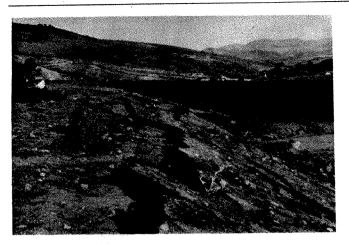


Fig. 2 The thrust surface break north of Oued Fodda (looking north-east).

The new fault scarp is 3.2 m high.

weak pre-existing plane. If such a plane exists the slip direction will not necessarily define the broader-scale direction of shortening⁷.

Tensional faulting

Although the reverse faulting is clear and unambiguous in the section we discuss here, the most extensive and striking features of the surface deformation are tensional. In other regions, for example north of El Attaf thrusting becomes difficult to identify but tensional faulting remains obvious.

Figure 4a shows some of the tensional features to be seen when looking south-west from the vicinity of A-A' (Fig. 1b). The faults have produced a graben which seems to be reflected in the existing topography, suggesting previous motion on similar faults. Figure 4b shows a normal fault in a similar environment at the southern end of the fault system. The stratigraphic offset here is several times the displacement of 1 m caused by the present earthquake. Other examples of normal fault reactivation were also found.

Matching of opposite sides of the new tensional cracks^{6,8} showed that, despite wide variation in dip, most of the new slip vectors (including many not shown on Fig. 1b) had horizontal components close to N 140°E.

Two traverses were made across the fault zone to measure slip vectors on fault segments. One of these traverses is indicated by A-A' on Fig. 1b and the information is summarized in Fig. 5d. If no block deformation or rotation occurs between faults then a rigid-body kinematic model can be described using a displacement vector diagram as in Fig. 5c. This suggests that the net effect of hanging wall ruptures is to modify the slip vector (and hence the fault plane) such that its dip increases with depth.

Although a rigid block hypothesis is adequate to describe the surface ruptures alone, it does not explain evidence for rotation and continuous deformation; we therefore reject it. Evidence for rotation and continuous deformation is better explained by a hypothesis of fold formation. This alternative model does not demand a change in fault dip with depth and accounts for all of the observed features.

Evidence for active fold development

The deformation zone is traversed by the Chelif river (Fig. 1a,b). After the earthquake water levels reportedly rose by 5 or 6 m and formed a lake $\sim 5 \text{ km}^2$ in area. The lake was dammed not by the thrust fault scarp but by a gentle upwarp 1 km further down the valley. Before the earthquake, upwarping had already disturbed the otherwise regular trend of the river gradient. This may be observed by plotting the river profile from the 1/50,000 maps (Fig. 5a) and was noted in 1957 by Boulaine⁹, who also ascribed it to active uplift. No lake is indicated in the 1934 edition of the topographic map (Oued Fodda sheet) but a marsh is shown in the 1890 sheet occupying roughly the same area. The

marsh probably represents the remains of a lake produced in a similar way, which was completely drained by downcutting of the river channel before the 1934 map revision.

The present longitudinal profile of the river is shown in Fig. 5a which also shows the profile of a river terrace that forms a clear, continuous and dateable feature except in the region of maximum uplift. Here the river flows in a gorge¹⁰ and the terrace cannot be traced continuously. Possible terrace fragments exist within the gorge section. Boulaine identified a terrace covered with immature soils at a height of 30 m above the river⁹ (Fig. 5a) but its relation to the terrace outside the gorge cannot be established. Yet even without this information it is clear that the terrace is more uplifted than the present river profile. The uplift must have occurred after the river ceased flowing at the terrace level.

A maximum age for the alluvial deposits of the terrace is provided by Roman sites they have buried in the Chelif basin. The age of the terrace surface is at present less certain but it could be 500 yr (ref. 11). A comparison of the terrace height

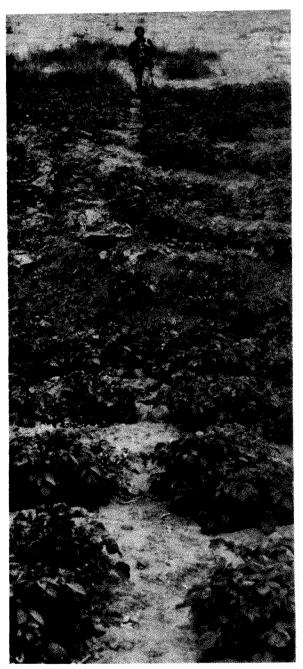
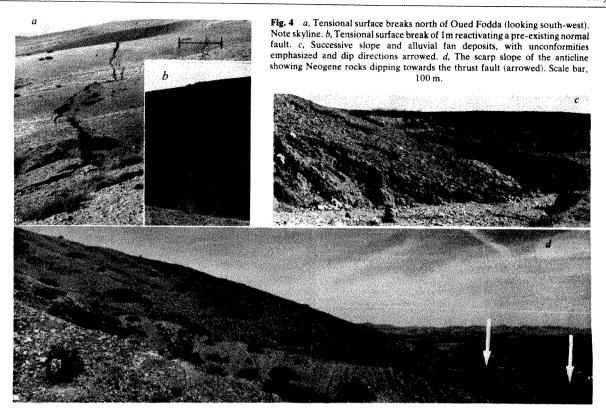


Fig. 3 The thrust scarp, here 1.8 m high, crossing a potato field. Photograph was taken along a line used for laying irrigation pipes. The line was not offset by the earthquake.



with the apparent uplift resulting from the 1980 earthquake suggests that one or possibly two earthquakes of similar magnitude have occurred since the end of deposition.

Figure 5b shows a representative structural section across the ridge. The surface breaks we have described occur at the crest and on the south-east flank of an asymmetrical anticline composed principally of late Tertiary (7 Myr) rocks. The deformation displayed by the bedrock (Fig. 5d) echoes that of the river profile and of the Chelif terrace. The steeper flank is mantled by scree and alluvial fan material. Ravines have cut through these deposits near the Chelif river to reveal three successive units (Fig. 4c), of which the oldest is nearly vertical, the middle dips about 45° and the youngest is in places nearly horizontal. The two oldest deposits contain artefacts that are Lower Palaeolithic, perhaps Acheulean, in type (Fig. 6) and therefore date roughly from the period 1-0.1 Myr ago¹². The youngest deposit contains Mousterian artefacts, which can be ascribed to the period 0.1-0.03 Myr ago¹². Deformation was therefore already in progress between these two time spans. The sequence closely resembles that of the Gafsa area in Tunisia, where conglomerates and sands containing late Acheulean artefacts with dips ranging up to 80° are overlain by alluvial deposits containing Mousterian and later industries. It is no coincidence that the Gafsa structure should also be a 'faulted fold'13

The close relationship between the tilted gravels, the thrust and the anticline north of Oued Fodda suggests that they are co-genetic. If we accept that the deformation of the Chelif terrace indicates one event and a recurrence interval of 500 yr all the topography shown in Fig. 4d could have been formed in 15,000 yr by 30 events each with an uplift of 5 m. A similar rate has been demonstrated for anticlinal uplift in the coastal Zagros¹⁴. The long chronology favoured by some archaeologists would, of course, imply longer recurrence intervals. In due course radiometric dating will remove the uncertainty.

Earthquake faulting and surface deformation

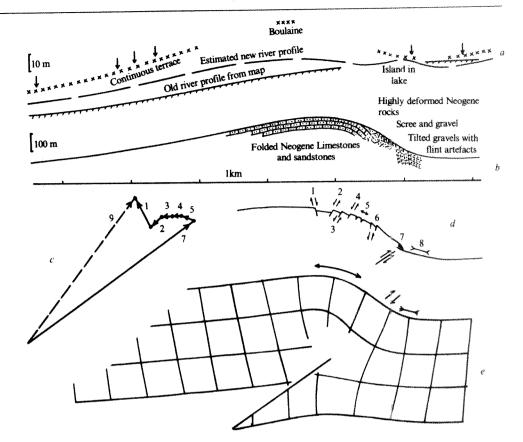
Earthquakes result from failure in rock in elastic—brittle conditions. Localized rupture at the hypocentre extends catastrophically as the rupture front expands over the fault plane until it reaches a region where the progressively increasing stress intensification resulting from increasing fault area is none the

less insufficient to fracture more rock¹⁵⁻¹⁷. The general nature of such regions remains enigmatic but certain conditions must prevail as the ruptured front of a crustal earthquake propagates downwards or towards the surface (see Fig. 7).

In regions both near the surface and at depth the rupture enters rock that is relatively stress-free before the earthquake, and fracture is driven into these zones only as a consequence of crack-tip stress. We emphasize that, so far as the rupture processes are concerned, the medium behaves in an elasticbrittle fashion and it is only over long time scales that creep relieves stress. Near the surface, stress is slowly relieved by chemical processes assisted by water flow in cracked or porous rock. These processes are suppressed at depth by confining pressure; at even greater depths thermally activated creep processes relieve stress in metamorphic conditions¹⁸. The depths that bound the elastic-brittle and elastic-brittle-ductile regions are shown in Fig. 7 as 5 and 20 km respectively. These depths are, of course, arbitrary and depend on such diverse factors as rock type, grain size, groundwater flow, thermal gradient and the recurrence period of earthquakes. The behaviour is also modified by complex fault geometry and the presence of highly incompetent layers such as salt. But, in so far as continental earthquakes rarely have hypocentres outside the range of 5-20 km and the surface ruptures of large earthquakes broadly reflect the source parameters determined from seismic observations, the model in Fig. 7 is adequate.

An implication of the model is that the rapid deformation at the time of an earthquake is elastic-brittle in nature. The ductility relieves stress created by the earthquake over a longer time scale and hence 'freezes in' the elastic deformation field produced at the time of the main shock. Following the earthquake, the relaxation of stress in the elastic-brittle-ductile zones will tend to reload the elastic-brittle zone. This reloading provides an additional or an alternative explanation for after shocks to that proposed by Das and Scholz¹⁶ who consider that stress corrosion in a brittle environment permits crack extension which leads to failure; essentially a microscopic process. The process described here will produce similar aftershock time histories but is a bulk process and depends on creep distant from the crack tip. In either case the release of strain in the aftershocks is small compared with the main shock and puts an upper limit on strain change associated with post-seismic creep. Thus

Representative sections showing faulting and deformation near the Chelif river. a, Old river profile from the topographic map, and the new river profile deduced partly from the form of the new lake. The post-Roman (Chelif) river terrace is indicated; arrows show where the height was calibrated with respect to the river profile by levelling. The terrace is a clear continuous feature arrowed the positions hetween upstream and downstream of the gorge. b, Topographical and geological section through the anticline showing the relation between the tilted gravels (as screes and alluvial fans) and the folded Neogene rocks. c, Vector diagram showing the effect of the observed tensional surface features on the slip vector at depth assuming a rigid, non-rotating, block The vectors correspond to motion on features shown in d. 1, 0.9 m, dip 60°, azimuth 100°; 2, 0.3 m dip 45°; 3, 0.3 m, dip 30°, azimuth 140°; 4, 0.5 m, dip 30°, azimuth 145°; 5, 0.2 m over many small tension cracks; 6, small bedding thrusts with negligible offsets in this particular section; 7, main thrust estimated to dip at 50°; 8, region of compression without faulting; irrigation channels in this region were telescoped by between 1 and 2m over a distance between 200 and 300m; 9, slip vector at depth. e, Deformation around a buried thrust fault with a dip of 35° and dimensionless fault parameters¹⁹ of d = 1.5, D = 1.25 I = 10 and slip of 1.15. Note that a and b have vertical exaggeration.



an elastic model approximates both the displacement at the time of the earthquake and the strain field following post-seismic stress relaxation. Modelling of the effect of ductility, which may produce long wavelength (but small strain) displacement fields will be discussed elsewhere.

Figure 5e shows the elastic deformation above a buried thrust fault. In this model the fault does not break the surface but the deformation field scarcely differs if the fault reaches the surface with diminished slip amplitude. The model is calculated using analytical finite integral expressions derived by Mansinha and Smylie¹⁹ for the displacement anywhere in a half space due to a buried dislocation surface on which displacement is specified. Figure 5e may be interpreted as representative of the deformation of a single earthquake in which strain has been exaggerated to produce a clear figure, or as an unexaggerated view of the cumulative effects of many similar earthquakes.

Assuming that the fractures represent only a small release of the total strain produced in surface materials, the position and nature of the fractures (Fig. 5d) are well explained by the model

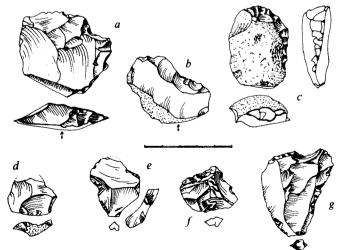


Fig. 6 Artefacts from the tilted gravels; a, b and c from the oldest unit; d, e and f from the intermediate unit; g from the youngest (ref. 11) unit. The scale is in centimetres.

in Fig. 5e. The only fractures poorly described by the model are those marked 6 in Fig. 5d and these had small displacement and were related to steeply dipping beds. This explanation of the surface features, based on a model of continuous deformation, contrasts with that offered earlier using a vector diagram (Fig. 5c), which assumes rigid unrotated blocks and requires the fault plane to steepen with depth. Although continuous deformation models in which fault dip steepens with depth would probably fit our observations, the data do not demand this refinement. The simple continuous deformation model also explains the form of the uplift of the river profile and terrace uplift (Fig. 5a). It also describes the deformation of the Neogene rocks and the present-day morphology (Fig. 5b).

Conclusions

Perhaps the most surprising aspect of the Algerian earthquake was the predominance of tensional surface faulting associated with a compressional event. This effect has been attributed to landslip motion or to changes in the dip of the main thrust plane with depth. The deformation model we present produces both extensional and compressional strain near the surface at the time of the earthquake; but, as surface materials fail more readily in tension than in compression, widespread tensional features are to be expected. The scale of the normal faulting at El Asnam is substantial and has produced major features in Neogene rocks. Thus normal faulting cannot be taken to be a fossil indicator of regional tension unless it is known to extend into the elastic-brittle zone.

The relation between structure, morphology and the location of surface breaks in the recent earthquake was clear and greatly assisted the task of fault mapping. The relation between topography and some of the surface features associated has been dismissed by many field workers as the product of large scale landslides. This view does not wholly contradict our interpretation. The elastic-ductile surface zone must retain sufficient shear strength to support the topography (or the mountains would rapidly fall down²⁰). The stresses that exist before the earthquake modulate the stress associated with the earthquake and thus, to some extent, determine where fault features are observed. In several places, tensional surface breaks increased

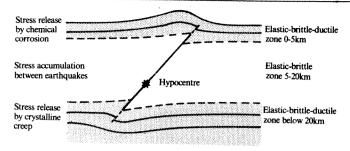


Fig. 7 The stress environment of a shallow continental earthquake.

in slip amplitude near the tops of hills and diminished in the valleys while the opposite was true of compressional surface breaks.

Although the rocks folded in this earthquake are mainly Neogene in age, the present evidence suggests that the observed folding is very much more recent. All of the folds in the region are unlikely to be this young. It is plausible that deformation has moved southwards with time and that the present fold is the frontal fold of an assemblage of serial folds21 (a view to be discussed more fully elsewhere), in which case the migration of fold activity should be regarded as the effect of serial faulting. In terms of the Jackson model of orogenic development²², this suggests that deformation in a closing sedimentary basin starts at the centre and spreads outwards.

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- Ambraseys, N. Geol. J. (in the press).
- Ouyed, M. et al. Nature 292, 26-31 (1981).
- Yielding, G. et al. (in preparation).
- Kanamori, H. & Anderson, D. Bull. seism. Soc. Am. 65, 1073-1095 (1975).
- King, G. C. P. Phil. Trans. R. Soc. A288, 197-212 (1978). King, G. C. P., Soufferis, C. & Berberian, M. Disasters (in the press). Bott, M. H. P. Geol. Mag. 96, 109-117 (1959).
- ., Mouyaris, N., Simeakis, K., Kondoyannis, Th. & Angelidhis, C. Nature 278, 45-48 (1979)
- Boulaine, J. Étude des Sols des Plaines du Chélif (Service des Études Scientifiques, Birmandreis, 1957).
- Anderson, R. van W. Mem. geol. Soc. Am. 4, 1-450 (1936). Vita-Finzi, C. Man 2, 205-215 (1967).
- Isaac, G. Ll. in Calibration of Hominoid Evolution (eds Bishop, W. W. & Miller, J. A.) 381-430 (Scottish Academic, Edinburgh, 1972).

Without suggesting that all folding is as intimately related to faulting as in the Algerian example, one is tempted by the simplicity of the proposed mechanism to regard it as widely applicable. Faults at depths formerly in the elastic-brittle region have been exhumed by erosion in sufficient numbers to account for a substantial proportion of surface folding.

Unlike the conventional view of fold formation as a plastic instability in a homogeneous stress field²³ or as a gradual process in incompetent rock above a competent faulted basement²⁴⁻²⁶ the observed folding occurs rapidly in conditions of inhomogeneous stress above and as a direct consequence of the properties of the elastic-brittle layer. The model also indicates fold formation beneath the brittle layer and, in so far as the upper and lower layers are arbitrary and time-dependent, within the brittle layer.

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Note added in proof: S. Wisnousky has drawn our attention to work related to ours being conducted at Tokyo University by Y. Ikeda and Y. Nobuyuki but at present published only in Japanese.

- Castany, G. Geol. Rdsch. 43, 196-203 (1955).
 Vita-Finzi, C. Nature 278, 632-634 (1979).
- Aki, K. & Richards, P. Quantitative Seismology (Freeman, San Francisco, 1980).
 Das, S. & Scholz, C. H. J. geophys. Res. (in the press).
- Madariaga, R. in Mécanismes et Prévision des Séismes (ed. Allègre, C. J.) 125-134 (CNRS, Paris, 1980).

- Ashby, M. F. & Verrall, R. A. Phil. Trans. R. Soc. A. 288, 59-95 (1977).
 Mansinha L. & Smylie, D. E. Bull. seism. Soc. Am. 61, 1433-1440 (1971).
 Jeffreys, H. The Earth, 4th edn (Cambridge University Press, Cambridge, 1959).
 Shearman, D. J. Geogr. J. 142, 393-404 (1976).

- Jackson, J. A. Nature 283, 343-346 (1980).
 Ramsay, J. G. Folding and Fracturing of Rocks (McGraw-Hill, New York, 1967).
 Stearns, D. W. 23rd A. Field Conf. Guidebook, 125-144 (Wyoming Geological Association, Laramie, 1971).
- Matthews, V. (ed) Mem. geol. Soc. Am. 151 (1978).
 Smithson, S. B., Brewer, J., Kaufman, S., Oliver, J. & Hurich, C. Geology 6, 648-652

Seismotectonics of the El Asnam earthquake

M. Ouyed, M. Meghraoui, A. Cisternas, A. Deschamps, J. Dorel, J. Frechet, R. Gaulon[‡], D. Hatzfeld[§] & H. Philip[§]

*Centre National d'Astronomie, d'Astrophysique et de Géophysique, Algeria, and Laboratoire de Géophysique Interne et Tectonophysique, ERA No. 603 IRIGM, BP No. 53 X, 38042 Grenoble Cedex, France

†Centre de Recherches et d'Applications en Géosciences, Algeria, and Laboratoire de Dynamique de la Lithosphère, Université de Paris VII, 4, place Jussieu, 75230 Paris Cedex 05, France

‡Laboratoire d'Etude Géophysique des Structures Profondes, LA No. 195, Institut de Physique du Globe de Paris, Université Pierre-et-Marie Curie, 4, place Jussieu, 75230 Paris Cedex 05, France

\$Laboratoire de Géophysique Interne et Tectonophysique, IRIGM, Université Scientifique et Médicale de Grenoble, BP No. 53 X, 38041 Grenoble Cedex, France

¶Laboratoire de Géologie Structurale, Université des Sciences et Techniques du Languedoc, Place E. Bataillon, 34060 Montpellier, France

The mechanism of the El Asnam earthquake of 10 October 1980 ($M_s = 7.2$), the strongest recorded in northwestern Africa is compatible with a convergence between the African and European plates. The thrust is also consistent with field observations and with long period data from the global network. Aftershocks cluster around the fault trace, with a maximum depth of 12 km. Maximum vertical displacement is \sim 6 m and there is some left lateral motion. The fault trace of the 1954 earthquake at Beni-Rached was also reactivated.

THE destructive earthquake in the region of El Asnam (Algeria) on 10 October 1980 (12.25 UTC) was of magnitude $M_s = 7.2$ according to the Europeo-Mediterranean Seismological Center (CSEM) and $M_s = 7.3$ according to the US Geological Survey. Previous earthquakes have occurred in this region on 9 September 1954 with magnitude 6.7 and epicentre at Beni-Rached, and on 7 September 1934 with

epicentre at El Abadia (Carnot) 5 km north of El Attaf $(m_b = 5.0).$

The event was the largest recorded in northwestern Africa, and because of its significance to the area geodynamics, a team of French and Algerian seismologists and geologists began recording the aftershocks and mapping surface breaks within 48 h. Some of their results are reported here.

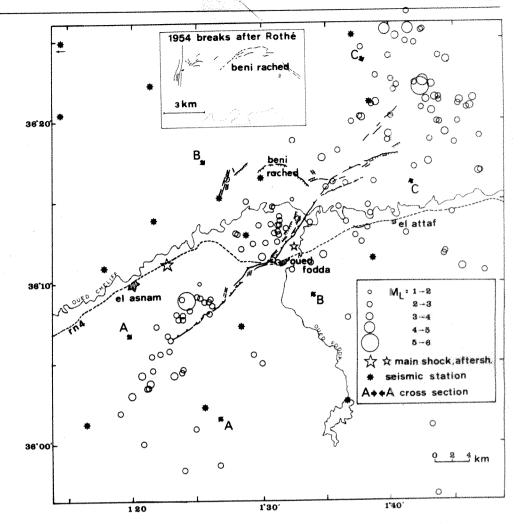


Fig. 1 Epicentres of 140 selected aftershocks (O) recorded by the local network up to 22 October 1980. A simplified map of the surface breaks is included for reference.

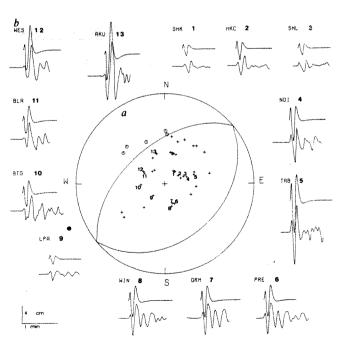


Fig. 2 Focal mechanism of main shock 10 October 1980 at 13h 25min, from long period data of the worldwide network (lower hemisphere, equal area projection). \bigcirc , Dilatations; +, compressions. The fault plane is well constrained and agrees with field observations, the auxiliary plane is less defined. The solution with no strike-slip is shown. Long period P-arrivals at different stations of the global network are shown. Theoretical seismograms (upper records) fit the first part of the signal in amplitude and phase. The numbers link seismograms to points on the focal sphere.

Geodynamics

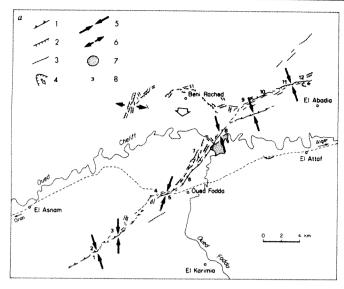
The Maghreb, like other sectors of the Western Mediterranean region has significant Quaternary tectonic movement. Recent neotectonic studies in the region have shown that the deformation is mostly compressional with associated folds, strike-slip faults, and thrusts, and a direction of shortening between N-S and NNW-SSE. This direction is compatible with the convergence of the European and African plates. Evidence of extension with direction E-W to ENE-WSW, orthogonal to the direction of shortening has also been found.

The large scale tectonics have been explained using plate tectonics and continuous deformation models^{2,3}. However, the distribution of intra-continental deformation suggested by the diffuse pattern of the seismicity and the focal mechanisms, indicate a plastic collision of continental masses without well-defined linear plate boundaries.

Effects of the earthquake

The main earthquake killed more than 3,500 people within the El Asnam district (population $\sim 900,000$). Most of the casualties occurred in the cities, although 80% of the people live in the countryside. The MKS intensity reached X in the cities of El Asnam, Oued Fodda and El Attaf. The inhabitants of the region felt small shocks as early as July 1980.

Many surface features accompanied the shock: the uplift of the northwestern block produced a natural dam ~ 4 km northeast of the city of Oued Fodda, where the Cheliff river formed a lake ~ 2.5 km² in area at the point where the Chelif river enters a canyon: landslides on the walls of the Canyon also contributed to the dam. This phenomenon shows that the river is older than many of the geological structures of the region: damming of the river may have occurred several times during the Quaternary, as shown by geomorphological evidence: for example, elevated meanders on the north-west block and thick sedimentation



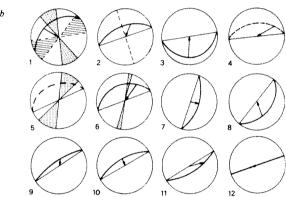


Fig. 3 a, Structural map of the epicentral region. 1, Thrust; 2, normal fault; 3, vertical cracks; 4, landslides; 5, direction of compression; 6, direction of extension; 7, flood; 8, site of slip measurements. b, Displacement vectors and striations. Stippling, extension cracks; hatching, pressure ridges. Lower hemisphere Schmidt projection shows the fault trace and striation or displacement vectors (4.5.6.).

during the Quaternary before entering the canyon due to successive inundations. Perturbations of the flow of the Chelif river reported⁴ after the 1954 earthquake can possibly be attributed to the same cause. Detailed observations of the Quaternary sediments under the lake⁵ should enable the recurrence record of large earthquakes along the fault to be studied.

Groundwater subjected to a sudden pressure increase was ejected to the surface leaving sand blars characteristic of large earthquakes. Many natural sources were interrupted (near the fault) and others were reactivated (away from the fault).

Main shock

Relocation was used with respect to a well determined reference shock (one recorded by our local network and by the global network (8 November 1980, at 7h 54min, $m_b = 5.3$)) to obtain more precise epicentres for the main shock and the largest aftershock: values of 36°11.1'N, 1°22.8'E for the main shock, and 36°12.5'N, 1°32.0'E for the largest aftershock (10 October 1980 at 15h 39min; $m_b = 6.0$) were obtained with standard error of 1.6 and 1.5 km respectively (Fig. 1). These differ by less than 8 km from values obtained by the USGS and the CSEM. Because our method fails to provide good depth control, we regard the vertical distribution of the aftershocks which give a maximum of 12 km for the depth of the main shock and of the largest aftershock as an important constraint. The data on the focal mechanism of the main shock, from the long period stations of the global network are shown in Fig. 2a. The fault plane can be determined accurately, but the auxiliary plane is

uncertain. Our solution has no strike-slip ($\phi = 230^{\circ}$, $\delta = 52^{\circ}$, $\lambda = 90^{\circ}$), but field observations suggest that a small component of left lateral motion is present, at least in the southern part of the fault zone. Field observations also allow the seismic moment to be estimated. If we assume an average displacement of 4 m, a fault length of 40 km and a fault width of 15 km from the aftershocks distribution $\mu = 3.10^{11}$ dyn cm⁻², we obtain a seismic moment $M_0 = 7.2 \times 10^{26}$ dyn cm. A similar seismic moment of $M_0 = 4.1 \times 10^{26}$ dyn cm was also obtained by modelling long period P-wave recorded at various stations. Figure 2b compares observed and theoretical seismograms using a simple point source in an homogeneous medium and the radiation pattern given by Fig. 2a. The source function is trapezoidal in shape with a total duration of 12 s. On some of the stations (PRE, GRM. ARO, BTG, LPA) a secondary arrival is observed some 3 or 4 s after the first one. This arrival is not seen at SHK, MAT, KEV and JKF and may be due to fault propagation. The arrival times of the reflected phases led us to work with a focal depth of 3.5 km; the position of the relocalization, however, meant that this was too shallow: A more complex time function of the source leads to a greater depth: the indroduction of a sedimentary layer above the source $(v_p = 4.5 \text{ km s}^{-1})$ has little effect on the waveforms.

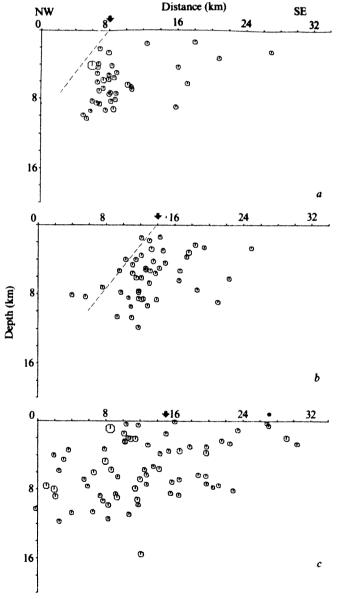


Fig. 4 Vertical distribution of the hypocentres of aftershocks at three different cross-sections (see Fig. 1). The complexity of the distribution increases from south-west (a) to the north-east (c) Dashed lines show the fault plane from teleseismic data.

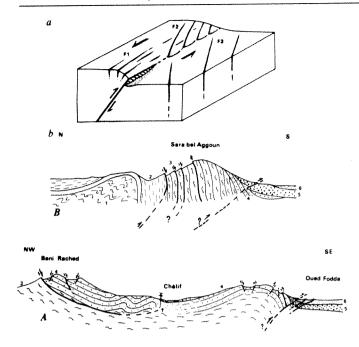


Fig. 5 a, Block diagram showing the structures at southern end of the fault. F₁, Flexure cracks; F₂, left lateral en échelon cracks; F₃, extension cracks parallel to pressure axis. b, Schematic crosssections. A, NW-SE section through Beni-Rached: 1, flysch; 2, medium Miocene marls; 3, higher Miocene marls; 4, Pliocene sandstone; 5, conglomerates; 6, recent Quaternary. B, N-S section through the anticline north-west of El Abadia (Fig. 3, site 10).

A stress drop of 70 bar can be estimated from the field observations using the procedure of Kanamori and Anderson⁶.

Aftershock activity

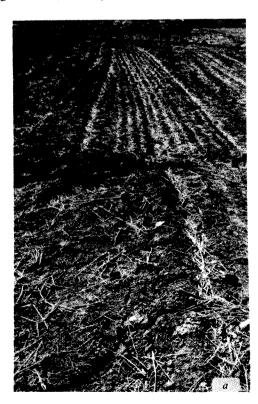
A network of 14 seismic stations (11 portable Sprenghneter MEQ-800 paper recorders and three 3-component analogue magnetic tape event recorders) were installed around the fault and operated for more than a month starting on 12 October. For clear records in conditions of strong aftershock activity, a low

gain was selected. A drum speed of 12 cm min⁻¹ was chosen and to obtain precise readings time marks were activated every second. Epicentres of 150 aftershocks within the period between 12 to 22 October 1980 (see Fig. 3) were determined using the HYPO 71 program with a layer of 3 km of 4.5 km s over an half space of 6 km s⁻¹. Most of the aftershocks correspond to local magnitude (calculated from duration) smaller than 3. There is a clear difference between the b-values at the north-east and those at the south-west side of the fault trace. For example, the station at El Tnine, (NE) recorded ~500 events per day, whereas that at Ouled Ben Abdel Kader (Massena) (SW) had only 50 events per day, the difference being smaller for the larger events located near these stations. There is clear indication of migration of seismicity at this stage of the analysis. A striking feature of the seismicity pattern is the gap in activity between the epicentres of the main shock and the city of Oued Fodda. This gap may be due to the fact that, after the main shock, most of the stress was released. Note that there is almost no seismicity around the trace of the curved fault at Beni-Rached which was activated during the 1954 earthquake. Overall aftershocks are concentrated towards the ends of the fault trace, but there is also a cluster towards the centre, north of the city of Oued Fodda.

Figure 4 shows cross-sections drawn at three places along the main fault and aftershocks contained within 11 km on each side of the cross-section projected on a vertical plane. Almost all the aftershocks have depths < 12 km, with a standard error of 2-3 km. Sections a at the south-west and b at the centre of the fault suggest a steeper fault plane than that given by the focal plane solution of the main shock. This slope is more pronounced in section a, indicating either a discontinuity or a twisting of the fault surface. Seismic activity is concentrated on the southeastern block. On the other hand the cross-section c shows a very diffuse pattern of seismicity. This area has the most complex surface breaks. The fact that a fan of surface cracks is observed suggests a multiple branching of the fault at depth at the end where the tearing stopped. This complexity might also explain the abundance of microearthquakes there compared with the smaller number of events recorded at the southwestern end.

Many records provided clear first readings and an attempt was made to construct mechanisms for the different clusters of aftershocks. Those located to the southwestern side of the fault

Fig. 6 a, The 1.30-m left lat slip measured on a ploughed at Zebadja near the centre of fault. b, Southern segment of fault. Typical pressure ric showing the uplift of the nowestern block (to the left), collapse due to gravity. Exten cracks follow the compression





trace, show a coherent composite mechanism that agrees rather well with the NW-SE compression and associated thrust of the main shock. But the data from the centre and the north-east give incoherent composite solutions. The only inference that we may draw at present is that the fault complexity seen at the surface also occurs at depth.

Tectonic structures

A large area was affected by surface fracturing (Fig. 3). The fault is relatively linear on the southern segment (N050) but it becomes wider and more complex towards the north as it approaches the hills on the right bank of the Chelif. This complex fault is superimposed on the complex geological structure of this area: the southwestern Miocene monocline gradually becomes a strongly folded and faulted structure towards the north-east (see cross-section, Fig. 5). The combined effect of rugged topography and the strong dip of layers of different strength in the folded area, may generate landslides of considerable extension and it is not easy to separate structures due to faulting from mass displacements produced by gravity. This is the case at Beni-Rached (see Fig. 3).

(1) Southern segment of the fault: the fault trace seems to be continuous in this region but a detailed analysis shows a succession of segments connected by *en échelon* cracks. There were several notable common features observed in this region.

A topographic flexure, some metres in size, with extension cracks parallel to the axis of the fold (see Fig. 5b, A) commonly occurred with the main break at the inflection point of this flexure. A striated fault plane is sometimes present. The striations indicate thrusting with an occasional left lateral motion component. At the sites with soft topography (small inclination of the fault plane near the surface) cracks (Fig. 3), some metres to tens of metres long are disposed en échelon indicating a left lateral displacement (see Fig. 5a). In all cases, outside the flexured area, we may see extension cracks that seem to be parallel to the pressure axis (Fig. 5a). In many places, where the surface soil is thick and unconsolidated (cultivated fields) the fault is recognized by a succession of pressure ridges (10-150 cm high) with the axis parallel to overall fault strike. The pressure ridges are shifted along extension cracks en échelon corresponding to transform faults. Similar surface features, which are very common along surface breaks during large shocks, were observed for the Tabas-e-Golshan earthquake7, and the Pariahuanca earthquake⁸ (Peru) (Fig. 6b).

(2) Northern segment: near the city of Oued Fodda the fault changes direction from N050 to N070 for ~3 km, and returns to N050 for ~ 10 km. At this point the fault spreads into several branches, some of which again have a N070 direction. Northwest of Oued Fodda the faulted zone is superimposed on an important topographic step, corresponding to an anticline affecting a Mio-Plio-Quaternary terrain. This anticline zone becomes N080 north of El Adabia. Here the faulting becomes more diversified and there are a large number of breaks with the aspect of normal faults. These normal faults are localized on the anticline, while below the relief there is a region of compression folds and thrusts with important displacements (up to 2.5 m) north of Oued Fodda (see Fig. 5b, A). On the other hand, motion along the segment at N070, between the lake formed by the Cheliff river and El Abadia, is modest (15-30 cm of horizontal displacement).

Normal faults within this region show different characteristics. In many cases they are the result of the extension of the surface layers of the fold (Fig. 3, site 7) or they may also correspond to the gravitational collapse of the upper block of the thrust. Those observed north of El Abadia, may result from slip along bedding planes with amplitudes of up to 3 m. This case occurs frequently in this zone, and steeply dipping beds seem to guide the deformation. Also, a fault with a 'normal' aspect on the surface may become a thrust at depth. This could be the case in Fig. 3 at points 10, 11 and 12, and Fig. 5b, A. Analogous cases of normal faults that show an aspect of reverse faults at the surface, have been demonstrated for the Thessaloniki earthquake.

More complex deformation mechanisms are observed in the folded region at the north, the whole anticline structure being affected. The left lateral component of the displacement at the south may be absorbed here. This hypothesis agrees with the progressive increase of the vertical slip towards the north-east. (3) Dislocations at Beni-Rached and Sidi Djilali: a continuous system of normal faults with large offsets extends for several kilometres near Beni-Rached in an arcuate shape near the interface between the thick limestone series of the Miocene marls and the Pliocene sandstones. This geometry suggests a large gravity slide. Several workers have described important water jets at the base of the relief at the level of the Cheliff valley, together with slow motions of the soil for several days after the main earthquakes. Note that these were the same structures that were activated during the 1954 earthquake¹⁰. The cross-section in Fig. 5b, A shows that the folds in the Pliocene layers on the right bank of the Cheliff valley may be explained by successive repetitions of the large landslides at the base of the Pliocene sandstones during the Quaternary, with the folds at the Cheliff level accounting for the extension in Beni-Rached. However, the complexity and size of the deformation mechanism means that a tectonic origin for these features is also possible. A detailed study of the aftershocks may throw some light on the nature of the tectonics at depth.

At Sidi Djilali (5-km west of Beni-Rached), the interpretation of the structures becomes more difficult. Some of the structures may be the continuation of those present at Beni-Rached, but others, corresponding to a graben parallel to the line of steepest slope have a tectonic origin. The direction of the grabens is roughly parallel to the compression axis as measured at the level of the main fault. The same interpretation may be given to certain graben to the east of Beni-Rached near the intersection with the main fault (Fig. 3). Also, a single focal mechanism corresponding to the only aftershock (8 km in depth) located at Sidi Djilali (Fig. 1), although constrained by the small number of stations, is compatible with a WNW extension of the graben.

Slip amplitude

A topographic survey conducted by the Algerian SNTF revealed important movements across the fault along the rail track from Algiers to Oran. The mean slope was 2% before the earthquake and is now 2% over 300 m. The levelling 3 days after the main shock shows that the changes are discontinuous, the largest being 6 m. The vertical displacements decrease to the South of the fault zone and disappear near the RN 19 highway (Fig. 3, site 1). To the north, vertical displacements are often larger than 1 m (2.5 m north of Oued Fodda) and reach 4 m north of El Abadia.

The amount of horizontal displacement increases from the south to the centre of the fault. It is zero at the level of RN 19 and amounts to $\sim 1.30 \,\mathrm{m}$ of left lateral slip at the centre (Zebadja) (Fig. 6a). It may reach 2.70 m to the north of Oued Fodda. It was measured by the offset of linear structures traversing the fault.

A measure of ground shortening was possible using a water duct orthogonal to the fault trace north of Oued Fodda. We obtained a total shortening of 2.2 m over 300 m—following the relative displacement of the duct with respect to the soil would give a separate confirmation of this observation—this figure provides a lower limit on shortening across the fault as the aqueduct did not cross the entire zone.

Conclusions

We have shown that, except for Beni-Rached and Sidi Djilali where gravity has a role, the nature of observed dislocations is consistent with a thrust with some left lateral slip in the South. The focal mechanism determined from long period data agrees with surface tectonics and aftershock distribution. Seismic moment calculations determined independently from local seismotectonic evidence and from long period P-data also give similar values.

The complexity of the fault increases from the south-west to the north-east, as indicated by surface evidence, aftershock distribution, the difficulty to obtain composite mechanisms, and b-value variations. The main fault, some 40 km in length which has been active during the Quaternary, is associated with folding of the Miocene, Pliocene and Quaternary formations, and is composed of segments of two different directions N050 and N070: flexure on the northwestern side of the main rupture produces a set of normal faults.

The 1980 earthquake, in contrast with the 1954 earthquake, shows a remarkable concordance between surface tectonics and seismic focal mechanisms giving a stronger constraint for plate motions. In particular, the shortening of ~2.50 m gives an upper limit to the relative motion between Africa and Europe of

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- 1. Bousquet, J. C. & Philip, H. Sedimentary Basins of Mediterranean Margins, URBINA (in the press).
- 2. McKenzie, D. P. Geophys. J. R. astr. Soc. 30, 109-185 (1972).
- Tapponier, P. Bull. Soc. geal. Fr. 7, 437-460 (1977). Theyenin, J. Terre Eaux. Alger 24, 14-23 (1955).
- Sieh, K. E. J. geophys. Res. 83, 3907-3939 (1978).

the order of 0.8 cm yr⁻¹, as there has not been such a large event in the past 300 yr.

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- Kanamori, H. & Anderson, D. L. Bull. seism. Soc. Am. 65, 1073-1095 (1975).
 Berberian, M. Bull. seism. Soc. Am. 69, 1861-1887 (1979).
- Philip, H. Megard, F. Tectonophysics 38, 259-278 (1977) Mercier, J. L., Mouyaris, N., Simeakis, K., Kondoyannis, Th. & Anghelidhis, C. Nature 278,
- Rothé, I. P. La Nature 3237, 1-9 (1955).
- Groupe de recherche Néotectonique de l'Arc de Gibraltar. Bull. Soc. geol. Fr. 7, 575-614

A human onc gene homologous to the transforming gene (v-sis) of simian sarcoma virus

Riccardo Dalla Favera, Edward P. Gelmann, Robert C. Gallo & Flossie Wong-Staal

Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The human genome contains a single and constant locus (c-sis) related to the transforming gene (v-sis) of simian sarcoma virus. The isolation of a recombinant phage containing the entire human c-sis gene has allowed the study of its genomic organization: c-sis extends over a region of \sim 12 kilobases (kb) which includes 1.2 kb of v-sis-related sequences interrupted by four intervening sequences.

RETROVIRUSES have been identified as the aetiological agents of naturally occurring tumours in several animal species (see ref. 1 for reviews). Furthermore, recent evidence indicates that they can sometimes be isolated from certain human T-cell leukaemias and lymphomas (see refs 2, 3 and review in ref. 4). Some of these agents rapidly induce tumours when inoculated into animals and can transform cells in vitro5. The genomes of these viruses contain sequences, called viral onc genes, which are directly responsible for transformation both in vitro and in vivo5 Substantial evidence indicates that these onc genes originated from normal cellular genes by recombination between a parent non-transforming virus and host cellular DNA5. Molecular hybridization experiments have shown that the cellular genes that gave rise to viral transforming genes are conserved throughout evolution⁵ and that their rate of evolutionary divergence parallels that of globin sequences⁶. This evolutionary conservation suggests that cellular onc genes may code for important functions in cell growth or tissue differentiation.

As these normal cellular genes are homologous to viral transforming genes, their potential role in tumorigenesis is of great interest. Several examples suggest that transformation could, in some cases, be due to an increased level of onc gene expression. In the case of the Rous sarcoma virus, the protein product of the vital transforming gene (src) is indistinguishable from that of its cellular homologue (sarc)⁷⁻⁹. The protein (pp60^{src}) is present at low levels in uninfected cells and its amount is elevated 100-fold in transformed cells⁷⁻⁹. Another cellular onc gene, c-myc, related to the avian myelocytomatosis virus (MC29) is also implicated in neoplastic transformation. B-cell lymphoma

induction in chickens by the non-acute leukosis virus (RAV-2) is sometimes associated with 'downstream' promotion of c-myc 10,11

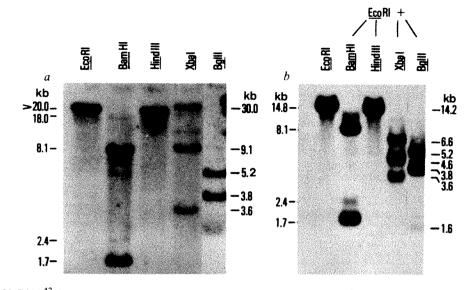
These observations underline the importance of studying the structure and function of human onc gene. Reports of preliminary experiments have confirmed the presence of some of these cellular onc genes in the human genome 6.12-14. Here we report the detection, molecular cloning and genomic organization of a newly described human onc gene (c-sis) related to the transforming gene (v-sis) of simian sarcoma virus (SSV).

The human genome contains v-sis-related sequences (c-sis)

SSV is a defective transforming virus isolated from the fibrosarcoma of a woolly monkey15,16. The genomes of both SSV and of its helper, simian sarcoma-associated virus (SSAV), were recently cloned in our laboratory¹⁷. The recombinant phages containing SSV (λ -C60) and SSAV (λ -B11) were characterized and compared with each other. The SSV genome was found to have a 1.2-kilobase (kb) insert, not shared by SSAV, which represents the viral onc gene (v-sis)17. Molecular hybridization experiments suggest that v-sis is a unique viral onc gene originating from woolly monkey DNA (F.W.-S. et al., unpublished

To investigate whether the human genome contains sequences related to v-sis, we analysed human DNA by restriction enzyme digestion and Southern blotting 18. Both SSV and SSAV were subcloned in the plasmid vector pBR322 (ref. 19) and the resultant recombinant plasmids (pC60 and pB11,

Fig. 1 Restriction pattern of c-sis in total human DNA (a) and in clone A-L33 (b). a, DNA from the human cell line HL60 (ref. 38) was prepared by cell lysis, proteinase K digestion, phenol extraction and ethanol precipitation³⁹; 20 µg were digested with 40 units of the appropriate restriction endonuclease in standard conditions recommended by the supplier. Fragments were separated by electrophoresis on a 0.8% agarose gel. DNA was denatured and transferred to nitrocellulose as described by Southern¹⁸ and hybridization was performed using dextran sulphate⁴⁰. The probe used was 2×10⁶ c.p.m. of nick-translated 18 SSV DNA (clone pC60). pC60 is a recombinant plasmid derived from λ -C60, the recombinant λ phage in which the entire SSV genome was originally cloned¹⁷. For subcloning into plasmid, the \u03b1-C60 insert was excised by EcoRI digestion, isolated on agarose gel and ligated into EcoRI-cleaved and bacterial alkaline phosphatase-treated pBR322 (ref.



41). Subclone selection was performed by colony hybridization to 32 P-labelled SSV/SSAV cDNA 42 . b, A partial AluI-HaeIII human DNA library (from T. Maniatis) 21 was screened using 32 P-labelled pC60 as a probe: 2.5×10^5 plaques were screened and one positive signal was obtained. The corresponding phage (λ -L33) was plaque-purified three times and grown in large scale 21 . λ -L33 DNA was purified and 2 μ g analysed as detailed in a. Molecular weights are in kilobases (kb).

respectively) were used to probe genomic blots of human DNA. Twelve different samples of human DNA from normal tissues, cell lines, leukaemic leukocytes and solid tumour tissues were digested with several restriction enzymes, run on agarose gel and hybridized in duplicate to pC60 and pB11 DNAs labelled with ³²P by nick translation²⁰. In all cases the same set of bands was detected by pC60 (an example is shown in Fig. 1a) but no hybridization was obtained using pB11 (not shown). The fact that v-sis is the only region of pC60 not present in pB11 indicates that human DNA contains a cellular homologue (c-sis) of the SSV transforming gene. To confirm that the hybridization was specific for v-sis, different plasmid subclones of pC60 were used as probes. Only those known to contain *v-sis* (sequences) detected some or all of the bands identified with pC60 (data not shown). Analogous results were obtained with DNAs from several vertebrate species, confirming the evolutionary conservation of c-sis 14. In all human DNAs tested c-sis is contained in a 20-25-kb fragment delimited by EcoRI and HindIII restriction sites. Within this region of DNA there are internal cleavage sites for several other restriction enzymes (BamHI, XbaI and Bg/II, Fig. 1a) and these are also conserved among different DNA samples. These data suggest that c-sis is a single-copy gene with a constant locus in the human genome.

Isolation of a recombinant phage containing human c-sis

A clone of the c-sis gene was isolated from a recombinant phage library of human DNA²¹. ³²P-labelled pC60 was used as a probe to screen 2.5×10^5 phage, corresponding to about one genome. A single recombinant phage (λ -L33) was isolated by the Benton and Davis plaque hybridization technique²². After EcoRI digestion to release the insert from the Charon 4A (Ch 4A) vector arms, the DNA was analysed by Southern blotting with the same enzymes used in the analysis of the genomic DNAs. The results are shown in Fig. 1b. Digestion with EcoRI showed a single 14.8-kb band. Ethidium bromide staining of the gel (not shown) revealed three fluorescent bands: the same 14.8-kb insert and the two vector arms (20 and 10.5 kb), indicating that λ -L33 insert has no *EcoRI* internal cuts. Thus, the size of the human DNA insert in λ-L33 is 14.8 kb. Digestion with HindIII yielded a band of 14.1 kb; an additional band of 0.7 kb visible in the gel did not hybridize to pC60. Therefore, comparison with Fig. 1a suggests that most of the EcoRI-EcoRI and HindIII-HindIII regions are present in the 14.8-kb λ-L33 insert. Within this region all the v-sis-related fragments generated by BamHI and BgIII are the same size as genomic DNA fragments generated by the same enzymes. The two XbaI sites of c-sis in genomic DNA are also present in λ -L33 as shown by the presence of three bands in λ -L33. One band (3.6 kb) in the genomic blot is identical in size to a band in λ -L33 (Fig. 1b) and the other two, corresponding to \sim 40 kb of genomic DNA, are partially represented in the 14.8-kb λ -L33 insert.

In all the enzymes tested (XhoI, SstI and PstI; not shown) there were no bands visible in the genomic blot that did not have equivalent bands in the λ -L33 blot. These data suggest that the entire c-sis is included in the clone. This was further confirmed by the facts that: (1) 32 P-labelled λ -L33 detected all the v-sis-specific fragments in a Southern blot filter containing a restriction digest of pC60 (data not show); and (2) heteroduplex analysis between λ -L33 and λ -C60 showed that the region of homology between the two clones is \sim 1.2 kb, the estimated size of v-sis (see Figs 4, 5). The availability of a clone containing the entire c-sis allowed us to study its genomic organization.

Genomic organization of c-sis

Two techniques were used to locate the regions in λ -L33 homologous to the 1.2-kb *v-sis* sequence. *v-sis*-related sequences in λ -L33 were mapped by restriction enzyme analysis

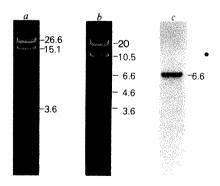
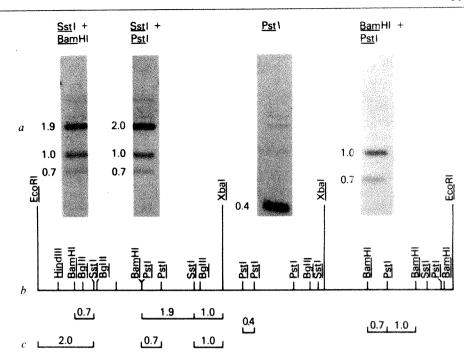
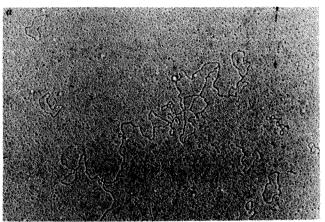


Fig. 2 $5' \rightarrow 3'$ orientation of c-sis in λ -L33. a, XbaI-digested λ -L33 DNA visualized by ethidium bromide staining. b, EcoRI + XbaI-digested λ -L33 DNA (ethidium bromide staining); 20- and 10.5-kb bands represent the left and right arms of Ch 4A, respectively. c, Hybridization of DNA from b to a probe containing the 5'-specific region of v-sis (plasmid pBG3). pBG3 was constructed by ligation of a BgIII fragment of pC60 (ref. 17), containing 0.35 kb of SSAV-related sequences and the first 5' 0.3 kb of v-sis, into BamHI-cleaved pBR322.

Fig. 3 v-sis homologous regions are fragmented in c-sis. λ-L33 DNA (100 ng)was digested simultaneously with EcoRI and XbaI restriction endonuclease. Fragments were run on a 1% agarose preparative gel. Bands at 6.6, 4.6 and 3.6 kb, corresponding to the human DNA insert, were purified from agarose by the NaI-glass powder binding method²⁴ and 100 ng from fragment were digested separately, run on a 1.4% agarose gel and transferred to duplicate nitrocellulose filters. One set of filters was hybridized to ^{32}P -pC60 DNA, the second to ^{32}P -labelled λ -L33 DNA. a, Hybridization to pC60; b, reconstructed restriction map of λ-L33 derived from hybridization of each XbaI fragment to ³²P-labelled λ-L33 DNA (blots not shown); c, brackets indicate the position of fragments (shown in a) which hybridize to pC60.





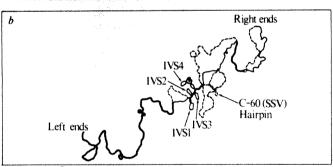


Fig. 4 Heteroduplex analysis of c-sis and v-sis. a, Electron micrograph of heteroduplex structure formed by annealing λ -L33 DNA and λ -C60 DNA. Heteroduplex formation was carried out in 50% formamide and 25 °C after alkali denaturation of caesium-banded phage particles⁴³. Heteroduplex spreading used a hyperphase of 50% formamide and a hypophase of 20% formamide⁴³. Magnification $\times 14,000$. b, An interpretive drawing of the heteroduplex structure. Intervening sequences are designated IVS 1-4 in the 5' \rightarrow 3' direction of c-sis and SSV DNA.

and heteroduplex molecules were formed between λ -L33 and the recombinant phage containing the SSV genome (λ -C60).

Restriction enzyme analysis: The orientation of c-sis in λ -L33 was determined using the endonuclease XbaI which does not cut either arm of Ch 4A (ref. 23). Digestion of λ -L33 with XbaI yielded three fragments of 26.6, 15.1 and 3.6 kb (Fig. 2a). When the human DNA insert was released from the vector arms by EcoRI digestion, the 26.6- and 15.1-kb fragments were reduced

to 6.6 and 4.6 kb, respectively, which suggests that they are linked respectively to the left (20 kb) and right (10.5 kb) arm of Ch 4A (Fig. 2b). The 3.6-kb band is conserved in both digestions; thus this fragment is not linked to vector arms and is located centrally in the λ -L33 insert. To determine the $5' \rightarrow 3'$ orientation with respect to the sis coding sequences, EcoRI-XbaI-digested λ-L33 DNA was hybridized to a probe specific for the 5' region of v-sis. Only the 6.6-kb fragment hybridized to this probe (Fig. 2c). Thus the data suggest that the orientation of XbaI fragments in λ-L33 is: (Ch 4A left arm)-5'-6.6 kb-3.6 kb-4.6 kb-3'-(Ch 4A right arm). For simplicity we mapped each XbaI fragment separately after isolation on a preparative agarose gel²⁴. Restriction digests of each fragment were used to make duplicate Southern blots to hybridize to 32P-labelled λ-L33 or p-C60 DNA. λ-L33 bands (not shown) allowed the construction of the map shown in Fig. 3b.

The v-sis-related bands are shown in Fig. 3a for each of the three fragments. These bands represent sequences of homology in five noncontiguous regions (bracketed in Fig. 3c). Fragments detected by λ -L33 but not by pC60 represent DNA consisting only of sequences unrelated to v-sis. In the case of the internal XbaI fragment these must be due to intervening sequences interrupting v-sis-related regions. Both intervening sequences (that is, between hybridizing regions) and flanking cellular sequences are present in the 5' and 3' XbaI fragments of c-sis.

The restriction enzyme analysis suggests the presence of five v-sis homologous regions interrupted by four intervening sequences. To analyse further this structure, electron microscopic studies were performed.

Heteroduplex analysis: Human c-sis, cloned in Ch 4A, and the 6.3-kb SSV genome, cloned in Ch 21A (Ch 21A) (ref. 17), were compared by heteroduplex formation. A typical molecule is shown in Fig. 4. The left arm of Ch 21A (C60) is 21.2 kb, of which 19.8 kb from the end are identical to the entire left arm of Ch 4A (L33) (ref. 23). Two portions of the right arms are the same²³ and form an interrupted duplex. The hairpin loop structure in λ -C60, formed by the self annealing of a direct and inverted long terminal repeat (LTR)17, provides a convenient marker for locating v-sis at the 5' side of the hairpin. Here a region of hybridization (~1.3 kb) between λ -C60 and λ -L33 can be seen. This region contains four deletion loops which represent sequences in the λ -L33 insert nonhomologous to v-sis. As they are between regions of homology they are most likely the four intervening sequences identified in the restriction enzyme map. Between the left arm area of the vectors and the region of homology there is a substitution loop formed by the 5'-flanking cellular sequences of λ -L33 (which do not hybridize to the SSV helper sequences 5' of v-sis) and the remaining 1.4 kb of the Ch 21A left arm not included in the Ch 4A left arm. A large substitution loop 3' of v-sis includes SSAV-related sequences of C60 and the Ch 21A right arm facing λ -L33 flanking cellular sequences and the Ch 4A right arm. Measurements and orientation of the left substitution loop, duplex regions and the deletion loops (Fig. 5) are consistent with the map derived from the restriction enzyme analysis. A general scheme of the genomic arrangement of c-sis is shown in Fig. 5.

Repeated sequences in c-sis introns

In heteroduplex experiments, λ-L33 single-stranded molecules showed various secondary structures. Furthermore, when λ-L33 was used as a probe in genomic blot experiments with human DNA, a hybridization smear was visible (data not shown). These observations prompted us to check the presence of repeated sequences in λ -L33, specifically, the Alu family of interspersed repeats because most repeated sequences in the human genome belong to this family²⁵. Accounting for 3-5% of human DNA, the Alu family consists of several 300-base-paired fragments bearing a single AluI site25. A recombinant plasmid (BLUR 8) containing a 300-base pair Alu repeat insert25 was used as a probe to ascertain the presence of these repeats in λ -L33. Several regions of hybridization were seen in restriction digests of λ -L33 DNA (data not shown). To locate the repeats more precisely, each XbaI fragment was mapped as previously described. Results are shown in Fig. 6b. Segments containing Alu repeats are located in three separate regions included in two different intervening sequences. Their approximate positions are also shown in Fig. 5.

Discussion

Vertebrate genomes contain nucleotide sequences homologous to the transforming genes of oncogenic retroviruses $^{5.6.12-14.26.27}$. Our study extends this observation to the cellular homologue of v-sis, a newly characterized viral transforming gene 17 , most likely originated from woolly monkey DNA (F.W.-S. et~al., unpublished data). We report elsewhere 14 that c-sis is conserved among vertebrate species and here we describe the characterization of the c-sis locus in the human genome. Our data suggest that v-sis homologous sequences are present in a single and constant locus in human DNA. This locus showed no polymorphism in our analysis of 12 different DNA samples. c-sis consists of a DNA sequence 12 kb long where 1.2–1.3 kb of v-sis homologous sequences are interrupted by four intervening

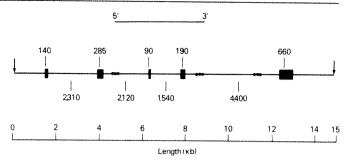


Fig. 5 A schematic representation of the organization of c-sis sequences in $\lambda\text{-}L33$. The lengths of the v-sis homologous regions (black boxes) and intervening sequences (solid lines) are mean values derived from measurements of at least six heteroduplex structures. Standard deviations range from $\pm 5\%$ for the larger to $\pm 15\%$ for the smaller measurements. Charon 4A DNA sequences are not shown here; arrows indicate the ends of the cloned genomic fragment. $\bullet \bullet \bullet$ Indicate the approximate position of repeated sequences of the human Alu family (see text and Fig. 5). kb, Kilobases.

sequences. The presence of intervening sequences has been shown in all but one case by studies of cellular *onc* genes from the species of origin of the respective retroviruses. Examples are the cellular *onc* genes of Abelson leukaemia virus in mice, of Harvey sarcoma virus in rats, Rous sarcoma virus and avian erythroblastosis virus in chickens, and feline sarcoma virus in cats ^{13,26}. Although *v-sis* is not originated from human DNA, the general structure of *c-sis* has been preserved throughout evolution, which suggests that it may represent a functional gene in man. If *c-sis* and *v-sis* code for a similar protein as in the Rous sarcoma system⁹, the intervening sequences would represent noncoding regions (introns) which would be excised during RNA processing.

A separate observation reported here is the presence of repeated sequences related to the Alu family of repeats in c-sis introns. The significance of this finding is unknown. Members of this family of repeats are estimated to be present $\sim 300,000$ times throughout the human genome²⁵. They have been found at the 5'- or 3'-flanking region of several genes and in the intergenic region of gene clusters, as in the case of the human β -globin gene family²⁸. However, their inclusion in intervening sequences has not been reported. Preliminary data from our laboratory suggest that the human onc genes related to feline sarcoma virus (c-fes) and avian myelocytomatosis virus (c-myc) also contain Alu repeated sequences. Further experiments will be required to determine the exact location of these repeats in

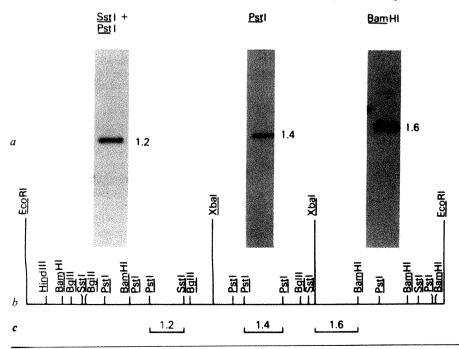


Fig. 6 Mapping in λ-L33 of repeated sequences homologous to the human Alu family. a, λ-L33 Xba fragments of DNA were prepared as described in Fig. 2 legend and each fragment was hybridized to BLUR 8 DNA²⁵. This recombinant plasmid contains a 300-bp insert corresponding to one copy of the human Alu family of interspersed repeats²⁵. b, λ-L33 restriction map; c, brackets indicate the position of hybridizing fragments (shown in a).

the different cellular onc genes and their role, if any, in onc gene expression.

Thus far, the protein product of either v-sis or c-sis has not been fully characterized. Many of the known retroviral transforming proteins are kinases which have the unusual property of phosphorylating tyrosine residues²⁹⁻³⁴. Furthermore, recent studies indicate that some of these viral onc gene products are structurally and functionally similar to cellular protein kinases involved in the regulation of the (Na⁺ + K⁺)ATPase pump (M. Spector and E. Racker, personal communication). It is of interest to test whether other onc genes, including c-sis, are involved in this basic metabolic pathway. In preliminary experiments we used cloned SSV DNA bound to nitrocellulose to select v-sisspecific mRNA from SSV-transformed non-producer cells. In vitro translation of this mRNA resulted in the synthesis of a protein of molecular weight 20,000 (V. Manzari et al., unpublished data). This product could account for the coding potential of c-sis and experiments are now being done to characterize the protein.

Substantial evidence suggests involvement of cellular onc genes in neoplastic transformation. Increased levels of expression of any of these genes may be the common mechanism in pathogenesis of different types of tumours. This hypothesis is

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- 1. Klein, G. Viral Oncology (Raven, New York, 1980).
- Poiesz, B. J. et al. Proc. natn. Acad. Sci. U.S.A. 77, 7415-7419 (1980).
 Reitz, M. S., Poiesz, B. J., Ruscetti, F. W. & Gallo, R. C. Proc. natn. Acad. Sci. U.S.A. (in the
- 4. Gallo, R. C., 3rd a. Bristol Myers Symp, Cancer Res. (eds Kaplan, H. S. & Rosenberg,

- Duesberg, P. H. Cold Spring Harb. Symp. quant. Biol. 44, 13 (1980).
 Roussel, M. et al. Nature 281, 452-456 (1979).
 Oppermann, H. D., Levinson, A., Varmus, H. E., Levintow, L. & Bishop, J. M. Proc. natn. Acad. Sci. U.S.A. 76, 1804-1808 (1979).

 8. Karess, R. E., Hayward, W. S. & Hanafusa, H. Proc. natn. Acad. Sci. U.S.A. 76, 3154-3158
- 9. Collett, M. S., Erikson, E., Purchio, A. F., Brugge, J. S. & Erikson, R. L. Proc. natn. Acad Sci. U.S.A. 76, 3159-3163 (1979).

 10. Hayward, W. S., Neel, B. G. & Astrin, S. M. Nature 290, 475-480 (1981).

 11. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. Cell 23, 323-334

- 12. Spector, D. H., Varmus, H. E. & Bishop, J. M. Proc. natn. Acad. Sci. U.S.A. 75, 410-4106
- Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. Cell 22, 777-785 (1980).
 Wong-Staal, F., Dalla Favera, R., Franchini, G., Gelmann, E. P. & Gallo, R. C. Science (in the press).
- 15. Theilen, G. H., Gould, D., Fowler, M. & Dungworth, D. L. J. natn. Cancer Inst. 47, 881-889 (1971)
- Wolfe, L. G. et al. J. natn. Cancer Inst. 47, 1115-1120 (1971).
- 17. Gelmann, E. P., Wong-Staal, F., Kramer, R. A. & Gallo, R. C. Proc. natn. Acad. Sci. U.S.A. (in the press).

substantiated by both in vitro and in vivo experiments. In vitro experiments performed with mouse c-mos, the cellular homologue of Moloney sarcoma virus²⁷, showed that this gene acquires transforming activity when linked to promoter sequences from the murine leukaemia virus genome²⁷. In vivo, B-cell lymphomas in chickens are characterized by abnormal expression of another cellular onc gene, c-myc 10-11. As these genes are conserved structurally and functionally among diverse species it is conceivable that they are also targets for tumorigenesis in man. There is evidence that mutation at certain loci is associated with specific human malignancies35. Moreover, it has been recently reported36,37 that DNA from certain human tumours have transformation activity in vitro involving specific, possibly unique genetic loci. Complete definition of the family of human onc genes will allow us to investigate whether any are involved in the pathogenesis of human aumours. The detection and molecular cloning of the human c-sis gene has allowed us to initiate these studies.

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18. Southern, E. M. J. molec. Biol. 98, 503-517 (1975).

19. Bolivar, F. et al. Gene 2, 95-113 (1977)

Rigby, P. N., Dieckmann, M., Rhodes, C. & Berg, P. J. molec. Biol. 113, 236–251 (1977).
 Maniatis, T. et al. Cell 15, 687–701 (1978).

- Benton, W. D. & Davis, R. W. Science 198, 180-182 (1977). Williams, B. G. & Blattner, F. R. J. Virol. 29, 555-575 (1979)
- Vogelstein, B. & Gillespie, D. Proc. natn. Acad. Sci. U.S.A. 76, 615–619 (1979).
 Jelinek, W. R. et al. Proc. natn. Acad. Sci. U.S.A. 77, 1398–1402 (1980).
- Franchini, G., Even, S., Sherr, C. S. & Wong-Staal, F. *Nature* 290, 154–157 (1981).
 Oskarsson, M., McClements, W. L., Blair, D. G., Maizel, S. V. & Van de Woude, G. F. Science 207, 1222-1224 (1980).
- 28. Duncan, C. et al. Proc. natn. Acad. Sci. U.S.A. 76, 5096-5099 (1979)
- Witte, O. N., Dasgupta, A. & Baltimore, D. Nature 283, 826-831 (1980). Van de Ven, W. J. M., Reynolds, F. H. & Stephenson, J. R. Virology 101, 185-197 (1980). 31. Barbacid, M., Beemon, K. & Devare, S. G. Proc. natn. Acad. Sci. U.S.A. 77, 5158-5162
- 32. Feldman, R., Hanafusa, T. & Hanafusa, H. Cell 22, 757-765 (1980)
- Collett, M. S. & Erikson, R. L. Proc. natn. Acad. Sci. U.S.A. 78, 2021-2024 (1978).
- Sefton, B. M., Hunter, T., Beemon, K. & Eckart, W. Cell 20, 807-817 (1980).
 Knudson, A. G. New Engl. med. J. 301, 606-607 (1979).
- Shilo, B. Z. & Weinberg, R. A. Nature 289, 607-609 (1981).
 Shih, C., Padhy, L. C., Murray, M. & Weinberg, R. A. Nature 296, 261-264 (1981).
- Collins, S. J., Gallo, R. C. & Gallagher, R. E. Nature 270, 347-399 (1977).
 Wong-Staal, F., Reitz, M. S. & Gallo, R. C. Proc. natn. Acad. Sci. U.S.A. 76, 2032-2036
- 40. Wahl, G. M., Stern, M. & Stark, G. R. Proc. natn. Acad. Sci. U.S.A. 76, 3683-3687 (1979).
- Ulrich, A. et al. Science 196, 1313 (1977). Grunstein, M. & Hogness, D. S. Proc. nam. Acad. Sci. U.S.A. 72, 3961 (1975)
- 43. Davis, R. W., Simon, M. & Davidson, N. Meth. Enzym. 24, 413-428 (1971)

Inflation in the Universe

John D. Barrow*† & Michael S. Turner*‡

- * Institute for Theoretical Physics, University of California, Santa Barbara, California 93106, USA
- † Department of Physics, University of California, Berkeley,
- California 94720, USA ‡ Astronomy and Astrophysics Center, University of Chicago, Chicago, Illinois 60637, USA

During the past 15 yr a small but remarkable collection of cosmological conundrums have been identified; each apparently independent, but probably all inter-related at a deeper level than existing theories have penetrated. The outstanding problems are of explaining the observed isotropy, homogeneity, flatness and specific entropy of the Universe¹. Good explanations for the last of these have recently emerged^{2,3} from the study of C, CP and baryon nonconserving interactions which arise in grand unified gauge theories (GUTs) of the strong and electroweak interactions. Recently, Guth has discussed the inflationary Universe as a possible natural explanation for the observed large-scale homogeneity and near critical density ('flatness') of the Universal expansion⁴. We show that one cannot ignore the first conundrum—the isotropy; a Universe

with a large amount of anisotropy will not undergo the inflationary phase. A Universe with only moderate anistropy will undergo inflation and will be rapidly isotropized. Other consequences of the inflationary Universe are discussed.

The three specific problems which we shall refer to are: the isotropy problem; the homogeneity-horizon problem; and the flatness problem.

The isotropy problem is exhibited by the observed structure of the 2.9 K microwave background radiation⁵. The temperature distribution of this primordial radiation field is isotropic to high precision⁶, $\Delta T/T \leq 10^{-4}$ over large angular scales, $\theta \geq 7^{\circ}$. All attempts to explain this present isotropy as a consequence of classical dissipative processes acting during the early stages of the Universe have been unsuccessful and they are opposed in principle by various general arguments⁷⁻⁹. Despite being an unstable property of physically realistic cosmological initial conditions, isotropic expansion is a property of the observed Universe. It is not yet clear whether particle production at $t \sim 10^{-43}$ s will help resolve why the present level of isotropy exists and when it arose because some anisotropies grow up again after the Planck time and some anisotropic plane wave universes create no particles 10-12.

The homogeneity-horizon problem falls into two parts. On the one hand, the microwave and X-ray radiation backgrounds,

and the radio source counts^{6,13}, all provide evidence for a striking level of large-scale spatial homogeneity on scales >~1 Gpc. But on the other, we see that luminous material is clustered into discrete inhomogeneities of characteristic sizes. Statistical studies⁶ of galaxy clustering indicate the existence of a universal clustering scale, $\sim 10^{15} M_{\odot}$, which divides the developed (non-linear) small-scale structure of galaxies and clusters from the smooth background. On dimensions exceeding the encompass of $\sim 10^{15} M_{\odot}$ the observed structure fades away into increasing homogeneity on larger and larger scales. Explaining the origin of small-scale inhomogeneity in the form of galaxies whilst simultaneously explaining the smooth underlying background on very large scales is the homogeneity problem. This problem is compounded by the presence of horizons¹⁴ in known cosmological solutions to Einstein's equations. In the Friedmann model during the radiation-dominated epoch, the total mass of baryons within a causally coherent region of volume $\sim (ct)^3$ at time t after the 'bang' is only $M_{\rm H} \sim (t/1{\rm s})^{1.5} M_{\odot}$. This ensures that causal processes can only smooth out irregularities over the small scale $M_{\rm H}(t)$ at early times. By the same token, the spontaneous generation of small inhomogeneities during phase transitions or other non-linear events is limited to the scale $M_{\rm H}(t)$. To explain the large-scale homogeneity or small-scale inhomogeneity, rather than treat them as initial data, it is necessary (but not sufficient) to find mechanisms which can remove or considerably inflate the horizon size during the very early $(t \le 10^{-4} \text{ s})$ stages of the Universe. This is the horizon problem.

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It is still an open question whether or not the Universe contains sufficient material to begin recollapsing at a finite time in the future. One reason why this question has proved so difficult to resolve observationally is the 'flatness' problem⁴. The Universe is expanding very close to the intermediate (Einsteinde Sitter) model which contains the critical matter density and so just sufficient expansion energy to expand to infinity. To evolve the observed proximity to the flat (=Euclidean space sections) Einstein-de Sitter model, deviations from the critical density must have been smaller than one part in 10^{57} at the Planck time,

$$\left(\frac{\rho - \rho_{\rm cr}}{\rho_{\rm cr}}\right)_{\rm fp} \leq 10^{-57}$$

This remarkable degree of fidelity constitutes the flatness problem. Another way of phrasing it is: although the only natural time scale in the gravitation theory is $t_{\rm p} \sim 10^{-43}$ s, the Universe has expanded for $\sim 10^{60}$ $t_{\rm p}$ without either recollapsing or entering the Milne (negative) curvature-dominated phase.

Recently, Guth⁴ has suggested a possible resolution of the horizon-homogeneity and flatness problems—the so-called inflationary Universe. In his picture, owing to the slowness of the GUT phase transition at $t_{\rm GUT} \sim 10^{-35}$ s the Universe undergoes a period of de Sitter expansion during which the (particle) horizon grows exponentially with time and the spatial curvature decreases relative to the matter density, also exponentially, thereby explaining two of our conundrums.

In the Friedmann model the expansion scale factor, R(t), is governed by⁵, $(\hbar = c = k_B = 1)$;

$$\frac{\dot{R}^2}{R^2} = \frac{8\pi G}{3} (\rho_r + \rho_0) - \frac{k}{R^2}$$

$$= \frac{4\pi^3 G}{45} g_*(T) T^4 + \Lambda - \left(\frac{k}{R^2 T^2}\right) T^2 \tag{1}$$

where ρ_r is the radiation energy density which equals $g_*(T)\pi^2T^4/30$, $g_*(T)$ is the total effective number of degrees of freedom of all relativistic species at temperature T (= $\Sigma_{\rm bosons}$ g + 7/8 $\Sigma_{\rm termions}$ g), ρ_0 is the vacuum energy density associated with the cosmological constant, $\Lambda = 8\pi G \rho_0/3$, and $k = \{\pm 1, 0\}$ is the space-time curvature signature⁵. We know today that the cosmological term is small, $\rho_0 < 10^{-30}$ g cm⁻³. However, in particle physics theories which undergo spontaneous symmetry breaking (SSB) the Lorentz invariant energy density associated

with the vacuum changes during a phase transition and creates an effective cosmological constant, $\rho_0 \sim {\rm O}(T_{\rm c}^4)$, where $T_{\rm c}$ is the critical temperature of the phase transition. In GUTs the symmetry of the theory breaks down at a temperature ${\rm O}(10^{15}~{\rm GeV})$ leaving an induced cosmological constant and associated vacuum energy density ${\rm O}(10^{77}~{\rm g~cm}^{-3})$ —unless one specifies an initial compensatory cosmological term of exactly the same magnitude but opposite sign. In this case, at temperatures above $T_{\rm c}$ we have $\rho_0 \sim (10^{15}~{\rm GeV})^4$, but below $T_{\rm c}$ the cosmological term is zero—the initial cosmological term and the vacuum energy density associated with SSB precisely cancel.

As the Universe cools below T_c , bubbles of the low-temperature (broken) phase nucleate and grow, and eventually the entire Universe is in the broken phase. However, Guth⁴ pointed out that if the nucleation rate is sufficiently small, then the Universe will remain in the high-temperature (symmetric) phase for a non-negligible period: the 'false vacuum' is metastable. During this interval the initial cosmological term, ρ_0 , remains uncancelled and soon begins to dominate the expansion dynamics, $\rho_0 > \rho_r$. The Universe undergoes a phase of de Sitter expansion, $R \propto \exp\left(\Lambda^{1/2} t\right)$ with $T \propto R^{-1} \propto \exp\left(-\Lambda^{1/2} t\right)$ and 'supercools' (the Universe remains in the symmetric vacuum until a temperature $T_s \ll T_c$). Finally, the transition is completed $(t = t_* \geqslant t_{\rm GUT}$ and $T_s \sim T_c \exp\left(-\Lambda^{1/2} t_*\right) \ll T_c$); the initial ρ_0 term is cancelled and the Universe is reheated to nearly T_c by the latent heat release. The expansion then resumes Friedmann behaviour. (The graceful return to a Friedmann model remains a difficulty⁴.)

Just before the transition occurs, $(T \ge T_c)$, one might expect that because t is not too different from $t_p \sim 10^{-43}$ s, (the only natural time scale in the theory), the curvature term (k/R^2) would be $\sim O(\rho_r)$ (the flatness problem). In addition, if the baryons were created (or already present) at this time, then there would be only about one baryon per horizon volume. Guth⁴ points out that if the inflationary phase lasts long enough to supercool by a factor $\sim 10^{28}$ ($t_* \sim 65 \Lambda^{-1/2} \sim 65 t_{\rm GUT}$), then when Friedmann behaviour recommences: (1) the curvature term will have been reduced relative to the radiation density by a factor $\sim 10^{56}$ ($T_* \sim T_c$, while R has inflated by 10^{28}) so solving the flatness problem; and (2) the horizon has grown large enough to encompass the portion of the Universe accessible to us today $\ge 10^{22} M_{\odot}$. Thus if the Universe was smooth on scales of the horizon at $t_{\rm GUT}$, the horizon problem is solved.

The question naturally arises as to whether nucleation is slow enough to allow such supercooling. The probability that a given point remains in the unbroken phase is (ref. 4, equations 3.14/.15)

$$p(t) = \exp\left\{-\int_{0}^{t} dt_{1} \lambda(t_{1}) R^{3}(t_{1}) V(t, t_{1})\right\}$$
 (2)

where

$$V(t, t_1) = \frac{4\pi}{3} \left(\int_0^t \frac{\mathrm{d}\theta}{R(\theta)} \right)^3$$

and λ is the nucleation rate which, if one assumes that $\lambda(t) = \lambda_0$ and $R \propto \exp(\Lambda^{1/2} t)$, then implies $p(t) = \exp(-t/\tau)$ where $\tau = 3\Lambda^{3/2}/4\pi\lambda_0$. In principle λ_0 can be computed and Guth states that because $\lambda_0 = A\rho_0 \exp(-B)$, one can easily obtain $\tau \approx t_* > 65\Lambda^{-1/2}$; (note, $A \sim 0(1)$ and B is a barrier penetration term).

This analysis relies on the assumption of Friedmann expansion for R(t); that is, it resolves the homogeneity-horizon and flatness problems by ignoring the isotropy problem. The Friedmann model is already isotropic and homogeneous. It is argued that particle physics processes homogenize the Universe on scales $\leq ct_{\text{GUT}}$ so the Universe can be assumed 'patchwise' homogeneous. However, the tacit assumption of some degree of isotropy remains. We shall show that if it is relaxed the inflationary phase can be prevented.

If the very early Universe were anisotropic then a good description of its expansion dynamics is provided by a generalized Friedmann equation¹⁵ for the (geometric) mean scale factor

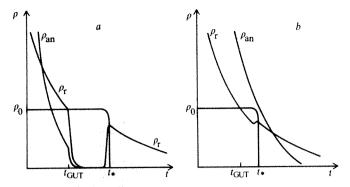


Fig. 1 The contributions to the right-hand side of equation (3) are shown as a function of time. a, If $\rho_{\rm an} \leq \rho_0$ at $t_{\rm GUT}$, the inflationary phase will ensue and both $\rho_{\rm r}$ and $\rho_{\rm an}$ decrease exponentially. When the phase transition is complete, $t=t_*$, the latent heat release increases $\rho_{\rm r}$ to $\sim T_{\rm c}^4$. $b_{\rm r}$ If $\rho_{\rm an} \geq \eta^{1/2} \rho_0$ at $t_{\rm GUT}$, the inflationary phase does not occur, and the Universe continues to be anisotropic. When the transition is complete, there is a latent heat release increasing $\rho_{\rm r}$ slightly. The anisotropy is always decaying adiabatically $(\rho_{\rm r}/\rho_{\rm an} \sim R^2)$, eventually becoming negligible.

R(t),

$$\frac{\dot{R}^2}{R^2} = \frac{8\pi G}{3} (\rho_r + \rho_0) - \frac{k}{R^2} + \frac{\Sigma^2}{R^6}$$
 (3)

where $\rho_{\rm an}=\Sigma^2R^{-6}$ is the anisotropy (gravitational wave) 'energy density'. Equation (3) is exact for space-times which possess isotropic three-curvature and an excellent approximation to those that do not over short-time intervals. In equation (3) the constant Σ is arbitrarily fixed by the initial data. When $8\pi G\rho/3 < \Sigma^2R^{-6}$ the cosmological model is called anisotropic and will have an overall expansion rate, $R \propto t^{1/3}$, which differs from the isotropic radiation model, $R \propto t^{1/2}$. We can choose a class of anisotropic models which have Σ large enough to remain anisotropic until any pre-specified epoch. The isotropy problem can be thought of as explaining why Σ must be chosen to be 'small'. All anisotropic models described by equation (3) eventually isotropize as $\rho_r/\rho_{\rm an} \propto R^2$. They will only have isotropized by $t_{\rm GUT}$ if Σ is specially chosen.

If the Universe expands anisotropically at $t_{\rm GUT}$ then we can solve equation (3) to obtain a description of the competition between anisotropy and vacuum energy (Λ)

$$R^3 = \frac{\Sigma}{\Lambda^{1/2}} \sinh \left(3\Lambda^{1/2} t \right) \tag{4}$$

We obtain the Kasner anisotropic behaviour, $R \sim t^{1/3}$, when the anisotropy dominates $(t \to 0)$ and de Sitter, $R \sim \exp{(\Lambda^{1/2} t)}$, when the vacuum energy dominates $(t \to \infty)$.

If the anisotropy energy density $\rho_{\rm an}$ is less than $\rho_0(\sim \rho_r)$ at $t_{\rm GUT}$, then the inflationary phase will proceed (Fig. 1a). In addition $\rho_{\rm an}$ will decay adiabatically as exp $(-6\Lambda^{1/2} t)$, rapidly degrading any anisotropies. However, if $\rho_{\rm an} > \rho_0$ at $t_{\rm GUT}$, then the Universe will continue to be anisotropic with $R \propto t^{1/3}$ and $\rho_{\rm an} \propto t^{-2}$. If the transition is slow enough, then $\rho_{\rm an}$ may fall below ρ_0 and an inflationary phase would ensue. To address this issue consider p(t) given by equation (1). For $R \sim t^{1/3}$ we find

$$p(t) = \exp(-t/\tau) \tag{5}$$

$$\tau = \frac{80}{9\pi\lambda_0 t^3} \tag{6}$$

In computing τ we have assumed spherical geometry, although the actual geometry is nonspherical. With Kasner geometry τ is smaller by a geometric factor of 0(10). Therefore, we shall use $\tau = 1/\pi\lambda_0 t^3$ rather than equation (6). Unlike the inflationary case, where $\tau = 3\Lambda^{3/2}/4\pi\lambda_0 \approx 3/4\pi\lambda_0 t_{\rm GUT}^3$ the nucleation time scale τ decreases as $\sim t^{-3}$ so that $t_* \sim (\pi\lambda_0)^{-1/4}$. Suppose that in the absence of anisotropy $t_* = \eta t_{\rm GUT}$, then in the presence of anisotropy $t_* \approx \eta^{1/4} t_{\rm GUT}$. During the time between $t_{\rm GUT}$ and t_* , $\rho_{\rm an}$ will have decreased by a factor $(t_*/t_{\rm GUT})^2 \approx \eta^{1/2}$. For $\eta \sim 65$,

(the value required to resolve the horizon problem in the isotropic model⁴), $\rho_{\rm an}$ has only decreased by a factor ~ 10 by t_* . That is, if $\rho_{\rm an} \ge 10\rho_0$ at $t_{\rm GUT}$, then the anisotropy will prevent the occurrence of the inflationary phase (Fig. 1b). The value of the nucleation rate λ_0 is very uncertain, so that η might be $\gg 65$ or $\ll 65$; in any case, one must not separate the question of isotropy from the homogeneity-horizon and flatness problems.

The inflationary Universe was motivated, at least in part, by the flatness and homogeneity-horizon conundrums. However, the isotropy problem must also be considered. The presence of a large amount of initial anisotropy $\rho_{\rm an}(t_{\rm GUT}) \ge \eta^{1/2} \rho_0(t_{\rm GUT})$, will prevent the de Sitter phase from occurring and will decay away adiabatically thereafter ($\propto R^{-6}$). The pressure of this anisotropy at $t_{\rm GUT}$ will not prevent baryosynthesis, and in fact allows for the production of isothermal perturbations highly desirable for galaxy formation highly desirable for galaxy formation of the 2.9 K background.

If the inflationary phase were to occur there would be some interesting consequences. Any relics from an earlier epoch are effectively erased. For example, any initial baryon or lepton number is deleted (hence baryosynthesis must occur after the inflation which is not a difficulty as the Universe is reheated to $\sim T_c$); any gravitons emerging from t_p will have their density degraded relative to photons by a massive factor $\exp(3\Lambda^{1/2}t_*) \sim 10^{83}$ because the latent heat release will not couple to the collisionless graviton sea. Recently, Starobinskii 18.19 has pointed out that the graviton background arising from a cosmological model with a de Sitter expansion before t_p (not arising from a phase transition) is potentially detectable at a frequency of $\sim 10^{-4}$ Hz. If this radiation were detected it would exclude the inflationary process by showing that the primordial graviton density is not $\sim 10^{80}$ times smaller than the photon density.

It also seems that, whilst solving the horizon problem, the inflationary Universe may compound the part of the homogeneity problem which seeks an explanation for the existence of small inhomogeneities and thereby galaxies. Any curvature fluctuations (spatial variations in k/R^2) imprinted on the dynamics before t_{GUT} would have their effective amplitudes reduced by $\sim \exp(2\Lambda^{1/2} t_*) \sim 10^{56}$. A new spectrum of adiabatic inhomogeneities would have to be produced when the phase transition is completed (note, anisotropies in the spatial three curvature are also reduced during the de Sitter phase). In essence the inflationary Universe cleans the slate, and the Universe is reborn. Elsewhere 16 we have described a way of generating isothermal fluctuations during this epoch. The process of bubble nucleation and percolation is inhomogeneous and might provide a mechanism for creating new fluctuations: for example, bubble wall collisions might produce large-scale inhomogeneous shear, which in turn can give rise to isothermal perturbations16. However, owing to its extremely chaotic nature these fluctuations might be of such large amplitude on all scales up to the inflated horizon size $\ge 10^{22} M_{\odot}$, that a profusion of black holes would result, rather than a regular Poisson spectrum.

In the Guth hypothesis⁴ the explanation of the flatness, and homogeneity, problems is tied to that of the isotropy problem. The web of interconnections may be far more extensive: a theory of quantum gravity may reveal them all to have a common origin^{10,11}. An interesting inter-relationship is already known at the classical level. It has been shown^{8,20} that the set of homogeneous initial data giving rise to open isotropic universes asymptotically is of measure zero in initial data space but the set which gives rise to flat, isotropic Universes is not of measure zero. When the initial anisotropy of the Universe is not too large, the inflationary Universe can exist and explain the flatness we observe. This case is an interesting contrast to the result of Collins and Hawking: inflated universes will be flat if they are isotropic.

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- 1. Barrow, J. D. Sci. Prog. 65, 129-60 (1978); Problems of the Cosmos (Encic. Italiana, Rome,
- Kolb, E. & Wolfram, S. Phys. Lett. 91B, 217 (1980); Nucl. Phys. B172, 224 (1980).
- Fry. J. N., Olive, K. A. & Turner, M. S. Phys. Rev. Lett. 45, 2074 (1980); Phys. Rev. D22, 2953; D 22, 2977 (1980).
 Guth, A. Phys. Rev. D23, 347 (1981).
- Weinberg, S. Gravitation and Cosmology (Wiley, New York, 1972).
- Peebles, P. J. E. The Large Scale Structure of the Universe (Princeton University Press,
- Darrow, J. D. & Matzner, R. A. Mon. Not. R. astr. Soc. 181, 719-28 (1977). Collins, C. B. & Hawking, S. W. Astrophys. J. 180, 317 (1973). Stewart, J. M. Mon. Not. R. astr. Soc. 145, 347 (1969).
- Lukash, V. N., Novikov, I. D., Starobinskii, A. A. & Zeldovich, Ya. B. Nuovo Cim. 35B, 293-397 (1977).
- Hu, B. L. & Parker, L. Phys. Rev. D17, 933 (1978).
 Gibbons, G. W. Commun. Math. Phys. 45, 191–202 (1975).
- Webster, A. Mon. Not. R. astr. Soc. 175, 61; 71 (1976).
 Rindler, W. Mon. Not. R. astr. Soc. 116, 663 (1956).
- 15. Ellis, G. F. R. Cargese Lectures in Physics Vol. 6, (ed. Schatzman, E.) (Gordon & Breach, New York, 1973).
- 16. Barrow, J. D. & Turner, M. S. Preprint (80/03 ITP, 1981); Nature 291, 469 (1981).
- Barrow, J. D. Phil. Trans. R. Soc. A**296**, 273 (1980).
 Starobinskii, A. A. Soviet JETP Lett. **30**, 719 (1979).
 Lukash, V. N. Soviet JETP Lett. **31**, 596 (1980).

- 20. Barrow, J. D. & Tipler, F. J. Nature 276, 453 (1978).

Evidence for sulphur implantation in Europa's UV absorption band

Arthur L. Lane, Robert M. Nelson & Dennis L. Matson

Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91109, USA

The International Ultraviolet Explorer (IUE) spacecraft has obtained observations of the galilean satellites over the past 2 years which fortuitously span the periods of the Voyager encounters with the jovian system. Our IUE observing programme is designed to determine the UV spectral characteristics of the galilean satellites as a function of orbital position, largescale areal variability and temporal dynamics. During the past year we have concentrated on the albedo variations of each body^{1,2}. We report here the discovery of an absorption feature at 280 nm in Europa's reflection spectrum. Observations with the IUE show that this absorption is strongest on Europa's trailing hemisphere (central longitude 270°). We identify the feature as an SO₂ absorption band and hypothesize that SO₂ may form when energetic jovian magnetospheric sulphur ions are injected into Europa's water-ice surface.

Initial analysis of the Europa spectra² showed a substantial near-UV (250-320 nm) albedo difference when comparing the leading (central longitude 90°) and trailing (central longitude 270°) hemispheres. This effect has been reported previously on the basis of ground-based observations³⁻⁵. In view of these earlier discoveries we did not consider the near-UV behaviour as anomalous. A more detailed examination of the low-resolution (~1.1 nm FWHM) IUE spectra showed a pronounced decrease of disk-averaged albedo towards shorter wavelengths (3,250-2,600 Å). The decrease was observed for both the leading and trailing hemispheres. To improve the signal-to-noise ratio, several spectra from the same hemispheres (orbital longitudes) were summed. These pairs of summed spectra represent the extrema of the visible and UV albedo variations. These sets were then ratioed to each other. For Europa, spectra at orbital longitudes between 84° and 104° were used for the leading side and between 270° and 281° for the trailing side. For comparison, a set centred near 90° and 270° was selected for Ganymede (see Fig. 1). Noise which results from partial pixel misregistration and from small wavelength drifts in the extracted data from the IUE image tube has not been filtered or smoothed. Therefore,

we present a 'worst-possible' case for the credibility of the ratioed spectra. Unlike the trailing/leading side comparison for Ganymede which demonstrates an additional darkening with decreasing wavelength on the trailing side relative to the leading side, the Europa ratio has a definite absorption band centred near 280-290 nm. Modest amplification of the Ganymede ratio to the noise limit of the data shows no similar band.

The shape of the Europa absorption band suspiciously similar to the absorption spectrum of the $\tilde{A} - \tilde{X}$ UV band of SO₂ centred between 280 and 290 nm (ref. 6). This portion of the SO₂ absorption spectrum is also presented in Fig. 1. The alignment and shape of the vapour-phase SO₂ absorption spectrum is an excellent match to the observed absorption on the trailing side of Europa. We suggest that on Europa this spectral feature is caused by a sulphur atom-oxygen atom interaction within the water-ice lattice. It is not caused by an SO₂ surface frost, because the frost has a very different UV spectrum that does not match what has been observed⁷. Also, unlike Io, where a 4.08-μm absorption band assigned to SO₂ frost has been observed, no SO₂ IR band has been detected on Europa^{8.5} There is no evidence for a low-pressure SO₂ atmosphere, from either Voyager observations (A. L. Broadfoot, personal communication) or light curves of mutual satellite eclipses and occultations. If the imbedded sulphur atoms are sufficiently distant from each other in the ice lattice, they may acquire the bulk spectral character of a 'gas' in which the molecular interactions are small. This is the case for the present model. For the concentrations of sulphur detected, each deep-imbedded sulphur atom has between 120 and 200 Å³ of volume, at least an order of magnitude greater than the molecular volume found in a frost.

The possibility for the presence of the element sulphur is supported by other evidence. Data from the Voyager plasma experiment 10,11, and the low-energy charged particle (LECP) experiment¹², show that the galilean satellites are in an ion plasma composed primarily of sulphur and oxygen in which the co-rotating ions reach keV energies. The Voyager plasma experiment (J. Sullivan, personal communication) provided a measurement of about 7 sulphur ion cm⁻³ for the 'lower-energy' ions at Europa's orbit distance of 9.4 R_i. In this region their mean velocity is ~100-115 km s⁻¹. If we remove Europa's orbital velocity of ~ 13.7 km s⁻¹, then the mean flux of sulphur ions onto Europa's trailing side is $\sim 7 \times 10^7$ ion cm⁻² s⁻¹. Data from the LECP experiment indicate that there are also higherenergy sulphur and oxygen ions present, with energies in the hundreds of keV (ref. 12). An estimate of the integrated flux of these S and O ions is ~108 cm⁻² s⁻¹ with a mean energy of 100 keV (ref. 13, R. Johnson, personal communication). Thus, we can model the sulphur ion flux at Europa with two distinct distributions: a 1-keV source with a flux of $\sim 7 \times 10^7$ ion cm⁻² and a 100-keV source with a flux of $\sim 3 \times 10^7$ ion cm⁻² s⁻¹. Although laboratory data for heavy ion bombardment of water ice are meagre, R. Johnson and P. Haff (personal communications) have independently estimated that the low, 1-keV sulphur particles should penetrate the ice surface to a depth of \sim 35-40Å (about 10 molecular layers of water ice in the I hexagonal form). The 100-keV ion penetration is more difficult to estimate, but a linear model for depth versus energy seems reasonable until laboratory data are available. The faster ions may penetrate to between 3,000 and 4,000 Å.

In addition to deposition, the heavy ion impacts produce sputtering of the surface ice and over long periods of time there is erosion and the possibility for removal of a significant mass of water ice from Europa. Sputtering in water ice is anomalous when compared with processes for metals; there is an unexpectedly large yield of sputtered fragments from ice 14,15. The referenced studies showed that the sputtering yield is dependent on the mass of the ion as well as its energy. Johnson et al. 13 and Eviatar et al.16 have modelled the erosion at Europa caused by sulphur and oxygen ions diffusing outwards from Io's plasma torus. The erosion rates estimated are as high as 100 m of ice per 10° yr, which corresponds to 10° Å yr

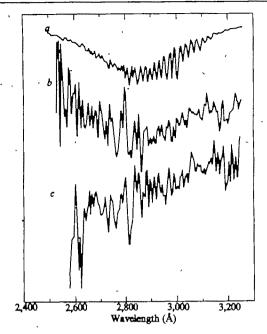


Fig. 1 a, The gas-phase absorption spectrum of the A-X band of SO_2 (ref. 6). The 10% attenuation illustrated in the depth of the band corresponds to a column density of 1×10^{17} molecules cm⁻². b, Three separate Europa trailing side and leading side spectra were added before the ratio spectrum was formed. Each separate trailing side spectrum shows ~3% absorption. Note that the brightness ratio of the two hemispheres is approximately constant at 2,600 and 3,200 Å. Thus, ratio spectrum is not seriously distorted. c, This trailing side/leading side ratio spectrum of Ganymede was formed the same way as the Europa ratio spectrum. The additional darkening of Ganymede's trailing side is seen in the gradual decrease of the ratio. No absorption minimum is detectable within the noise limit of the data. We conclude that this indicates on Europe the presence of S-O bands in a water-ice matrix, originating from sulphur ion implantation.

The ability to see sulphur deposition depends on the balance between injection/deposition and erosional loss to space or ballistic migration to the opposite side of Europa. The leading side of Europa has no detectable absorption band; the absorption would be marginally detectable, in our present limits, at a factor of about 4 less than what is present on the trailing side. Loss to space may be significant, but no atmosphere or strong outgasting signature was detected by either Voyager 1 or 2. The lack of a positive atmospheric detection by the Voyager UV spectrometer makes high erosion rates unlikely. Also, high erosion rates would reach the 3,000-4,000 A deep sulphur ions within days to months of their initial injection into the ice surface. Within that time ~1012-1014 new 'deep-seated' ions per cm⁻² would have been injected into the ice, and all the lowenergy ions lying in the upper 50 Å would have been removed. Even 10¹⁴ sulphur atoms (as SO₂) would not be detectable.

The depth of the Europa absorption band can be estimated from the individual spectra that make up the trailing side data set: ~3% attenuation within a factor of 2. To produce such an absorption, ~2×10¹⁶ molecules of SO₂ per cm² are required. Erosion rates of 104-106 Å yr-1 are about 2-4 orders of magnitude too large to retain sufficient sulphur atoms at a depth where an S-O molecular spectral band can be established and maintained. In fact, not all sulphur ions will make spectroscopically active bonds, so the inequality could be even larger. The fate of the lower-energy sulphur ions nearer the surface is unknown. They may be lost to space through energetic sputtering, or more probably, they co-deposit with any water molecules and oxygen atoms somewhere else on Europa after following ballistic trajectories from the sputtering site. Whether these sulphur atoms end up as sulphur dioxide molecules buried in a deposited

oxygenated water ice is also unknown. No laboratory data seem to be available for 2,800 Å light penetration into highly irregular water ice in which the many optical scattering centres far exceed the number of SO₂ molecules. If this ice deposit is relatively opaque, any real SO₂ could be hidden from detection. High erosion rates seem to be a problem for the detection of this absorption band. L. J. Lanzerotti has indicated that laboratory experimental studies seem to indicate that energetic particle bombardment of amorphous ice causes a conversion to a crystalline structure. We have assumed that the UV penetration into the surface ice is one or two wavelengths deep (3,000-5,000 A) because of the optical scattering centres and lattice defects. For an amorphous ice, this assumption should be adequate. Regular crystalline ice may permit UV penetration to 10,000 Å or more based on the fact that liquid water is quite transparent to 2,800 Å light. If the surface were crystalline ice, we would be seeing absorptions caused by the deepest implantations, and would bias our sulphur-ion source to a higher-energy distribution.

The lower rates of Johnson et al. 13 and the revised rates of Eviatar et al.16 seem more promising. At 103 Å yr-1, it would take 3-4 yr to reach the deeper-lying sulphur atoms in the damaged water-ice lattice. The equilibrium implanted sulphur concentration would be of the order of 3-4×10¹³ cm⁻², or only a factor of 5 less than what was detected. Johnson et al. 13 state that the rate could be as high as 100 m per 10° yr. If it were closer to 20 m, then an approximate equilibrium between deposition and erosion is possible. Even in this case, the fate of the low-energy, 1-keV sulphur ions that are stopped in the upper 40 Å is unclear. About 5×10^{14} sulphur ions cm⁻² should be an equilibrium value with an erosion rate of 20 m per 10° yr (0.2 yr to deplete), and this number is about 10 times smaller than the current limit of IUE detectivity. These sulphur atoms could be buried in waterice fragments that strongly scatter UV light and thus, not be detectable by UV absorption techniques.

We conclude that the IUE has detected an absorption band on the trailing side of Europa that resembles SO₂, and seems to result from S-O bond formation between deeply implanted sulphur atoms and the adjacent, damaged water-ice lattice. The sulphur detected seems to come from the energetic (hundreds of keV) sulphur ions that are present in the jovian magnetosphere. Given the current state of knowledge about the physical processes involved in heavy ion bombardment in water ice, we cannot be precise about the fate of much of the sulphur which undergoes sputtering along with the dominant water-ice fragments. An appropriate equilibrium condition can be found to match the observed spectral data if sputtering erosion occurs at no greater than 20 or so metres per 10° yr.

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- Nelson, R. M. et al. Spance 216, 784 (1980)
- Nelson, R. M., Matton, D. L., Lans, A. L., Motteler, F. C. & Ochert, M. E. Bell. Am. astr. Sec. 12, 713 (1980).
- loison, R. M. & Hapko, B. W. Journs 36, 304 (1978)
- Morrason, D. & Morrason, N. D. Plenestry Satellites (ed. Burne, J.) 363 (University of **4,** 1977) Johnson, T. V. Josnet 14, 94 (1971).
- Herzberg, G. Molecular Species and Molecular Structure Vol. 3, 605 (Rombold, New York,
- Nash, D. B., Panalo, F. P. & Nalson, R. M. Geophye. Rev. Lett. 11, 665 (1980) Nash, D. B. & Nalson, R. M. Nature 286, 763 (1979)
- Panalo, F P et al. Nature 286, 761 (1979)
 Bridge, H S et al. Science 284, 987 (1979), 286, 972 (1979)
- Bridge, H. S., Sulliven, J. D. & Bagonal, F. Nature 288, 798 (1979)
 Krimight, S. M. et al. Science 284, 998 (1979); 266, 977 (1979)
- Johnson, R. E., Lanzsrotti, L. J., Brown, W. L. & Arasstrong, T. P. Sessics (submitted).
 Brown, W. L., Lanzsrotti, L. J., Posts, J. M. & Augustymsk, W. M. Phys. Rev. Lett. 46, 1027.
- (1978).
 15. Brown, W. L. *et al. Nucl. Instrum. Meds.* 178, 321 (1980)
 16. Bvistar, A., Secce, G. L., Johnson, T. V. & Matson, D. L. *Instru* (substitted).

Vapour deposition polymerization of butadiyne

Arthur W. Snow

Chemistry Division, Naval Research Laboratory, Washington DC 20375, USA

In seeking preparative routes to highly conjugated polymeric systems, we have found that butadiyne, C₄H₂, the simplest diacetylene, may be polymerized from the vapour state by selective deposition onto an organic substrate polymer surface, such as polyethylene or Teflon. This polymerization is sensitive to the nature of the substrate surface, occurring most rapidly on a hydrocarbon surface. The polymer is characterized by IR spectroscopy as having a polyconjugated main chain with pendant acetylenic functional groups. ESR spectroscopy shows the polymer to be highly paramagnetic which is interpreted as indicating the presence of a large number of defects within the conjugated polymer. Structurally, this butadiyne polymer appears to be more closely related to substituted diacetylene polymers prepared by melt polymerization as opposed to those prepared by solid state polymerization.

In the solid state, mechanistic investigations with single crystal diacetylene polymerizations have elucidated monomer molecular and crystal structure requirements as well as a 1,4 polymer repeat unit structure¹⁻³:

$$nR - C \equiv C - C \equiv C - R \rightarrow C - C = C - C \Rightarrow_{n}$$
 (1)

In the melt^{4,5} or in solution^{5,6}, the polymerization is more complex, and polymer structures are less extensively characterized with combination of structures having been proposed^{4,5}:

$$R-C \equiv C-C \equiv C-R$$

$$R \qquad R \qquad R \qquad R \qquad R$$

$$\parallel \qquad \parallel \qquad \parallel \qquad \parallel \qquad \parallel$$

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The presence of the pendant group obscures much characterization information with regard to the polymer chain structure. The butadiyne (R=H) polymer, in addition to being conveniently prepared and handled as a vapour-deposited film, presents a unique opportunity for characterizing the chain structure.

Depending on the nature and composition of the substrate surface, these polymerizations may be conducted by thermal

initiation at temperatures ranging from 25 to 100 °C. Polymerization occurs most rapidly on a hydrocarbon surface but fluorocarbon surfaces can also be coated at reasonable rates. For example, in an atmosphere of 700 mm C₄H₂ at 25 °C, polyethylene film 3.7 thousandths of an inch thick, will increase its weight by 20% from polymer deposited over a 6-h period while a Teflon film 2.1 thousandths thick, subjected to a similar exposure for 36 h, increased its weight by 10%. Other surfaces such as quartz, glass, sodium chloride or aluminium oxide are highly inert showing only trace or no polymerization at all after a month's exposure to the same conditions. However, at 100 °C it is possible to deposit films on their surfaces. It is interesting that a simple hydrocarbon monomer should selectively polymerize on hydrocarbon or fluorocarbon surfaces. The polymer conforms well to the substrate surface, and, in the case of organic substrates, examination of a cut cross-section of the sample. some penetration of the substrate film the polybutadivne does occur. It is also noteworthy that the deposited polymer like the C₄H₂ monomer is a hydrocarbon, and, as might be anticipated, an accelerating polymerization rate is observed with a substrate such as Teflon. As might also be expected, the monomer will polymerize rapidly in the bulk liquid state. At high degrees of conversion this bulk liquid polymer has a brass-like metallic lustre similar to some substituted diacetylene single crystal polymers.

Characterization is made difficult by total insolubility in all organic solvents as well as concentrated sulphuric acid and 10% hydroxide. Consequently, it is necessary to use solid-state techniques.

In this polymerization system the growth of a very intense singlet ESR signal accompanies the polymerization of the liquid or gaseous monomer. The signal has a free electron g-value, a linewidth of $10 \, \mathrm{G}$ and a lorentzian line shape. Similar ESR spectra have been reported for solid state⁸⁻¹⁰, melt^{4.5} and solution^{6.7} polymerized substituted diacetylenes. However, the spin density of the melt polymerized diacetylene is significantly greater than that observed for solid state $(10^{19} \, \mathrm{spin}/g \, \mathrm{compared})$ with $10^{16} \, \mathrm{spin}/g$. A free electron ESR singlet is frequently observed in polyene systems and in usually ascribed to a defect in the conjugated polymer chain¹¹. The higher spin density may be correlated with a larger number of defects. Vapour deposited polybutadiyne has a spin density of $8 \times 10^{19} \, \mathrm{spin}/g$.

IR spectroscopy yields the most structural information. The collection of bands at 3,315, 2,090 and $640\,\mathrm{cm}^{-1}$ indicate the presence of a high concentration of pendant terminal acetylenic functional groups. The broad bands at 1,600, 1,210 and 885 cm⁻¹ suggest that the polymer chain is unsaturated and polydisperse. In the context of this polymerization, the band at 885 cm⁻¹ could be correlated with a central acene structure or, alternately, with the C-H vibrations of a 1,3,5-trisubstituted benzene structure¹². In describing the melt polymerized diphenyldiacetylene and dimethyldiacetylene systems, the former correlation in addition to other data has been used to propose a block polyene-polyacene copolymer structure composed of the products in equation (2). In the present case of unsubstituted butadiyne with direct detection of a high density of terminal acetylenic functional groups, a substituted polyphenyl is being considered as well.

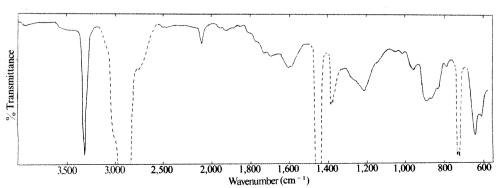


Fig. 1 Transmission IR spectrum of polybutadiyne deposited on a polyethylene film. The broken line corresponds to absorptions from the polyethylene.

The observation that the polybutadiyne penetrates into the substrate film suggests that absorbed monomer may diffuse through the surface before polymerizing. Polymer formation might be envisioned as involving formation of a substituted linear polyene structure followed by a partial intramolecular cyclization yielding structures represented in equation (2) or involving a trimerization of terminal acetylenic groups of two monomers and of a growing polymer molecule to produce a polymer with phenyl linkages in the main chain and pendent unreacted terminal acetylenic groups. The intense ESR signal suggests many defects exist in such a conjugated system. The kinetics, structure and properties of this deposition polymeric system are currently under study and will be reported elsewhere.

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- 1. Wegner, G. Makromolec. Chem. 154, 35-48 (1972); 145, 85-94 (1971); J. polym. Sci. B 9, 133-144 (1971).
- Baughman, R. H. J. polym. Sci. A212, 1511-1535 (1974).
- Bloor, D., Koski, L., Stevens, G. C., Preston, F. H. & Ando, D. J. J. mater. Sci. 10,
- 4. Berlin, A. A., Cherkashin, M. L., Chauser, M. G. & Shifrina, R. R. Vysokomolek, Soedin A9,
- Chauser, M. G., Cherkashin, M. I., Kurhnerev, M. Ya., Protsuk, T. I. & Berlin, A. A. Vysokomolek. Soedin. A10, 916-924 (1968).
 Wiley, R. H. & Lee, J. Y. J. macromolec. Sci. A5, 513-527 (1971).
- Teyssie, P. H. & Korn-Girard, A. C. J. polym. Sci. 2, 2849-2858 (1964).
 Baughman, R. H., Exarhos, G. J. & Risen, W. M. J. polym. Sci. A212, 2189-2193 (1974).
- Stevens, G. C. & Bloor, D. J. polym. Sci. A213, 2411-2427 (1975); Chem. phys. Lett. 40, 37-40 (1976).
- Eichele, H., Schwoerer, M., Huber, R. & Bloor, D. Chem. phys. Lett. 42, 342-346 (1976).
 Bishop, A. R. Solid State Commun. 33, 955-960 (1979).
- 12. Avram, M. & Mateescu, G. H. Infrared Spectroscopy, 211; 218-220 (Wiley-Interscience, New York, 1972).

Negative exotic particles as low-temperature fusion catalysts and geochemical distribution

Christian K. Jørgensen

Département de Chimie Minérale, Analytique et Appliquée, University of Geneva, CH 1211 Geneva 4, Switzerland

Minute concentrations of unfamiliar particles (X+ or X-) with atomic weights M between 10^2 and 10^6 (10^{11} and 10^{15} eV) may be remnants¹⁻⁴ of the Big Bang or may originate in recent events (their expected properties are discussed elsewhere⁵). The adduct of X with a Z-nucleus acts as a superheavy isotope of (Z-1). It is shown here that although their geochemical separation would be less obvious than that of fractionally charged species containing unsaturated quarks^{6,7} it should still be possible to concentrate these exotic isotopes. Exceptional cases are the neutral adducts pX and ²DX, which may form tiny molecules with a second proton, or a polymer with a density $M \times 10^{10}$ g cm⁻³ which could act as a low-temperature fusion catalyst and explain the excess heat irradiated by Jupiter.

The fusion of four protons to 4He in most stars (including our Sun with a central temperature close to 15 × 10⁶ K) does not go⁴ mainly via the C-N-O catalysis proposed by Bethe, but rather takes place along a more direct path starting with two protons forming a deuteron, a positron and a neutrino^{8,9}. Zweig ted out that unusual quarks with charge (-4e/3) could bring together two deuterons, providing a one-stage fusion catalysis, if the quark has a much longer lifetime than the negative muon known11 to promote fusion in liquid hydrogen. If the stellar interior contains a small concentration of X which does not become inactivated by trapping on unreactive nuclei, the neutral pX starts by capturing a second proton to form a 'molecule' pXp+ having a wave function analogous to the hydride anion containing two electrons with a binding energy 1,836 times the electron affinity 0.75 eV of the hydrogen atom, not much above the prevailing kT. However, its ephemerial (but repeated) existence may help the otherwise low rate of forming 2DX and the two leptons. Although Coulomb barriers could be entirely avoided by subsequent reactions (pX+3He) or (2DX+2D) with increasing cross-sections at lower temperature due to longer DeBroglie wavelengths, the major problem in stellar interiors is the trapping of X on helium, carbon and so on. The nonrelativistic Schrödinger energy for the ground state of a system consisting of X and a nucleus with atomic weight A and charge Ze (where 1,823 is the reciprocal atomic weight of the electron)

$$-AZ^2 \cdot 1,823 \cdot 13.6 \text{ eV}$$
 (1)

almost independent of the atomic weight M of X^- (if $M \gg A$ the nucleus becomes the 'easily mobile' particle). For pX, equation (1) gives the binding energy as 25 keV, about 20 times $k\hat{T}$ at the solar centre, whereas ³HeX would have 300 keV and ¹²CX 10.8 MeV, although the spatial extension 6 of X^- and ^{12}C would decrease the latter value⁵. Exotic lithium may contain⁵ not only BeX but also BeX (where the X prevents the almost instantaneous fission in two α -particles) removing the bottleneck⁸ of nucleosynthesis.

Jupiter has an unaccounted heat production 12 slightly above 10²⁴ erg s⁻¹, which is of the same order of magnitude as the insolation (with an albedo 0.42). As the mass of Jupiter is 1.9×10^{30} g, this corresponds to an average energy production of 6×10^{-7} erg g⁻¹ s⁻¹ in comparison with 1.87 erg g⁻¹s⁻¹ for solar material. The temperature (128 K) doubles¹³ when looking down in the atmosphere through the 'window' at 2,000 cm where the IR lines are weak. If this excess heat production had continued at the present rate for 3×10^9 yr, then 10^{-8} of the Jupiter mass would have had to be transmuted from hydrogen to helium. A simpler alternative would be that the excess heat was due to the gravitational energy released by radial contraction. Indeed, the same amount of heat could be evolved by the radius decreasing ~400 km or 1%. But although such a contraction may seem insignificant, this slow re-adaptation of the radius causes several problems, one being that if there was a time when the temperature was double its present value, the radiative cooling would have been 16 times more rapid.

Although the rotational ground state of pX does not have an electric dipole moment (if X is a fermion, the hyperfine splitting between I = 0 and 1 may be several eV) it is important to know whether pX (having the average distance 3 bohr/ $2 \times 1,836 = 4.3 \times 10^{-12}$ cm) dimerizes, or polymerizes at room temperature to a highly compact liquid. The electrostatic dimerization energy of pX is ~10-15 keV. If droplets of such an exotic liquid exist (with a density close to M times 10¹⁰ g cm⁻³) they would form a general catalyst for nuclear reactions, and because of $M \gg 1$, they lose X by evaporation much less readily than protons. Such a material would be rather similar to a metal which has had its conduction electrons replaced by protons.

At first, one would expect irreversible trapping of X in CH₄, NH₃, and so on to create a worse problem in Jupiter than in the Sun, but the core of Jupiter is under such strong pressure that it may contain huge volumes of metallic hydrogen fairly separate from gaseous helium, and from the heavier elements, which may

not be soluble in metallic hydrogen. On the other hand, pX is expected to be soluble, and even effectively extracted. No fusion process is observed in hydrogen bubble chambers but all terrestrial hydrogen may have been stripped of X in early stages of the formation of Earth. An experiment at the Rutherford laboratory¹⁴ has put extremely low limits (10⁻²⁹-10⁻³⁰ X per proton if M is between 20 and 1,400). Direct mass spectrometric higher limits are close to 10^{-22} . Unfortunately, no comparable data have been presented for M between 104 and 106.

The relative concentration of exotic isotopes of a given element Z would be expected to be proportional to the ratio between the abundances of (Z+1) and of Z. Exotic boron, scandium, manganese and thallium would be good candidates from this point of view⁵. As boron isotopes ¹⁰B and ¹¹B can be separated on a commercial scale it might be useful to attempt to detect 12CX in the extreme fractions, although most boric acid has already been evaporated once from volcanoes. On the other hand, isotopes with A even as high as 106 should not be strongly fractionated in amorphous or crystalline solids by terrestrial gravitation, again except for polymeric pX. The latter should behave in a similar manner to that predicted 15 for high-M magnetic monopoles concentrating at the centre of the Earth. As technetium and promethium possess only β -unstable isotopes, it might be useful to look for them in minerals as adducts of X and ruthenium or samarium. In concentrated uranium minerals, spontaneous and neutron-induced fission maintain¹⁶ concentrations of 10^{-17} promethium and 10^{-12} (of the longer lived) technetium. The search has only 16.17 been for the specific isotope 147Pm but it would be interesting to know whether it is accompanied by comparable amounts of SmX.

Cahn and Glashow⁵ point out that the presence of X decreases the energy of α -decay to the extent that ²⁴⁴PuX and CmX may be long-lived on a geochemical time scale. Such exotic isotopes would show up as the (ultramicroscopic amount) neptunium and (as yet unreported) americium in minerals. These authors do not contemplate the capture of X by the heavy nuclei, which would be likely to induce fission, but consider the buildup of ⁸BeX (not representing a barrier to nucleosynthesis8 like the instantaneous decay of 8Be) by adding many consecutive 4He. Such a process may have occurred shortly after the Big Bang and give products normally only obtained⁸ in supernova explosions. One of the attractive candidates would be exotic actinium ²³²ThX. Because of radioactive equilibria, there are 10⁻¹⁵ actinium, 10⁻¹² protactinium (slightly below the concentration of ²²⁶Ra) to be compared with 12 p.p.m. of thorium, 16 p.p.m. of lead and 4 p.p.m. of uranium in the Earth's crust. If actinium is separated from a thorium mineral containing <0.3% uranium, the amount should be $<10^{-12}$ of the thorium. If a percentage of such a sample were 232 ThX rather than ²²⁷Ac, it would establish a concentration of 10⁻¹⁴ X⁻ per nucleus or $5 \times 10^{-17} \text{ X}^-$ per nucleon. The same argument would apply to a rare-earth mineral (containing <0.3% uranium) where exotic actinium may be more likely to be situated by the geochemical fractionation. These limits are far lower than the 10⁻¹⁰ first suggested and not much higher than the ratio between unsaturated quarks and nucleons, which is likely^{6,18} to have an order of magnitude of 10^{-20} (6,000 per g) if the existence of such species is not excluded by dogmatic quark confinement. It is possible that the very high atomic weights of exotic isotopes are concentrated on definite spots in large-scale mass spectrometers (like Oak Ridge calutrons) but there has been little effort to find them. A tandem accelerator 19 was recently used to detect oxygen isotopes with (not necessarily integral) atomic weights between 20 and 54, and upper limits for abundances 10⁻¹⁶-10⁻¹⁸ were found. However, the exotic ¹⁹FX discussed here would be heavier, and not particularly abundant8. A few atoms of ²³⁵UX may be detected by irradiating a photographic plate (or transparent plastic), having been exposed to the ultrahigh M-region of the mass spectrum of protactinium, with slow neutrons at high flux inducing fission. In such an experiment, the beam of Pa⁺ should be as free of neutral atoms (and molecules) as possible. The asymmetry of the short and intense track of the

fragment carrying X and the long track of the other, much more rapid, fragment should be conspicuous.

If positive X⁺ can be bound to nuclei (as predicted⁷ for positive quarks) by short-range interactions compensating for the electrostatic repulsion, separation of lithium would become of major interest as it would contain the adduct of ⁴He with X⁺; and the exceedingly low concentrations of polonium and of neptunium in minerals might contain the adducts of 209 Bi and 238 U with X^+ .

The two most promising concentration techniques seem to be ultracentrifugation and fractional distillation of liquid air. In aqueous, quite concentrated solutions, ultracentrifugation is an excellent way of separating proteins or synthetic polymer molecules according to molecular weight. Supposing kilogramme quantities of dissolved lithium chloride were suspected of containing traces of ⁸BeX or ⁹BeX, then by adding a separation buffer, such as gramme quantities of caesium chloride, any exotic lithium would show up in the almost vanishing, heavier fraction. On the other hand, if caesium nitrate is suspected of containing adducts of X^- with barium, $N(C_4H_9)_4^+NO_3^-$ or thallium nitrate may be used as separating agents.

The technology of separating the constituents of atmospheric air is highly developed. The boiling points of ³He and ⁴He are sufficiently different that 6LiX may boil at a higher temperature than neon. There is an enormous gap between the boiling point 27 K of the (18 p.p.m. of volume) neon and 77 K of the major constituent N2 without any known, or conceivable, minor constituent. Exotic neon ²³NaX would be situated in this gap. There is much less neon in our atmosphere than extrapolated from the cosmic abundance8 and a preliminary concentration by a factor of several thousand is obtained by the Earth having a gravitational field strong enough to retain exotic (but not conventional) neon over 109 yr.

Holt et al.20 wanted to investigate a possible superdense modification of nuclear matter by looking for the noble gas radon (of which the longest living isotope known ²²²Rn has the half-life 3.8 days) in xenon fractions from liquid air. Their negative result (with the higher limit 10^{-16} atom per xenon atom) is relevant to our problem. Because francium is even less abundant than radon, the search was really for exotic xenon formed by 133CsX, and caesium has a rather low cosmic abundance. Gaseous diffusion or centrifugation of krypton, which contains²¹ 0.01% CF₄, or collecting the almost absent fractions between krypton and xenon might enrich exotic krypton formed from the more abundant rubidium.

As pointed out by Fairbank⁶, exotic isotopes might be detected in dilute gaseous systems by the laser techniques developed22 for detecting single atoms. An especially clear case is the atomic spectrum of lithium, where the $2p \rightarrow 2s$ transition in the red has 0.2 cm^{-1} lower wavenumbers in ⁶Li than in ⁷Li. If this isotope shift is exclusively an effect of the electron moving the centre of gravity, it is 42 times smaller than the shift expected for any heavy isotope and the line positions then hardly depend on the explicit M value. Also exotic rubidium and caesium atoms should be quite good candidates for accurate monochromatic laser excitation but here the isotope shift is not due to a modified Rydberg constant. For our purposes, it is important that single xenon atoms23 have been detected.

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- Dover, C. B., Gaisser, T. K. & Steigman, G. Phys. Rev. Lett. 42, 1117-1120 (1979).
 Farhi, E. & Susskind, L. Phys. Rev. D20, 3404-3411 (1979).
 Eichten, E. & Lane, K. D. Phys. Lett. 908, 125-130 (1980).
 Rajpoot, S. Phys. Rev. D22, 2244-2254 (1980).
 Cahn, R. N. & Glashow, S. L. Chemical Signatures for Superheavy Elementary Particles, LBL-12010 (Berkeley, 1980).
 Iarvensen, C. K. Strutt. Bond. 34, 19-38 (1978); 43, 1-36 (1981).
- Dørgensen, C. K. Struct. Bond. 34, 19-38 (1978); 43, 1-36 (1981). De Rújula, A., Giles, R. C. & Jaffe, R. L. Phys. Rev. D17, 285-301 (1978). Trimble, V. Rev. mod. Phys. 47, 877-976 (1975).
- 9. Kuchowicz, B. Rep. Prog. Phys. 39, 291-343 (1976).

10. Zweig, G. Science 201, 973-979 (1978).

Zweig, G. Science 201, 973-979 (1976).
 Alvarez, L. W. et al. Phys. Rev. 105, 1127-1128 (1957).
 Chase, S. C. et al. Science 183, 315-317 (1974).
 Terrile, R. J. et al. Science 204, 1007-1008 (1979).

CERN Courier, 18-21 (January 1981)

Carrigan, R. A. Nature 288, 348-350 (1980).

Larrigan, K. A. Nature 288, 348-350 (1980).
 Kenna, B. T. & Attrep, M. J. inorg. nucl. Chem. 28, 1491-1500 (1966).
 Attrep, M. & Kuroda, P. K. J. inorg. nucl. Chem. 30, 699-703 (1968).
 Wagoner, R. V. & Steigman, G. Phys. Rev. D20, 825-829 (1979).
 Middleton, R. et al. Phys. Rev. Lett. 43, 429-431 (1979).

Holt, R. J. et al. Phys. Rev. Lett. 36, 183-186 (1976). Gassmann, M. Naturwissenschaften 61, 127 (197-

Hurst, G. S. et al. Rev. mod. Phys. 51, 767-819 (1979).

23. Chen, C. H., Hurst, G. S. & Payne, M. G. Chem. phys. Lett. 75, 473-477 (1980).

²⁴¹Am from the decay of ²⁴¹Pu in the Irish Sea

J. P. Dav & J. E. Cross

Department of Chemistry, University of Manchester, Manchester M13 9PL, UK

Americium-241 is a long-lived α -emitting nuclide which has become one of the more important marine contaminants arising from the discharge of effluents to the Irish Sea from the nuclear fuel reprocessing plant at Windscale, Cumbria¹⁻³. These discharges contain ²⁴¹Am, currently $\sim 200 \text{ Ci yr}^{-1}$, and also the β -emitting nuclide, ²⁴¹Pu (half life 14.7 yr), which decays in the environment to ²⁴¹Am. Both americium and plutonium seem to be largely retained in sediments relatively close to Windscale 4-7 and a reservoir of 241 Am is building up both from direct deposition and from the radioactive decay of sedimented 241 Pu. Here we attempt to estimate quantitatively the size and rate of growth of this reservoir. Using data for Windscale discharges have estimated the probable sedimentary deposition of 241Am and 241Pu over the past 20 yr, and calculated the consequent additional ingrowth of 241 Am. The latter is now ~600 Ci yr rising towards a steady state ~1,300 Ci yr⁻¹ if present rates of discharge of 241 Pu are maintained. The present sedimentary reservoir of ²⁴¹Am is ~18,000 Ci, of which about 4,800 have resulted from 241 Pu decay in situ. Measurement of the 241 Pu/Puα isotope ratio in coastal sediments confirms our estimates of ²⁴¹Pu deposition. Outside the Irish Sea, in the dispersal plume into Scottish waters, we have concluded that the observed ²⁴¹Am concentration⁸ can be accounted for largely by ingrowth from the 241Pu transported in the water mass.

It has been established^{4.5}, by measurement of the activities of the α emitters ²³⁸Pu and ²³⁹⁺²⁴⁰Pu, in Irish Sea sediments, that >95% of the plutonium released from Windscale during the past 20-25 yr has collected relatively close to the point of discharge. In many locations, these sediments have remained largely undisturbed and the sedimentary record with respect to several isotopes, such as plutonium^{4,5,9}, caesium and other nuclides 10 can be related satisfactorily to the history of the Windscale discharges. Although levels of ²⁴¹Pu in sediments have not been measured systematically, the accumulation and subsequent decay of ²⁴¹Pu to ²⁴¹Am can readily be calculated if the quantities of ²⁴¹Pu initially deposited can be estimated.

It also seems likely that the marine discharges of ²⁴¹Am from Windscale will have largely accumulated in sediments in the region, although this possibility has not yet been established from measurement of activity levels in cores. The relative adsorption of ²⁴¹Am by suspended particulates in seawater in the Windscale area has been found^{6,7} to be greater than for ²³⁹Pu, and the settling out of such material would, therefore, be expected to remove americium more rapidly than plutonium. Consequently, almost the entire inventory of ²⁴¹Am, both discharged and ingrown, for the 20-25 yr of plant operation will probably be present in the sediments of the region. Interpretation of the sedimentary record for ²⁴¹Am can only be achieved if due allowance is made for ²⁴¹Pu deposition and decay.

Table 1 Marine discharges from Windscale and environmental accumulation of ²⁴¹Pu and consequent production of ²⁴¹Am

	Windscale	discharges (Ci yr ⁻¹)	Accumulated ²⁸¹ Pu (Ci)	Production of ²⁴¹ Am (Ci yr ⁻¹)
Year	Pu-α	²⁴¹ Pu	²⁴¹ Am	ner'	
1960	78	2,255		2,203	2
1961	104	3,012		5,044	6
1962	186	5,413	-	10,099	12
1963	233	6,780	water.	16,257	21
1964	285	8,294		23,610	32
1965	292	8,497		30,823	44
1966	292	8,497		37,704	55
1967	499	14.521	*****	50,152	70
1968	828	24,095	576	71,379	97
1969	816	23,920	396	91,458	130
1970	936	27,092	540	113,711	164
1971	1.128	32,970	1,020	140,680	204
1972	1,548	45.163	2,172	178,319	255
1973	1,776	51,944	2,952	220,847	320
1974	1,248	36,491	3,192	246,322	374
1975	1,257	36,579	1,052	270,708	414
1976	1,266	35,048	323	292,477	451
1977	981	26,517	99	304,909	478
1978	1,567	47,928	214	337,684	514
1979	1,335	40,383	212	361,579	560
1980				381,527	595

Pu- α includes ²³⁸⁺²³⁹⁺²⁴⁰Pu (refs 1, 2, 4). ²⁴¹Am data are not available before 1968, although discharges presumably occurred. ²⁴¹Pu data were not published before 1976; we have estimated these releases (shown in italics) from the Pu-α activities, using the mean 241 Pu/Pu- α isotope ratio (29.1) between 1976 and 1979. The environmental accumulation of 241 Pu, and consequent production of 241 Am, resulting from the Windscale discharges and corrected for radioactive decay were calculated by numerical approximation, using equations (1) and (2) and computed on a daily basis (assuming 1/365 of the annual discharge for each day). For calculation, the annual discharge of ²⁴¹Pu subsequent to 1979 has been taken as 37,469 Ci yr⁻¹, the average for the years 1976-79

The potential radiological importance of 241 Am, which reaches human consumption largely through fish and shellfish, became particularly apparent1 in about 1975-76, following several years of relatively large discharges of ²⁴¹Am from Windscale (Table 1). The relative contributions of discharged and ingrown 241Am to the environmental pool available to marine organisms has been examined¹¹, and the potential radiological significance of sediment held ²⁴¹Am is thought to be small^{11,12}. However, the conditions in which americium could be remobilized from sediments have not yet been fully investigated.

The ingrowth of 241 Am in sediments has been calculated from the estimated discharges of ²⁴¹Pu (Table 1), assuming total deposition of discharged plutonium. Plutonium-241 decays as follows:

241
Pu $\frac{^{6}}{^{14.7}yr}$ 241 Am $\frac{^{\alpha}}{^{433}yr}$ 237 Np $\frac{^{\alpha}}{^{-10^{6}yr}}$

The quantities of ²⁴¹Am formed and ²⁴¹Pu remaining, following the decay of a given initial quantity of 241Pu, are given by equations (1) and (2):

$$[^{241}\text{Am}]_{t} = [^{241}\text{Pu}]_{0} \frac{T_{\text{Pu}}}{(T_{\text{Am}} - T_{\text{Pu}})} \exp\left(-\frac{t \ln 2}{T_{\text{Am}}}\right) - \exp\left(-\frac{t \ln 2}{T_{\text{Pu}}}\right)$$
(1)

$$[^{241}Pu]_t = [^{241}Pu]_o \exp\left(-\frac{t \ln 2}{T_{Pu}}\right)$$
 (2)

where T_{Pu} and T_{Am} are the radioactive half lives of ²⁴¹Pu and ¹Am, respectively.

The sedimentary accumulation of ²⁴¹Pu by the end of each year, and the corresponding annual ingrowth of 241 Am, is given in Table 1. The rate of generation of 241Am has increased steadily, and by 1980 had reached a level ~600 Ci yr⁻¹. The continued discharge of ²⁴¹Pu at a constant annual rate would eventually give rise to a steady-state condition in the sediments in which the rates of deposition, and loss by radioactive decay, would have become equal. The steady state would be

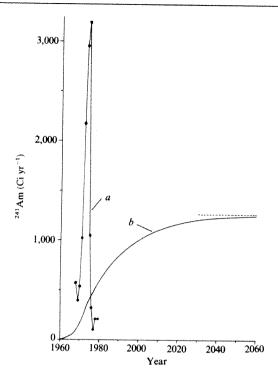


Fig. 1 Input of ²⁴¹Am to the environment. a, Direct discharge from Windscale; b, calculated production from radioactive decay of accumulated

241Pu in the environment from Windscale discharges.

approached exponentially (Fig. 1), with a half time of approach equal to the decay half life of ²⁴¹Pu. Assuming that discharges of ²⁴¹Pu remain at about their present levels, the ingrowth of ²⁴¹Am will level off at about ~1,300 Ci yr⁻¹. It is, of course, unlikely that discharges from Windscale will stay constant for this period in the future. However, the relative importance of the present rates of discharge of ²⁴¹Pu and ²⁴¹Am to future levels of ²⁴¹Am in the sedimentary environment is clear.

The sedimentary reservoir of ²⁴¹Am in the Irish Sea can be estimated if it is assumed (as seems likely) that effectively all the available americium, whether discharged from Windscale or ingrown, has been retained in the sediments. For the period 1960-80 we estimate that ~4,800 Ci of ²⁴¹Am will have ingrown from 241 Pu, whilst $\sim 13,000$ Ci has originated from direct deposition (this does not include any 241 Am discharged before 1968, for which we have no data, but it seems likely that the total released up to 1968 would have been relatively small, perhaps ~1,000 Ci). Correction for decay over this time period is insignificant, and the total reservoir is therefore ~18,000 Ci. To test these conclusions, we have measured the overall $^{241}\text{Pu/Pu-}\alpha$ isotope ratio in sediments taken from the Raven-

glass Estuary, adjacent to Windscale. Two cores, taken in 1978 to a depth sufficient to include the deposition for at least 20 yr, were homogenized and sub-samples analysed radiochemically. Plutonium was separated from the material by procedures ^{13,14} which exclude americium, and the activities of ²³⁸Pu, ²³⁹Pu and ²⁴¹Am determined by α spectrometry, both initially (²⁴¹Am = 0) and after about 14 months (Table 2). The measurement of ingrown ²⁴¹Am allowed the activity of the ²⁴¹Pu present in the original sample to be calculated15

The ²⁴¹Pu/Pu-α activity ratio (23.1) determined in these sediments is very close to that calculated (22.0) by summing the $Pu\text{-}\alpha$ and ^{241}Pu discharges (corrected for decay) up to the end of 1978. This value is significantly different from the current ratio at discharge, for which 241 Pu/Pu- $\alpha = 29.1$ (average, 1976–79). The close agreement between the measured and calculated values we take to confirm both that continuous accumulation of plutonium in sediments has occurred over that time, and also that our estimates of ²⁴¹Pu discharges for the years 1960-75 (Table 1) are essentially correct.

As an extension of these calculations, we have re-examined the data obtained by Murray et al.⁸ for the activities of ²⁴¹Am, ²³⁸Pu and ²³⁹⁺²⁴⁰Pu in Scottish coastal waters. It has been established ^{16,17}, largely from ¹³⁴Cs and ¹³⁷Cs measurements, that the main dispersal plume of radioactivity from the Irish Sea emerges northwards, passes along the west and north coasts of Scotland, and in part reaches the North Sea. The approximate average transit times for nuclides (1) to emerge from the North Channel of the Irish Sea¹⁶, and (2) to reach the sea area off the north-west corner of Scotland¹⁷ (Cape Wrath), following discharges from Windscale, are estimated at ~1.5 and 2 yr, respectively. The activity ratio, ²⁴¹Am/Pu-α, decreases from ~1.7 at discharge to ~0.06 in waters to the west and north of Scotland, as observed by Murray et al.8 who concluded that the Am and Pu- α are both of direct Windscale origin, the americium having been more rapidly removed from the water column. However, although qualitative reference was made^{8,17} to the possible contribution of ²⁴¹Am ingrown from ²⁴¹Pu, this was not estimated quantitatively.

We now suggest that these data, at least for the more northerly Scottish waters, can be interpreted on the hypothesis that the ²⁴¹Am arises almost entirely by ingrowth from ²⁴¹Pu during transport of the water mass. For the sea area to the north-west of Cape Wrath, the mean activities of ²⁴¹Am and ²³⁸⁺²³⁹⁺²⁴⁰Pu (Pu-α) from Murray et al.⁸ were 0.097 and 1.60 fCi l⁻¹, respectively. The inferred ²⁴¹Pu activity, ~44 fCi l⁻¹, would be sufficient to generate the observed ²⁴¹Am concentration over a period of ~1.4 yr. This is somewhat longer than the estimated transit time from the North Channel of the Irish Sea, but significantly less than the transit time from Windscale. If we assume that the ²⁴¹Am concentration results from an approximate steady-state condition, between rate of loss from the water mass (for example, by sedimentation) and rate of replacement by ingrowth, then the replacement time previously calculated (~1.4 yr) can be identified as the approximate mean residence time 18 for 241 Am in these waters. The probable existence of such a steady state is supported by the approximately constant value (between 0.05 and 0.08) for the ²⁴¹Am/Pu-α activity ratio round the Scottish coastline from the Minch to the North Sea8.

Although these conclusions are based primarily on the decay characteristics and observed activities in seawater of the plutonium and americium nuclides, the conclusions are not inconsistent with other facets of the marine behaviour of these elements, and may even help to remove an apparent anomaly. Thus, whilst most of the Windscale-discharged plutonium and americium seems to be rapidly removed to sediments, the remainder seems to be relatively well conserved in seawater, and to follow 137Cs in the dispersal plume. For plutonium, the probable explanation is that the sedimented and conserved fractions correspond to the element in the two different (environmentally stable) oxidation states, Pu(IV) and Pu(VI), respectively 19,20. For americium, the only plausible oxidation state in solution is Am(III), which is firmly and rapidly retained by sedimentary material within the Irish Sea^{6,7}. Thus, the

Table 2 Activities of ²³⁸Pu, ²³⁹Pu and ingrown ²⁴¹Am from sediment cores collected near Windscale at the end of 1978

Nuclide	Source activities (pCi)		
	Sample 1	Sample 2	
²³⁸ Pu	25.3	26.7	
²³⁹ Pu	102.2	107.7	
²⁴¹ Am	5.46	5.73	
²⁴¹ Pu	2,953	3,099	
Ratio ²⁴¹ Pu/Pu-α	23.2	23.1	

Activities were determined by a spectrometry 434 days after the preparation of Activities were determined by a spectrometry 4,34 days after the preparation of two sources from sediment cores collected from Ravenglass Estuary. The cores (~50 cm) correspond to a sedimentary deposition covering at least 20 yr, determined (J.E.C., unpublished) from the $^{134}\text{Cs}/^{137}\text{Cs}$ and $^{238}\text{Pu}/^{239}\text{Pu}$ isotope ratios^{4,9,10}. The ^{241}Pu activities were calculated¹⁵ from ingrown ^{241}Am . The measured activity ratio, $^{241}\text{Pu}/\text{Pu}-\alpha=23.1$, is very similar to that calculated by summation of the discharges up to 1978 (Table 1) that is $^{241}\text{Pu}/\text{Pu}-\alpha=22.04$. apparent maintenance of a rather constant Am/Pu ratio in Scottish waters is difficult to explain in chemical terms, as at first sight the phenomenon seems to imply a conserved fraction for americium, as well as for plutonium, in this water. However, we think a possible explanation, in terms of ingrowth from ²⁴¹Pu coupled with a rather short residence time for 241 Am in the water phase, is more plausible and is compatible both with the available environmental data and with the present understanding of the marine chemistry of americium.

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- Hunt, G. J. Radioactivity in Surface and Coastal Waters of the British Isles, 1978 (Directorate of Fisheries Research, Ministry of Agriculture, Fisheries and Food, Lowestoft,
- 2. Annual Survey of Radioactive Discharges in Great Britain, 1979 (Department of the
- Environment, London, 1980).

 3. Atherton, R. S. Annual Report of Radioactive Discharges and Monitoring of the Environment, 1979 (British Nuclear Fuels, Health and Safety Directorate, Risley, Warrington, 1978).
- Hetherington, J. A. in Environmental Toxicity of Aquatic Radionuclides; Models and Mechanisms (eds Miller, M. W. & Stannard, J. N.) Ch. 5 (Ann Arbor, Michigan, 1976).
 Hetherington, J. A. Mar. Sci. Commun. 4, 239-274 (1978).
 Hetherington, J. A. & Harvey, B. R. Mar. Pollut. Bull. 9, 102-106 (1978).
 Pentreath, R. J., Jefferies, D. F., Lovett, M. B. & Nelson, D. M. Proc. 3rd NEA Seminar on Marine Radioecology, 203-221 (Nuclear Energy Agency, OECD, Tokyo, 1979).
 Murray, C. N., Kautsky, H., Hoppenheit, M., & Domian, M. Nature 276, 225-230 (1978).
 Aston, S. R. & Stanners, D. A. Nature 289, 581-582 (1981).
 Aston, S. R. & Stanners, D. A. Estuar. coast. mar. Sci. 9, 529-541 (1979).
 Pentreath, R. J. Int. Symp. on the Impacts of Radionuclide Releases into the Marine Environment (IABR V Vienna. 1980).

- Environment (IAEA, Vienna, 1980).

 12. Hunt, G. J. & Jefferies, D. F. Int. Symp. on the Impacts of Radionuclide Releases into the Hunt, G. J. & Jeneries, D. F. Int. Symp. on the impacts of Radionuclide Releases into the Marine Environments (IAEA, Vienna, 1980).
 Hampson, B. L. & Tennant, D. Analyst 98, 873-885 (1973).
 Cross, J. E. & Day, J. P. Envir. Pollut. B 2, 249-257 (1981).
 Livingston, H. D., Schneider, D. L. & Bowen, V. T. Earth planet. Sci. Lett. 25, 361-367

- (1973).

 16. Jefferies, D. F., Preston, A. & Steele, A. K. Mar. Pollut. Bull. 4, 118-122 (1973).

 17. Livingston, H. D. & Bowen, V. T. Nature 269, 586-588 (1977).

 18. Holland, H. D. The Chemistry of the Atmosphere of the Atmosphere and Oceans, 5 (Wiley-Interscience, New York, 1978).
- 19. Nelson, D. M. & Lovett, M. B. Nature 276, 599-601 (1978).
- 20. Aston, S. R. Mar. Chem. 8, 319-325 (1980).

Binary mixing in ocean-ridge spreading segments

M. F. J. Flower

Department of Geological Sciences, University of Illinois, Chicago, Illinois 60680, USA

Compositional mixing trends in eruptive mid-ocean ridge basalt (MORB) seem to be confined to short time-space intervals, possibly corresponding to single fracture-zone-bounded segments of a spreading ridge axis. Binary mixing is discernible from the variation in Ta, Hf, Rb, Sr and rare earth elements (REEs), and may be interpreted as reflecting dual mantle sources for locally-defined units of the axis or associated transform fracture zones. These sources reflect contrasting degrees and types of incompatible (or 'low- K_D ') element enrichment relative to a 'normal' MORB source characterized by uniform depletion in these elements. Enrichment in low- K_D elements, radiogenic isotopes and normative nepheline has long been recognized in some fracture zone magmas 1-4 and it is important to speculate on the possible influence of fracture zones on the chemical character of spreading axes. Consideration of chemical and isotopic variation at a single Atlantic spreading segment (36-37 °N) in a regional context suggests that transform fracture zones may be instrumental in both generating compositional heterogeneity in the mantle and permitting its expression in eruptive crust. It is proposed here that hybridization of 'enriched' fracture zone-derived melt and less-enriched or depleted rift-derived melt occurs in sub-axial magma supply systems during an active spreading phase.

Between the Azores Plateau (40 °N) and ~30 °N the Mid-Atlantic Ridge (MAR) is 'transitional' in type and characterized by short offset spreading segments forming a SSW-trending zone of accretion. FAMOUS and AMAR studies of the axial region at 36-37 °N (refs 5-7) documented geophysical and petrological properties of such segments (Rift Valleys (RV) 2 and 3) and DSDP Leg 378 studied their stratigraphic expression at four sites (332-335) perpendicular to the ridge axis. The sites represent a spreading flow line between 3.4 and 13 Myr BP. Most DSDP/IPOD basement sites reflect negligible sourcerelated chemical differences between stratigraphically contiguous lava series9. In contrast, the source-related chemical diversity at Site 332 and RV-2 (refs 10-12) is apparently characteristic of transitional ridge axes^{2,4}, and precludes common derivation for these series through processes of fractional crystallization in small reservoir systems 10-12.

While some interpretations of 36-37 °N have failed to emphasize the transitional character of the ridge axis, Schilling et al. 13 explained REE variation in this region in the context of spatial and temporal trends, postulating influx of an Azores plume or mantle 'blob' 14. However, the case for binary mixing of sources on the scale required by this model, at least in simple form, is not supported by regional isotope and low- K_D element variation for either secular (0-13 Myr) or isochronic (zero age) trends15. Other explanations for anomalous geochemistry in 36-37 °N magmas appealed to zoned and/or periodically refilled magma chambers 16,17, and also to the process of 'dynamic melting'10 which involves incremental melting and incomplete extraction of the melt phase as a typical feature of dilating partial melting environments. These concepts have been questioned18, and in the absence of seismic recognition and petrological evidence for sub-rift reservoirs 19-21 and the lack of chemical characteristics ascribed to dynamic melting at 'normal' ridge axes (for example, 22 °N), it is reasonable to explore alternative models.

Evidence from lava and xenolith variation at single (such as island) localities suggests that mantle heterogeneity is possibly vertical in nature^{4,22,23} thus coalescence of dilating and transform fractures could allow differential tapping of the mantle. Thermal models^{24,25} predict progressively shallower partial melting with increasing spreading rates. A simplistic but reasonable synthesis could postulate preferential zones of partial melting, activated by either normal rift dilation (high degrees of partial melting at shallow depth), or (depending on

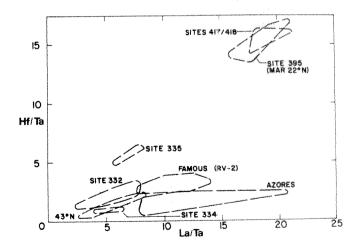


Fig. 1 Hf/Ta versus La/Ta diagram for basalts from the Atlantic/Azores region, data from refs 2, 8, 12, 36-39. Sites 395, 417 and 418 reflect 'normal' MORB depleted in low-KD elements. Note the absence of regional binary mixing; the colinear trend of FAMOUS RV-2 and Site 332; the lack of such a trend between 332, 334 and 335 (36-37 °N spreading flow line); and the diversity of 'enrichment' type compared with normal MORB.

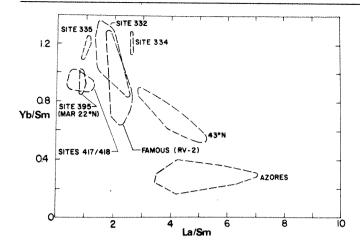


Fig. 2 Yb/Sm versus La/Sm diagram for basalts from the Atlantic/Azores region; data from same sources as for Fig. 1. Note the colinear RV-2-332 trend and lack of colinearity for 36-37 °N flow line; and the greater distinction of La/Sm (lower K_D) than Yb/Sm (higher K_D).

ridge axis kinematics) by transform fractures (low degrees of partial melting at greater depth), each environment constrained by a different range of pressure, temperature and hence, depth of melting. In turn, a given fracture zone-bound spreading segment might be characterized by (1) relative volumetric contributions from the two environments, and (2) the extent to which these hybridize during storage and/or transport to the surface.

Considering the range of variation at 36-37 °3N it seems that a combination of all factors is required. MgO-rich basalts are depleted in light REEs10 and there is a general correlation (not attributable to fractional crystallization) of low-K_D element enrichment, increases in La/Sm, Rb/Sr, Ta/La and so on and decreasing Mg/(Mg+Fe²⁺). This correlation may reflect the combined effects of source heterogeneity and fractional crystallization, and probably also variations in the extent of partial melting, and as a general effect may indicate relatively direct access to the surface from the rift-melting environment, but more lengthy transport/storage (hence fractionation) histories for enriched magmas derived from fracture zone melting, before hybridization. Most mixing models have considered ridgelongitudinal 'open' feeder systems²⁷ which are probably not typical for slow-spreading axes such as the MAR²¹. Possible roles for fracture zones have not been seriously examined. If transform fractures permit the confluence of different magma supply systems, the development of chemical gradients at midocean ridges may reflect the extent and nature of offset spreading as reflected by fracture zone frequency rather than regional mixing of different solid mantle fractions. This is apparent from recent data compilations²⁸ which show low-K_D element and radiogenic isotope peaks, together with lava temperature 'troughs', corresponding not only to positive gravity and thermal anomalies (proposed loci for mantle upwelling) but to major transform fractures. Mixing between fracture zone and rift systems is consistent with evidence for longitudinal magma flow at dilating ridges^{29,30} and with inferences from seismic and chemical data constraining magma systems to individual ridge segments 11,19,20

Low- K_D element ratio-ratio plots are a useful way to study source-characteristic mixing. If each ratio denominator is identical (for example, K/Rb and Sr/Rb) mixing produces straight line variation between the two³¹. Data for DSDP Sites 332 (3.4 Myr), 334 (10 Myr) and 335 (13 Myr), FAMOUS RV-2, the Azores and other Atlantic localities are depicted in two low- K_D element ratio diagrams: Hf/Ta-La/Ta and Yb/Sm-La/Sm (Figs 1 and 2). Hf/Ta-La/Ta plots for RV-2 and 332 reflect significant variation in source composition, distinct from that of 'normal' ridge segments (represented by Sites 395, 417

and 418) and consistent with binary mixing. The variation is also distinct from that of older sites at 36–37 °N and from other 'enriched' localities in the Atlantic. A similar, more diffuse, relationship is observed for Yb/Sm-La/Sm variation (Fig. 2). In general, variation for given localities such as spreading segments or individual islands is consistent with simple binary mixing, whereas variation on a regional scale reflects a complex array of fractionation processes in the mantle. The relation to small-scale rather than large-scale tectonic features is probably highly significant, and bears on fundamental problems concerning the chronology and origins of mantle heterogeneity.

 87 Sr/ 86 Sr and 143 Nd/ 144 Nd ratios for FAMOUS RV-2 lavas 18 indicate isotopic homogeneity for the source region at present. and, as for many low- K_D element-enriched magmas, reflect a time-integrated source depletion in Rb and LREE compared with chrondrites 18,32. White 18 suggests that this reflects that either Rb/Sr and Nd/Sm ratios in the source are uniform, or that heterogeneities in these ratios are young (≤80 Myr). Although White prefers the former interpretation, his data reflect Rb/Sr and Nd/Sm variation between 0.024-0.063 and 0.267-0.337 respectively (Fig. 3) and show a strong negative correlation. This clearly favours the interpretation that heterogeneity is of recent origin. The approximate coincidence of RV-2 and Site 332 Rb/Sr-Nd/Sm variation to bulk Earth estimates in Fig. 3 is probably fortuitous in view of the depletion history recorded by Sr and Nd isotopes. Together, the low-KD and isotope components suggest that generation of heterogeneity expressed in the 36-37 °3N lavas is coeval with, and may be complementary to, processes of magma generation as determined by the overall tectonic environment. From geophysical considerations³³ local binary mixing of mantle fractions is unlikely, suggesting that mixing of different mantle-derived magmas is a more plausible mechanism for producing mantle-related mixing trends of the type observed. The fracture zone-rift explanation for binary mixing is also appealing for its ability to explain the origins of low- K_D element enrichment in the mantle. Enrichment by metasomatic veining of fertile peridotite has been documented in terms of mineralogy, chemistry and isotopic equilibration^{34,35} and may be the result of incipient melting in garnet lherzolite. The thermal-kinematic nature of transform fractures and the correspondence of magmatic enrichment/mixing trends to local rather than regional tectonic features suggests that transform fracturing may actually provoke

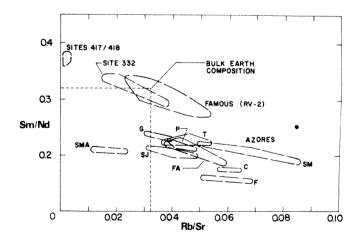


Fig. 3 Sm/Nd versus Rb/Sr diagram for basalts from the Atlantic/Azores region: data from refs 8, 10, 12, 18, 36-39. SMA, Santa Maria; G. Graciosa; T, Terceira; P, Pico; SJ, Sao Jorge; FA, Faial; C, Corvo; F. Flores and SM, Sao Miguel. Note the apparent correspondence of RV-2 and 332 to 'bulk Earth' composition (in contrast to Sr and Nd isotope compositions (ref. 18), 'depletion' of 'normal' MORB in Nd and Rb, and 'enrichment' of Azores in Rb (except Santa Maria) and Nd.

generation of low-percentage melt increments at depth, and provide upward access for these into depleted 'normal' mantle to form secondary 'enriched' sources for ridge axis magma. In this context, the evaluation of small variations in pressure, temperature, percent melting and disequilibrium in determining the nature of enrichment becomes critical in explaining the unique chemistries of enriched-magma associations.

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- 1. Bonatti, E., Chermak, A. & Honnorez, J. 2nd Ewing Ser. (eds. Talwani, M., Harrison, C. G. & Hayes, D. E.) (American Geophysical Union, 1979). Shibata, T., Thompson, G. & Frey, F. A. Contr. Miner. Petrol. 70, 127-141 (1979).
- Bonatti, E. & Hamlyn, P. R. Science 201, 249-251 (1978). Batiza, R. & Johnson, J. R. Init. Rep. DSDP 54 (in the press
- Battza, R. & Jonnson, J. R. Inti. Rep. D3DF 34 (in the press).

 Needham, H. D. & Francheteau, J. Earth planet. Sci. Lett. 35, 30-42 (1974).

 Hekinian, R., Moore, J. G. & Bryan, W. B. Contr. Miner. Petrol. 58, 83-110 (1976).

 Ballard, R. D. et al. EOS Union 59, 1198-1199 (1978).

 Aumento, F. et al. Init. Rep. D3DP Leg 37 (1977).

 Init. Rep. D3DP Legs 45, 46, 51-53 (1979-80).

 Langmuir, C. H. et al. Earth. planet. Sci. Lett. 36, 133-156 (1978).

- 10.
- Flower, M. F. J. et al. Contr. Miner. Petrol. 64, 167-195 (1977). Bougault, H. et al. Tectonophysics 55, 11-34 (1979).
- Schilling, J. G., Kingsley, R. & Bergeron, M. Init. Rep. DSDP Leg 37, 591-598 (1977). Schilling, J. G. Earth planet. Sci. Lett. 25, 103-115 (1975).

- Schilling, J. G. Earth planet. Sci. Lett. 25, 103-115 (1975).
 Flower, M. F. J. Lithos (submitted).
 Bryan, W. B., Thompson, G. & Michael, P. J. Tectonophysics 55, 63-85 (1979).
 O'Hara, M. J. Nature 266, 503-507 (1977).
 White, W. W. Yb. Carnegie Instn Wash. 78, 325-331 (1979).
 Fowler, C. M. R. Geophys. J.R. astr. Soc. 47, 459-591 (1976).
 Steinmetz, L., Whitmarsh, R. & Moreira, V. Geophys. J.R. astr. Soc. 50, 353-380 (1977).
 Flower, M. F. J. Nature 287, 530-532 (1980).
- Hofmann, A. W., White, W. M. & Whitford, D. J. Yb. Carnegie Instn Wash. 77, 548-562
- Zindler, A. et al. Earth planet. Sci. Lett. 45, 249-262 (1979).
- Sleep, N. H. J. geophys. Res. 80, 4037-4042 (1975). Kuznir, N. J. & Bott, M. H. P. Geophys. J.R. astr. Soc. 47, 83-95 (1976).
- Bottinga, J. & Allégre C. J. Phil. Trans. R. Soc. A288, 501-525 (1979). Rhodes, J. M. & Dungan, M. A. in 2nd Ewing Ser. (eds Talwani, M., Harrison, C. G., Hayes, D. E.) 262-272 American Geophysical Union, 1979) Schilling, J. G. & Sigurdson, H. Nature 282, 370-375 (1980).

- Schilling, J. G. & Sigurdson, H. Nature 224, 370–373 (1990).
 Vogt, P. R. Earth planet. Sci. Lett. 29, 309–325 (1976).
 Bjornsson, A. et al. Nature 266, 318–322 (1978).
 Langmuir, C. H. et al. Earth planet. Sci. Lett. 37, 380 (1978).
 O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. Earth planet. Sci. Lett. 34, 13–32 (1977).
- Forsyth, D. W. Tectonophysics 38, 89-118 (1977).

- Forsyth, D. W. Tectonophysics 38, 89-118 (1977).
 Menzies, M. & Rama-Murthy, V. Earth planet. Sci. Lett. 46, 323-334 (1980).
 Wood, D. A. Geology 7, 499-503 (1979).
 Blanchard, D. P. et al. J. geophys. Res. 81, 4231-4246 (1976).
 White, W. M., Tapia, M. D. M. & Schilling, J. G. Contr. Miner. Petrol. 69, 201-213 (1979).
 Hawkesworth, C. et al. Nature 280, 28-31 (1979).
- Jahn, B. M. et al. Earth planet. Sci. Lett. 48, 171-184 (1980).

Sanidine spherules at the Cretaceous-Tertiary boundary indicate a large impact event

J. Smit & G. Klaver

Geological Institute, Nieuwe Prinsengracht 130, Amsterdam, The Netherlands

The hypothesis that a catastrophic impact of an extraterrestrial body caused the terminal Cretaceous mass extinctions of dinosaurs, planktonic foraminfera and other species is now accepted as respectable following the discovery of a worldwide iridium enrichment in the Cretaceous-Tertiary (K-T) boundary clay¹⁻⁵. In the basal lamina of the K-T boundary clay of Caravaca (Spain)⁷ numerous spherules were discovered composed of finely crystallized, almost pure K-feldspar in the structural state of high sanidine. It is concluded here that these spherules solidified from a melt and were probably derived from the impacting body. This poses problems as high K-values are not reported from bulk analyses of meteorites⁶. The K-feldspar phenocrysts reported in some iron meteorites²³ suggest the body may have been a metal-sulphide-silicate planetesimal. A cometary body is suggested as an alternative.

The strongest geochemical extraterrestrial signals have been recorded in the basal lamina of the 10-cm thick K-T boundary clay of both the Barranco del Gredero section (Caravaca) and the 'Fish-clay' at Stevns Klint (Denmark)1-5. These basal millimetres show the highest concentrations of the siderophile elements Ir (44 and 86.7 parts per 109 respectively), Os, Cr, Co and Ni, and also a significant rare earth element (REE) depletion. (J.S and Ten Kate, in preparation). The REE depletion, if caused by the low concentration of REE in the impacting body, suggests a large meteoritic contribution in the basal lamina, regarded as 'fall-out' level of the impact event. Moreover, anomalously high amounts of the chalcophile elements Zn, As, Se, and Sb have been found, elements which are not present in high concentrations in known extraternestrial material⁶. The sanidine spherules occur as small (0.5-1 mm) globules, dumbbells and disks only in the 'fall-out' level of the K-T boundary clay at Caravaca; some 50-300 per cm3 sediment were found. X-ray diffraction analysis of the spherules has been carried out on a Guinier camera, using CuKα radiation and corundum as internal standard. The diffraction pattern shows only K-feldspar lines of good quality, which permit accurate determination of the 2θ angles ($\overline{2}04$; 50.78°, 060; 41.59°, $\overline{2}01$; 20.98° (± 0.1 °)) and lattice constants (Table 1, Fig. 1). Indexing of the Guinier pattern according to Borg and Smith8 as sanidine is unam-

Furthermore, the spherules were analysed chemically by electron microprobe and instrumental neutron activation analysis (INAA) (Table 1) and have been examined by scanning electron microscope and petrographic microscope (Figs 2, 3).

In good agreement with the X-ray diffraction, the microprobe analysis gives a K-feldspar composition of the formula $K_{0.95}Na_{0.01}Fe_{0.01}Al_{0.98}Si_{3.01}O_8$. The high K/Na ratio is remarkable: K/(K+Na) = 0.99. No difference in chemical composition has been observed between the centre and the edge of the spherules. Within the spherules occur numerous small opaque inclusions. Analysis of two of these inclusions (Table 1) shows a high Cr and Ni (9,400 p.p.m.) content, considerably more than the clay in which the spherules were found. As iridium generally correlates positively (above zero in impact melts) with Ni (refs 9, 10) we expect the inclusions to have a high Ir content. The whole rock INAA analysis of the spherules confirms the high K/Na ratio. Co, Cr, Ni, As and Sb occur in high concentrations and are presumably concentrated in the opaque inclusions. REE values are low. Similar low values in K-feldspar are rarely reported11

A preliminary K/Ar dating (E. A. Hebeda, personal communication) on handpicked and ultrasonically cleaned, but

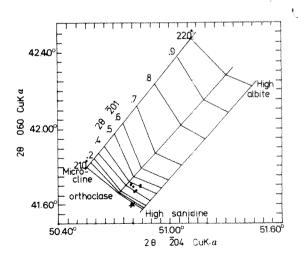


Fig. 1 Plot of 2θ angles ($\overline{2}04$) and (060) on Wright's ¹⁹ structural state diagram of the spherules (+) and reported ^{16,17} authigenic sanidine (lacktriangle). The 2θ angle (201) of the sanidine spherule predicted from this diagram (20.9°) hardly differs from the measured 2θ $(\overline{2}01)$: (20.98°) . Authigenic sanidine shows 2θ $(\overline{2}01)$ of 20.95° -21.01°, well outside the predicted range of 21.3°-21.42°. ×, Sanidine phenocrysts in Colomera iron-meteorite 23.



Fig. 2 Thin sections of the sanidine spherules from the Cretaceous-Tertiary boundary, showing dendritic microstructure and fibres radiating from the surface and from opaque inclusions. Scale bar, $75~\mu m$.

otherwise untreated sanidine spherules, gave a minimum age of 44 ± 2 Myr. This is ~ 21 Myr less than the generally accepted age of 65 Myr for the K-T boundary¹², and is probably due to loss of argon or to impurities within the spherules.

Figures 2 and 3 show the microstructure and crystal structure of the spherules. The microstructure consists of minuscule dendrites and fan-shaped fibres, radiating from the surface into the spherule, and from the opaque inclusions, acting as nuclei (Fig. 2a-c). This texture suggests that crystallization took place rapidly¹³.

But how did the spherules originate? Authigenic growth of K-feldspar as microcline or adularia occurs commonly^{14,15}, but little information on authigenic sanidine is available^{16–18}. Authigenic growth of the spherules, however, is considered unlikely, because of the crystal shape, texture and structural state of the sanidine.

Authigenic crystallization takes place slowly, which usually leads to equidimensional, euhedral crystals, in strong contrast with the texture shown in Figs 2 and 3. Figure 1 shows the difference in structural state¹⁹ between the sanidine of the spherules and authigenic sanidine—authigenic sanidine is clearly anomalous. The only argument for authigenic growth would be the high K/Na ratio, because of the limited miscibility of K- and Na-feldspar at low temperatures¹⁵.

A volcanic origin for the spherules is also considered unlikely, because of the lack of other volcanic products at the K-T boundary, and the high K/Na ratio.

A relation with the terminal Cretaceous impact event remains the most plausible explanation. We may consider the spherules either as microtektites, which implies a terrestrial source rock, or as ablation products of the incoming projectile(s). Mikrotektites are normally glassy objects with a terrestrial chemical composition, ejected from an impact crater²⁰.

The initiation of crystallization at the surface of the spherules indicates that the spherules were already in their present, spherical shape when crystallization started. This favours strongly rapid cooling, beginning at the surface of a molten silicate droplet. Perhaps the 'monomineralic' composition of the melt of the spherules has led to dendritic crystallization, instead of glass. For microtektites the high K/Na ratio would be difficult to explain, although K enhancements in crater ejecta and impact melts have been observed²¹.

The texture of the spherules resembles the texture of chondritic ablation spherules reported by Blanchard et al.²², although the chemistry of present spherules is completely different. Ablation products, rich in K are not yet reported; K is an accessory element in all types of meteorites (150–1,400 p.p.m., ref. 6), and only from a few iron meteorites are K-feldspar phenocrysts reported²³ with the same X-ray pattern as the spherules (Fig. 1). The enriched chalcophile elements As, Se and Sb may be derived from a comet, which is supposed to have preserved (part of) its volatile constituents. K may similarly be

Table 1 X-ray diffraction, microprobe and instrumental neutron activation analysis of sanidine spherules from the base of the Cretaceous-Tertiary boundary clay at the Barranco del Gredero, Caravaca, south-east Spain

	Microprobe			Instrumental neutron activation		X-ray diffraction (CuKα)		
	Mean of 5 spherules (%)		ark sions		Sanidine spherules (p.p.m.)	Clay between spherules (p.p.m.)	Lattice c	
SiO ₂ Al ₂ O ₃	$64.3 \pm 0.2 \\ 17.7 \pm 0.4$	41.0 17.8	36.75 17.7	Na K	700 10.2%	313.6 0.95%		
TiO_2	AND THE RESIDENCE OF THE PERSON OF THE PERSO	0.1	0.1	Sc	3.49	11.3		
Cr_2O_3		0.15	0.15	Cr	269.3	952	α	90.000°
FeO	(0.05 - 0.7)	15.05	19.55	Fe	1.77%	8.04%	β	116.026°
MnO		0.05	0.05	Co	184.1	720	Y	90.000°
NiO		0.8	1.2	Ni	810	2,980		
MgO		3.2	2.55	Zn	390	1,740		
CaO		1.25	1.2	As	166	751	а	8.61
Na ₂ O	0.15 ± 0.015	0.35	0.4	Se	< 3	6.0	b	13.02
K ₂ O	16.0 ± 0.2	0.8	0.65	Sb	4.8	17.2	c	7.19
ZnO	Angeman	0.25	0.2	La	1.35	5.5	v	724,058
				Ce	5.7	14.5	·	. = .,
Total	98,45%	80.8%	80.5%	Sm	0.27	0.9	20	9
	2011070	001010	0014	Eu	0.22	0.271	Ž01	20.98°
				Hf	1.9	4.8	131 (131)	
				Th	4.8	10.2	060	41.60°
				Ir	<11 p.p.b.	44.0 p.p.b.	204	50.76°

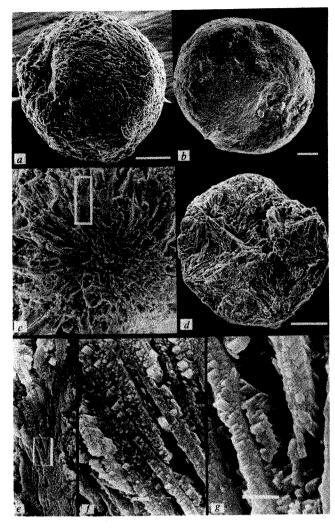


Fig. 3 Scanning electron micrographs showing the morphology of the sanidine spherules and dendritic sanidine crystals. Scale bars: a, b, d, 100 μ m; f, g, 1 μ m. e-g are enlargements of c.

derived from a comet. However, K-feldspar is a differentiated mineral, which probably does not occur in undifferentiated Solar System material, as comets are supposed to. Bandhari and Shah's report²⁴ of K-rich globules from Luna-20 soil is notable as the soil of the Moon is supposed to contain meteoritic and cometary material (J. Hertogen, personal communication). A third possibility is that the KAlSi₃O₈ spherules are condensates of more volatile constituents, which are vaporized on entry to the atmosphere or on impact on the Earth.

We suggest that sanidine spherules, restricted to the 'fall-out' lamina of the terminal Cretaceous impact event, are derived from a projectile, rich in chalcophile and moderately volatile elements like K, As, Se, Sb and Zn. Further chemical analysis of the spherules and their inclusions may shed light on the terminal Cretaceous impact event and on the composition of the impacting body.

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- Alvarez, L. W. et al. Science 208, 367-371 (1980).
- Smit, J. & Hertogen, J. Nature 285, 198-200 (1980). Ganapathy, R. Science 209, 921-923 (1980).
- 4. Kyte, F. T. et al. Nature 288, 651-656 (1980) Hsü, K. J. Nature 285, 201-203 (1980).
- 6. Mason, B. Handbook of Elemental Abundances in Meteorites (Gordon and Breach, New
- 7. Smit, J. Proc. Kon. Ned. Akad. Wet. 80, B. 280-301 (1977)

- 8. Borg, I. Y. & Smith, D. K. Geol. Soc. Am. Mem. 122 (1969)
- Palme, H. et al. Proc. 10th Lunar Sci. Conf. 2465-2492 (1979) Lambert, P. Impact and Explosion Cratering, 449-460 (Pergamon, Oxford, 1977).
- Smith, J. V. Feldspar Minerals (Springer, New York, 2974). Van Hinte, J. E. Bull. Am. Ass. petrol. Geol. 60, 498–516 (1976).
- Van Hinte, J. E. Bali, Am. Ass. perol. Geo. 60, 432–45 (1976). Lofgren, G. in Physics of Magmatic Processes, 487–551 (Princeton University Press, 1980). Kastner, M. Am. Miner. 56, 7–8, 1403–1442 (1971). Baskin, Y. J. Geol. 64, 132–155 (1956).

- Woodard, H. H. J. Geol. 80, 323-332 (1972). Brus, Z. & Rieder, M. Acta univ. carol. 1, 37-45 (1975)
- Lancelot et al. Init. Rep. DSDP Leg 11, 901-950 (1972) Wright, T. L. Am. Miner. 53, 88-104 (1968).
- O'Keefe, I. A. (ed.) Tektites (University of Chicago Press, 1963).
- 21. Parfenova, O. V. & Yakovlev, O. I. Impact and Explosion Cratering, 843-859 (Pergamon, Oxford 1977)
- Blanchard, M. B. et al. Earth planet. Sci. Lett. 46, 178-190 (1980).
- Wasserburg, G. J. et al. Science 161, 684-687 (1968).
 Bandhari, N. & Shah, V. G. Proc. Ind. nat. Sci. Acad. A 45/3, 199-200 (1977).

Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur

R. E. H. Reid

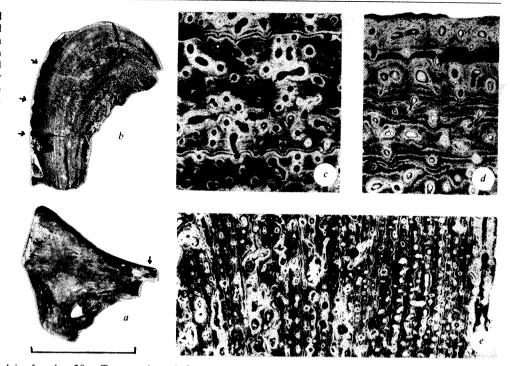
Department of Geology, The Queen's University of Belfast, Belfast BT7 1NN, UK

De Ricqlès¹⁻⁷ distinguishes two principal types of primary (unremodelled) compact bone (compacta), which he terms lamellar-zonal and fibro-lamellar. In the former, bone laid down by accretion at the periosteal (external) surface shows a general coarsely lamellated texture, and vascular canals (for blood vessels) are often sparsely developed and sometimes lacking. Primary osteons, formed by inward growth of bone inside vascular canals, may be present or absent. This type of compacta is seen in bones which grow slowly or only to small sizes, and is the only type seen in most ectotherms. In contrast, fibrolamellar bone is always rich in primary osteons, set in a matrix of woven bone which is typically laid down rapidly. This type of bone is seen mainly in medium- to large-sized endotherms, and reflects their ability to reach such sizes more quickly than comparable ectotherms. In ectotherms, lamellar-zonal bone may show a cyclical alternation of thin layers of dense bone. termed annuli, with thicker vascular layers known as zones, but it is claimed2 that such features are not developed cyclically in endotherms. The presence of fibro-lamellar compacta, without annuli and zones, in various dinosaurs is hence seen as evidence that dinosaurs were endotherms. However, I report here a case in which compact bone from the pelvis of a dinosaur is instead lamellar-zonal, and shows typical zones and annuli.

The material studied consists of the proximal parts of an incomplete left pubis (Fig. 1a) and ischium (anterior and posterior bones of the lower part of the pelvis) from an undescribed sauropod dinosaur, found in the Northampton Sands Formation (Middle Jurassic, Lower Bajocian) at Harlestone, near Northampton, UK. Both bones are broken across their shafts, and the compacta seen in section at the broken ends shows conspicuous concentric annulations (see Fig. 1b), which are due to its formation from a series of circumferential increments. The external surface shows fine longitudinal grooving, and the vascular canals exposed at broken surfaces are predominantly longitudinal. The compacta surrounds a core of spongy bone (spongiosa), containing tubular cavities which again run longitudinally.

In the pubis, the compacta is thickest on the outward-facing surface, and most of the increments are several times thicker on this side than on the inward side. Each increment is underlain by a sharply defined surface, which resembles the external surface. Where these increments are thickest (Fig. 1c), each one typically begins with a thin (for example, 25 µm thick) but well defined layer of bone, in which vascular canals occur mainly over

Fig. 1 Left pubis of a sauropod dinosaur (British Museum (Natural History) exhibit no. R. 9472) from the Northampton Sands Formation near Northampton, UK. a, Proximal part of the pubis, inner face, to show the dinosaurian character of the specimen and the source of the sectional material (arrowed), scale 30 cm. b, Polished section at the point arrowed in a, viewed distally, showing the annulated appearance; arrows mark the positions of traverse lines used in measuring from a parallel thin section (see text and below) \times_{4}^{3} . c, One increment of the primary compacta and parts of two others, as seen where the increments are thickest (b, left-hand side). showing two of the thin basal annuli and one complete vascular zone, ×30. Moving inwards, the outermost 11 such increments have average thicknesses (three measurements) of 0.30, 0.34, 1.06, 1.17, 0.47, 1.04, 2.27, 2.15, 1.14, 1.78 and 1.78 mm. respectively. d, The outermost three increments of the compacta as seen where the increments are thinnest (b,



right-hand side), and part of the underlying fourth, $\times 38$. e, Traverse through the compacta where its increments are thinnest, from the external surface (at right) to near the limit of the spongiosa (left), $\times 12$.

grooves in the underlying surface. A second similar layer sometimes follows, usually less distinct. The rest of the increment is formed by strongly vascular bone, showing little or no concentric layering, but packed with primary osteons. The vascular canals of this part of the increment are irregularly interconnected at rather wide intervals, and show no regular arrangement. The increment then ends with a surface which resembles the one underlying it. Up to 13 such increments were counted in a section cut parallel to the surface shown in Fig. 1b, with an average thickness (three measurements) of 1.23 mm in the outermost 11 (range 0.30-2.27 mm; see Fig. 1 legend). Where the increments are thinnest (Fig. 1d,e), there are usually several thin basal layers, but the vascular parts are reduced, and their vascular networks may be single layered. Bone under the terminal surface may also show a layered appearance, and this extends through the whole depth of some increments. Up to 17 such increments were counted, with an average thickness of 0.35 mm. Intervening parts of the compacta show conditions intermediate between these two types (Fig. 1c,d).

A sample from the ischium shows compact of the type seen in Fig. 1c, but only five or six increments remain between the surface and the spongiosa. (As usual, the spongiosa grew outwards by encroaching on the compacta from within, and these different counts of increments reflect different degrees of encroachment.) In both bones, Haversian systems (secondary osteons, formed from bone laid down in vascular canals cut though the primary structure by resorption) are mainly seen near the spongiosa. The bones are iron stained, with their fine structure partly masked as a result. Using cross-polarization, the fine banding of lamellar bone can be found in primary osteons, and in the Haversian bone and spongiosa, but it is not seen in the thin circumferential layers which seem to consist of parallel-fibred bone. Woven bone can be recognized in parts, between primary osteons.

Although these bones are pelvic, their shafts can be compared with those of limb bones, and the concentric structures seen in their compacta reflect the way in which new bone was added at the surface. These structures are not a result of internal remodelling, which is limited to replacement of the primary compacta by Haversian bone and spongiosa. The surfaces between successive increments mark pauses in growth represented by a resting line at the base of each increment as seen in section (see Fig. 1c). Each increment, as developed in

Fig. 1c, can be interpreted as consisting of a thin basal annulus with a vascular zone outside it; the modified pattern seen in Fig. 1d shows the condensing effect of slower growth. These features are repeated cyclically through the whole depth of the primary compacta, up to 17 times down to the level of internal replacement by spongiosa. There is no sign that this growth plan replaced an earlier non-cyclic pattern. Hence, they cannot be dismissed as a result of incidental formation of annuli, or their formation at the end of normal growth (compare ref. 2, p. 109 and ref. 4, p. 53). Annuli are more usually found under resting lines3, but annuli which follow resting lines, as in the material examined here, are known from the pelycosaur Dimetrodon, for example (Fig. 4 of ref. 8). The most similar material observed by de Ricqlès (Fig. 3 of pl. 19, ref. 6) is compacta from limb bones of the fossil amphibian Stenotosaurus (Subclass Labyrinthodontia, Order Temnospondyli, Suborder Stereospondyli, Lower Triassic), which may show thick vascular zones packed with primary osteons as found in parts of the material examined here (Fig. 1c), or a more condensed pattern (Fig. 9 of pl. 5, ref. 2) like that shown here in Fig. 1d,e. The compacta from Stenotosaurus was specifically shown^{2,6} as being lamellar-zonal bone with zones and annuli; therefore I take this to be the character of the compacta of the bones studied here.

The primary compacta of this sauropod pubis and ischium is thus not of the fibro-lamellar type, thought⁷ to be general in dinosaurs, and shows features (cyclically repeated annuli and zones) described^{2,4,6} as characteristic of ectotherms and absent² from known endotherms and dinosaurs. Their presence here cannot be dismissed as due simply to slow growth of the pelvic bones (compare p. 139, ref. 7), because the presence of resting lines between thick vascular zones implies that periods in which no bone was deposited periosteally occurred alternately with periods of more or less rapid growth. Where the zones are thick (Fig. 1c), they are more vascular than is usual in modern reptiles (compare with ref. 9), but can be closely matched in the amphibian Stenotosaurus, which is not a likely endotherm (all modern amphibians are ectothermic)—this condition has also been interpreted² as being related to aquatic modes of life. Thus the sauropod discussed here seems to have been ectothermic, unless annuli and zones can be cyclically developed in an endotherm. At first sight, this result is surprising but de Ricqlès' main work 8.10-13 on bone in fossil amphibians and reptiles contains no systematic study of dinosaurs, and his summary⁷ of their bone

histology makes use of work by other authors. Some material in the theropod Allosaurus is thought to show lamellar-zonal example if bone included by Seitz (Figs 53, 54 of ref. 14) from the theropod Allosaurus is thought to show lamella-zonal structure. This suggests that the material examined here only seems to be anomalous because no adequate systematic study of dinosaurian bone histology has yet been published.

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- de Ricqlès, A. J. C. r. hebd. Séanc. Acad. Sci., Paris 275, 1745-1749 (1972). de Ricqlès, A. J. Annis Paléont. 61(1), 49-149 (1975). de Ricqlès, A. J. Annis Paléont. 62(1), 71-126 (1976). de Ricqlès, A. J. Annis Paléont. 63(1), 33-56, 133-139 (1977).

- de Ricglès, A. J. Annis Paléont. 63(2), 133-160 (1977); 64(1), 85-111 (1978).
- de Ricqlès, A. J. Annis Paléoni. 64(2), 153–184 (1978).
 de Ricqlès, A. J. in Morphology and Biology of Reptiles Linn. Soc. Symp. Ser. 3 (eds Bellairs, A. d'A. & Cox, C. B.) 123–150 (London, 1976).
- de Ricglès, A. J. Annis Paléont. 60(1), 3-58 (1974)
- Enlow, D. H. in Biology of the Reptilia (eds Gans, C. & Bellairs, A. d'A.) 45-80 (Academic, London, 1969).
- de Ricqlès, A. J. Annis Paléont. 54(2), 131–146 (1968).
 de Ricqlès, A. J. Annis Paléont. 55(1), 3–72 (1969).

- de Ricqlès, A. J. Annis Paléont. 58(1), 17-80 (1972).
 de Ricqlès, A. J. Annis Paléont. 60(2), 171-234 (1974)
- 14. Seitz, A. L. Nova Acta Akad. Caesar. Leop. Carol. 87, 230-370 (1907).

New subclass of birds from the Cretaceous of South America

C. A. Walker

Department of Palaeontology, British Museum (Natural History), Cromwell Rd, London SW7 5BD, UK

Current classification of birds recognizes three subclasses which morphologically distinct: the Archaeornithes for Archaeopteryx, the Odontornithes for the Hesperornithiformes and the Ichthyornithiformes, and the Neornithes for all modern birds and their extinct immediate relatives. (Some authorities1 prefer different names for some of these taxa.) I have examined new material recently discovered in the Upper Cretaceous rocks of Argentina which indicates the existence of a group of birds having features so different from those of the currently recognized subclasses that they seem to represent a fourth subclass, here named the Enantiornithes ('opposite birds'). I describe unique features of the Enantiornithes which include a reduced outer metatarsal, in some forms an extreme modification of the remaining elements of the tarsometatarsus, a highly modified pectoral girdle, and sometimes a characteristic perforation in the proximal end of the humerus.

In the 1970s Dr Jose Bonaparte (Museo Argentino de Ciencias Naturales, Buenos Aires) conducted field excavations in the continental deposits of the Lecho Formation (Salta Group) of El Brete (Salta Province), northwestern Argentina, the age of which is probably Maestrichian (uppermost Cretaceous). The collection included ~60 individual bones, some associated, which are presumed to be avian; they show no similarity to any of the accompanying dinosaur remains (a titanosaurid, a coelurosaur and a carnosaur)2. This bird material consists of all the major postcranial elements and the posterior portion of a left lower jaw ramus; it will be housed in the Universidad Nacional de Tucumán, Argentina. I have described the material here as belonging to members of a single monophyletic group. Although

found separately, most of the elements are of a single unique form: for example, there are five coracoids, all very different from those of other birds, including that of Archaeopteryx. The same is true of the 10 humeri, 6 ulnae, 5 femora, 5 tibiotarsi and 5 tarsometatarsi. The hypothesis that all these remains belong to one group is supported by the fact that there is some degree of association between the elements: for example, one articulated group consists of scapula, coracoid and humerus, and in another case a very similar humerus was found with the whole of the rest of the forelimb skeleton. The fore- and hindlimb elements can be associated only tentatively, by size, and by a single associated ulna and tibia (4032).

However, within the highly characteristic structure, both size and shape vary considerably. The material falls into five categories, of which three have about the same dimensions (but differ from each other in form); in the other two categories the material is much smaller and much larger respectively. The morphological differences between the five tarsometatarsi are particularly striking; they clearly represent three lines of evolution so distinct that, had they still been extant, each would probably have been assigned a separate ordinal status. The differences between the humeri are less marked but are nevertheless significant.

The new specimens from Argentina possess certain characters common to all birds. These include a strap-shaped scapula; a coracoidal foramen (=supracoracoidal foramen of Ostrom3); a humerus with proximal and distal expansions almost in the same plane, well developed deltoid and bicipital crests, a pneumatic fossa and/or foramen and a well marked ligamental furrow; a radius with an expanded distal end; an opisthopubic pelvis; a pit in the head of the femur for the attachment of the round ligament; and some degree of fusion of the metatarsals.

A cladistic analysis of the remaining characters of this group, for which the new name Enantiornithes ('opposite birds') is proposed, results in the cladogram shown in Fig. 1. Table 1 lists the synapomorphies common to Enantiornithes and all other post-Jurassic birds (Odontornithes and Neornithes) in which they differ from the corresponding character-states of Archaeopteryx, while Table 2 lists the synapomorphies which are shared by the Odontornithes and Neornithes, in which they differ from the corresponding character-states of the Enantiornithes.

The positive character-states unique to the Enantiornithes (nos 3-6a, 8, 10 and 13 in Table 2) make it impossible to place them in any of the three major groups already recognized (Archaeornithes, Odontornithes and Neornithes). There are, however, plesiomorphic similarities with Archaeopteryx, including the length and slenderness of the femur and the imperfect fusion of the tarsometatarsus. As I do not support strictly Hennigian classifications I suggest that this group be assigned the status of a subclass, thus fitting it into the traditional classification as coordinate with the other three subclasses.

The coracoid (Fig. 2a, D and E) differs from those of typical birds, not only in having no procoracoid but also in its lack of a prominent sterno-coracoidal process. Perhaps the most fundamental and characteristic difference between the Enantiornithes and all other birds is in the nature of the articulation between the scapula (Fig. 2a, C) and the coracoid, where the 'normal' configuration is completely reversed. The humerus too possesses unique features: for example, the head is almost flat in anconal view (Fig. 2a, B) while there is a well marked depression

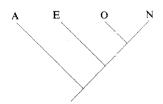


Fig. 1 Suggested cladogram for the class Aves. A, Archaeornithes; E, Enantiornithes; O, Odontornithes; N, Neornithes.

Table 1 List of the synapomorphies shared by the Enantiornithes and all other post-Jurassic birds, in which they differ from the corresponding character-states of Archaeopteryx

			•
	Character	Archaeornithes	Enantiornithes, Odontornithes and Neornithes
1	Vertebral centra	Not saddle-shaped	Saddle-shaped (? cervicals only in Enantiornithes)
2	Number of sacral vertebrae	Probably 6	At least 9
3	Caudal vertebrae	Not reduced	Reduced to a pygostyle (indication only in Enanti- ornithes)
4	Sternum	Not developed	Well-developed, with keel
5	Abdominal ribs	Present	Absent
6	Coracoid	Lunate	
7	Proximal carpals	Radiale and ulnare of simple shape	Elongate, spatulate at sternal end
8	Distal carpals	Lunate bone only	Scapholunar and cuneiform of complex shape Trochlea carpalis and extensor process fused together to form part of carpometacarpus, with
9	Metacarpals	Unfused II-IV (I-III)	pollical facet and well-developed metacarpal facet Fused
10	Ilium and ischium	Not fused distally	
11	Degree of fusion of astragalus and calcaneum to tibia	Nil or only partial (ref. 5)	Usually fused to enclose ilio-ischiadic fenestra Complete

Table 2 List of the synapomorphies shared by the Odontornithes and the Neornithes, in which they differ from the corresponding character-states of the Enantiornithes

	Character	Enantiornithes	Odontornithes and Neornithes
1	Saddle-shaped vertebral centra	Probably in cervicals only	In most cases, in cervicals and dorsals
2	Procoracoid	Absent	Present
3	Scapular articulation on coracoid	Boss	Facet
4	Coracoid articulation on scapula	Facet	Boss
5	Head of humerus	Flattened profile	Rounded profile, no flattening
6	Palmar surface of humerus:	F	Rounded prome, no nattening
	a, depression below head	Well marked	Absent
	b, brachial depression above	Absent	Well developed, surrounded by scar of brachialis
	distal end		anticus muscle
7	Internal condyle of humerus	Poorly developed	Well developed
8	External condyle of humerus	Transversely orientated	Less so
9	Femur	Relatively long and slender,	Much shorter than tibia, with well-marked
		without fibular groove	fibular groove
10	External cotyla of ulna	More pronounced	Less pronounced
11	Cnemial crests of tibiotarsus	Without flanges	With highly developed flanges
12	Tendinal groove of tibiotarsus	Absent	Present
13	Distal articulation of tibiotarsus:		riescht
	a, internal condyle	Bulbous	Less bulbous
	b, intercondylar fossa	Narrow	Wide
14	Fusion of tarsometatarsus	Only partial	Complete
			Complete

The characters of the new species *Enantiornis leali*, listed in 2-6a, are based on an associated coracoid, scapula and humerus (no. 4035). The species has been named after J. C. Leal, who discovered the locality and helped in the collection of material.

distal to it on the palmar side (Fig. 2a, A). At the distal end of the humerus the internal and external condyles (articulations for the radius and ulna respectively) are not well formed, the external condule being oriented more transversely (Fig. 2a, A) as in Alexornis antecedens⁴ from the Campanian (Upper Cretaceous) of Mexico. Furthermore, there is no indication of either a brachial depression or a scar for the brachialis anticus muscle. The most remarkable feature of the humeri is the variation in the area of the internal tuberosity, where four well preserved specimens show three distinct types. One has the large pneumatic foramen typical of many birds, another seems to have no such structure, while the third has what could be regarded as a pneumatic fossa with no foramen but with a canal running proximo-distally through the internal tuberosity. This third condition seems to be unique as it is unknown in any other bird or reptile. The remaining forelimb elements, although possessing unique features, are typically avian, especially metacarpals II-IV which are fused into a carpometacarpus (Fig. 2a, F). Very little can be said about the sternal fragment except that it has a keel and a large sternal notch.

The two preserved pelves are incomplete, but indicate an opisthopubic condition (Fig. 2b, C). This configuration,

generally believed to be confined to birds and ornithischian dinosaurs, has now been noted also in certain advanced coelurosaurs of the family Dromaeosauridae which, like the Enantiornithes, are of Maestrichian age. However, the large ilio-ischiadic fenestra is found only in birds. Furthermore, the smaller specimen has a synsacrum of at least nine vertebrae, a higher number than that found in coelurosaurs, and the last vertebral centrum is so tapered as to give a clear indication of a pygostyle.

The unusual morphology of the hindlimb elements might raise doubts about an avian identification. The femur (Fig. 2b, A), for example, could be said to show affinities with that of a very small theropod dinosaur in being long and slender, but in detail it seems to resemble more closely that of Archaeopteryx. The tibiotarsus (Fig. 2b, B) is very bird-like in having the astragalus and calcaneum fused to its distal end; the same is true of certain coelurosaurs, but the latter, unlike the Enantiornithes and all modern birds, still show marked suture lines between the bones involved. The distal articulation of the tibiotarsus is itself unique in that the internal condyle is larger and bulbous and the intercondylar fossa much narrower than in any modern bird.

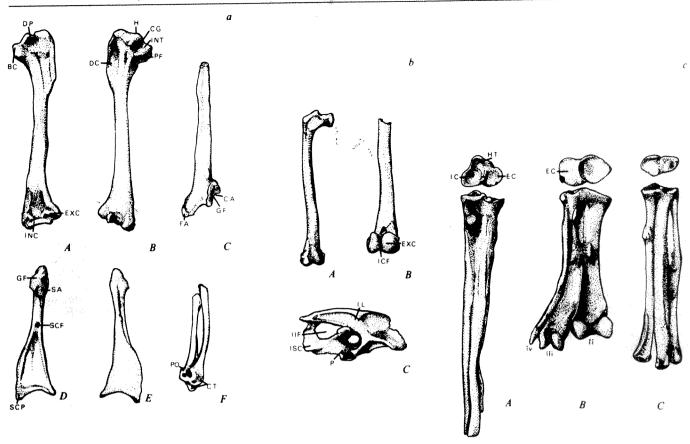


Fig. 2 a, New subclass, Enantiornithes: selected elements of pectoral girdle and forelimb. A, left humerus, palmar view; B, same, anconal view (4054); C, left scapula, external view (4039); D, left coracoid, internal view; E, same, external view (4035); F, left carpometacarpus, internal view (4049). F, ×0.6, all others ×0.3. BC, bicipital crest; CA, coracoidal articulation; CG, capital groove; CT, carpal trochlea; DC, deltoid crest; DP, depression; EXC, external condyle; FA, furcular articulation; GF, glenoid facet; H, head; INC, internal condyle; INT, internal tuberosity; PF, pneumatic fossa; PO, pollical facet; SA, scapular articulation; SCF, supra-coracoidal foramen; SCP, sterno-coracoidal process. b, Selected elements of pelvic girdle and hindlimb. A, right femur, anterior view (4037); B, distal end of right tibiotarsus, anterior view (4033); C, right side of pelvis, external view (4042). All ×0.3. EXC, external condyle; ICF, intercondylar fossa; IIF, ilio-ischiadic fenestra; IL, ilium; ISC, ischium; P, pubis. c, Three different tarsometatarsi. A, 4021 from right side (×0.5); B, 4053 from left; C, 4048 from ?right (both ×0.64). EC, external cotyla; IC, internal cotyla; HT, hypotarsus.

Three different types of tarsometatarsus are represented. The first (Fig. 2c, A), associated with a tibiotarsus, is the largest and possesses a 'primitive' avian hypotarsus (that is, one that is rectangular in proximal view). The second (Fig. 2c, B) is short and broad and lacks a hypotarsus; metatarsal II is more or less straight instead of having its distal end curving medially (the condition in most modern birds), while metatarsals III and IV are bent in the middle so that their distal ends are directed laterally. The third (Fig. 2c, C) also lacks the hypotarsus and is about the same length as the second but rather narrower, with the trochlea in a normal position; the general appearance of this third type-is distinctly coelurosaurian, except that the terminal phalanx associated with it lacks the bifurcated groove found in most-but not all-coelurosaurs (H.-D. Sues, personal communication). The most unusual feature of the first and second types of tarsometatarsus is the reduction of metatarsal IV to a lateral splint, which, although it may well have still had a toe, is attached firmly to its neighbour only proximally. The third type is somewhat different in that metatarsal IV is still substantial and attached to metatarsal III along almost its entire length. In all cases the metatarsals are not fused together completely, the sutures still being visible along much of their shafts; they are similar in this respect to Archaeopteryx and young modern birds.

It is too early to speculate as to the mode of life of these birds, but the largest forelimb elements indicate an animal with a wingspan of at least 1 m. The flying capabilities were probably limited or even non-existent as the sternum, although keeled, seems to have had a large sternal notch, and the distal articulation of the humerus is only weakly developed. The latter character and those of other forelimb elements suggest that this group of birds may have developed a form of flight, or some other method of propulsion, which differed from that of the Odontornithes (that is, Ichthyornis) and modern birds. This view is strengthened by the unique configuration of the scapulacoracoid articulation (Fig. 2a, C) and of the two ends of the humerus (Fig. 2a, A and B).

Of the hindlimb elements, only the tarsometatarsi allow one to speculate as to the possible habits of these birds. The length and narrowness of the first type (Fig. 2c, A) suggest either a wading or cursorial habit, whereas the extreme breadth and flattening of the second (Fig. 2c, B) suggest modifications for either propulsion through water and/or specialized support on land (as in the penguin). The third, more evenly broad type (Fig. 2c, C) appears to be analogous to the feet of Recent penguins; the strongly curved terminal phalanx associated with it could have been used for scrambling.

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- Brodkorb, P. Bull. Fla St. Mus. biol. Sci. 7, 180 (1963).
 Bonaparte, J. F. & Powell, J. E. Mém. Soc. géol. Fr. 139, 19-28 (1980).
 Ostrom, J. H. Smithsonian Contr. Palaeobiol. 27, 1-21 (1976).
 Brodkorb, P. Smithsonian Contr. Palaeobiol. 27, 67-73 (1976).
- Tarsitano, S. & Hecht, M. K. Zool. J. Linn. Soc. 69, 149-182 (1980).

Competition relatedness and efficiency

J. R. W. Harris

Natural Environment Research Council, Institute for Marine Environment Research, Prospect Place, The Hoe, Plymouth PL1 3DH, UK

Seger¹ has argued that, if a law of diminishing returns of personal fitness² with increasing consumption of a limiting resource applies, a greater increment to inclusive fitness³ may accrue to an individual by sharing the resource with its relatives than by excluding them. That is, from the point of view of an individual's inclusive fitness, there will exist an optimal relation between resource abundance, conversion efficiency (in terms of increment in personal fitness per resource unit consumed) and competitor abundance and relatedness to the subject. Here, this is rendered more concrete by deriving expressions for the optimum consumption rate for any one of a number of related individuals competing for a finite resource.

Let the selective universe consist of N individuals, among which the relatedness⁴ of the ith to the ith is z_{ij} . Let a total consumption x of a particular resource by the ith individual result in a total net reproduction for that individual of $g_i(x)$. Then, if this individual consumes an amount p_i , let the inclusive net reproduction, r_y^* , of the yth individual be defined by

$$r_y^* = \sum_{i=1}^N z_{iy} g_i(p_i)$$
 (1)

(Note that the use of 'inclusive' here does not correspond exactly to its use in the original definition of 'inclusive fitness' by Hamilton³. It avoids the necessity for a distinction between social and other effects and apparently corresponds to his earlier, heuristic usage⁵.) Differentiating and setting the derivative equal to zero yields a turning point with respect to p, when

$$g_{y}(p_{y}) = -\sum_{\substack{i=1\\i\neq y}}^{N} x_{iy}g_{i}(p_{i}) dp_{i}/dp_{y}$$
 (2)

Let the total amount of resource available to, and divided between, the N individuals be fixed, so that the p_i values are linearly dependent,

$$\sum_{i=1}^{N} dp_i/dp_y = 0, \text{ that is, } \sum_{\substack{i=1\\i\neq y}}^{N} dp_i/dp_y = -1.$$

If the individuals are ordered so that a number n, less than N, can be defined such that $dp_i/dp_y = 0$ if and only if i > n, this can replace N both in these summations and in equation (2) without loss of generality. Now, defining s_{iy} as $z_{iy}g_i'(p_i)$ and \bar{s}_{iy} as

$$\sum_{\substack{i=1\\i\neq i}}^{n} s_{iy}/(n-1)$$

equation (2) may be expanded to give

$$g'_{y}(p_{y}) = \bar{s}_{y} + \sum_{\substack{i=1\\i \neq y}}^{n} (\bar{s}_{y} - s_{iy}) dp_{i}/dp_{y}$$
 (3)

If $g_i''(p_i) < 0$ (that is, $g_i(x)$ is locally a law of diminishing returns) for $0 < i \le n$, then it is sufficient that $\mathrm{d}^2 p_i / \mathrm{d} p_y^2$ and s_{iy} are not negatively correlated for this to define a maximum. As long as $N \gg n$, so that $\sum_{i=1}^N g_i(p_i)$ is effectively independent of $g_y(p_y)$, this also defines a maximum in $r_y^* / \sum_{i=1}^N g_i(p_i)$, the proportion of the gene pool of the next generation formed by genes identical by descent to those of the yth individual.

Assumption of a particular form for $g_i(p_i)$ can yield an intuitively clearer relation. Thus, a reasonable and flexible assumption is that total net reproduction is a Michaelis-Menten function of consumption minus some maintenance factor (γ_i)

$$g_i(x) = \alpha_i \beta_i(x - \gamma_i) / (\alpha_i(x - \gamma_i) + \beta_i) : x > \gamma_i$$

$$g_i(x) = 0$$
; otherwise

so that

$$g_i'(x) = g_i^2(x)/\alpha_i(x - \gamma_i)^2$$
 (5)

In these equations, α_i , β_i and γ_i are positive constants. If $E_i(x)$ is defined by

$$E_i(x) = g_i(x)/(x - \gamma_i) \tag{6}$$

it may be conceived, in terms of Wiegert⁶, as a net efficiency of net reproduction per unit of consumption. If it is assumed that $p_i \ge \gamma_i$ for all i, that is, no individuals exist on a quota below maintenance, equation (5) may be substituted in equation (3). If, in addition, α_i , the maximum net efficiency, is constant over all individuals, the result may be expressed in terms of E_i

$$E_{y}^{2}(p_{y}) = \bar{c}_{y} + \sum_{\substack{i=1\\i\neq y}}^{n} (\bar{c}_{y} - c_{iy}) dp_{i}/dp_{y}$$
 (7)

where

$$c_{iy} = z_{iy} E_i^2(p_i)$$
 and $\bar{c}_y = \sum_{\substack{i=1 \ i \neq y}}^n \frac{c_{iy}}{(n-1)}$

If dp_i/dp_y and c_{iy} are uncorrelated, the second right-hand term of equation (7) disappears

$$E_{y}^{2}(p_{y}) = \sum_{\substack{i=1\\i\neq y}}^{n} z_{iy} \frac{E_{i}^{2}(p_{i})}{(n-1)}$$
 (8)

In the special case in which, in addition, $z_{iy} = z$ for $i \le n$ and $i \ne y$

$$E_{y}^{2}(p_{y}) = z \sum_{\substack{i=1\\i \neq y\\i \neq y}}^{n} \frac{E_{i}^{2}(p_{i})}{(n-1)}$$
(9)

That is, if an isolated population of equally related individuals can be defined, and success is to be judged in terms of some infinite, external gene pool, each should strive to raise its consumption until the ratio of its squared net efficiency to the mean square net efficiency of its competing relatives is equal to their relatedness.

In many cases, such a simple view would be inappropriate; correlations between dp_i/dp_y and c_{iy} (or s_{iy}) may be expected to be the rule. A positive correlation implies that the subject's consumption reduces most the consumption of competitors whose contribution to its inclusive fitness is thereby reduced least. As $g_{\nu}(x)$ is a law of diminishing returns, this will tend to increase optimum consumption and, under equation (4), to reduce optimum efficiency. Such an effect might be produced by a social system such as a pecking order in which the lowest members (least productive) are most affected by vagaries in consumption by those higher in the order. Hence, other things being equal, such a structure would be expected to be associated with lower population efficiencies. A spatial effect would be a common source of the converse, negative correlation, tending to raise optimum efficiency. For many organisms, consumption will tend to decline away from some 'centre of activity'. Competitor relatedness may be expected to decline similarly'; hence, the subject's consumption affects most that of those whose contribution to its inclusive fitness is most sensitive.

The negative relation of $g'_{y}(x)$ to $g_{y}(x)$ facilitates qualitative understanding of the implications of equation (3). For example,

it may be recognized that increased levels of relatedness between the individuals of a population will tend to reduce their optimal consumption, increasing their optimal net efficiency (the extent to which any one achieves this optimum depends, of course, on its competitors). Similarly, it may be seen that a reduction in the general level of individual consumption itself reduces the optimum level for any one. This immediately implies that it is adaptive for consumption to decline with increasing density of related competitors. Under equation (10), subsuming equation (5), the decline would be hyperbolic. Declines of this or a similar form have been reported for some predators and, equating hosts with the resource and oviposition with potential consumption, they are well known for insect parasitoids.

Parasitoids have also been found to emigrate in response to the presence of conspecifics⁸; as well as acting as a constraint on optimal consumption, the necessary relation of competitor numbers to individual consumption which is imposed by an overall resource limit implies that these may themselves be optimized. Thus, in the situation to which equation (9) applies, if it is further assumed that p_i is a constant, p_c , and that $g_i(x)$ is the same for all i < n $(i \neq y)$, then, from the point of view of any individual, there is an optimum density of competing relatives when $g'(p_c) = g(p_c)/p_c$. Where $g(p_c)/p_c$ may be interpreted as the gross efficiency of net reproduction per unit of consumption⁸. As $p_c \ge 0$ and $g''(p_c) < 0$ the implication is that this is maximized. Territoriality might be interpreted as the result of selection under some such relation for a population density which would be optimal for all competing individuals, although within which each will compete to increase its resource share in the manner indicated by equation (9).

Although a decline in the net efficiency of growth or reproduction at high consumption rates occurs widely 9-16, there are exceptions 17. These might indicate species for which, in response to the kind of coincidence of interests just outlined, it is the density of related competitors rather than their individual quotas which tends to vary with resource abundance, and for which there is an (evolutionary) expectation that a superfluity of resource will be utilized by an influx of related individuals, that is, inbreeding species exploiting idiosyncratic resources. In these cases, selection might act to produce an ingestive ceiling sufficiently low to veil the putative decline in efficiency.

It is the essence of these arguments that whereas in heavily outbreeding species selection will tend to maximize personal fitness, in more inbreeding ones personal fitness will tend to be sacrificed for increased 'population fitness'. It has been argued that species so adapted can only succeed if they have a fugitive way of life¹⁸. So, if the demands of inter- and intraspecies selection are to be reconciled, an association between this mode of existence and relative inbreeding would be expected; such an association seems to be found^{19,20}. In view of this, the relations derived here imply in addition that fugitive species will tend to exhibit higher net efficiencies of net reproduction per unit of resource depletion.

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    Seger, J. Nature 262, 578-580 (1976).
    Orlove, M. J. J. theor. Biol. 81, 577-586 (1979).
    Hamilton, W. D. J. theor. Biol. 81, 577-586 (1964).
    Michod, R. E. & Hamilton, W. D. Nature 288, 694-697 (1980).
    Hamilton, W. D. Am. Nat. 97, 354-356 (1963).
    Wiegert, R. G. Ecol. Monogr. 34, 217-241 (1964).
    Melecot, G. Les Mathematiques de l'Heredite (Masson et Cic, Paris, 1948).
    Hassell, M. P., Lawton, J. H. & Beddington, J. R. J. Anim. Ecol. 45, 135-164 (1976).
    Laybourn, J. E. M. & Stewart, J. M. J. Zool. Lond. 174, 277-283 (1974).
    Armstrong, J. T. Ecology 45, 361-365 (1964).
    Starkweather, P. L., Gilbert, J. J. & Frost, T. M. Oecologia 44, 26-30 (1979).
    Widdows, J. J. mar. Biol. Ass. U.K. 58, 109-124 (1978).
    Checkley, D. M. Limnol. Oceanogr. 25, 430-446 (1980).
    Griffiths, D. J. Anim. Ecol. 49, 99-125 (1980).
    Usher, M. B., Davis, P. R., Harris, J. R. W. & Longstaff, B. C. in Population Dynamics (eds Anderson, R. M., Turner, B. D. & Taylor, L. R.) 359-384 (Blackwell, Oxford, 1979).
    Paloheimo, J. E. & Dickie, L. M. J. Fish. Res. Bd Can. 23, 1209-1248 (1966).
    Lawton, J. H., Hassell, M. P. & Beddington, J. R. Nature 255, 60-62 (1975).
    Maynard Smith, J. Nature 201, 1145-1147 (1964).
    Levin, D. A. Am. Nat. 109, 437-451 (1975).
    Gelesener, R. R. & Tilman, D. Am. Nat. 112, 659-673 (1978).
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A potent cyclic hexapeptide analogue of somatostatin

Daniel F. Veber*, Roger M. Freidinger*,
Debra Schwenk Perlow*, William J. Paleveda Jr*,
Frederick W. Holly*, Robert G. Strachan*,
Ruth F. Nutt*, Byron H. Arison†, Carl Homnick*,
William C. Randall*, Monroe S. Glitzer†,
Richard Saperstein† & Ralph Hirschmann*†

Merck Sharp and Dohme Research Laboratories, *West Point, Pennsylvania 19486 and †Rahway, New Jersey 07065, USA

Conformational analysis has resulted in the design and synthesis of somatostatin analogues which show increased duration of action1-3. The introduction of covalent conformational constraints and elimination of amino acids that are not required led to the synthesis of the highly active bicyclic analogue I, cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)2.3, which showed potency equal to or greater than that of somatostatin for the inhibition of growth hormone release in vitro and in vivo, as well as for the inhibition of glucagon and insulin release in vivo (Aha, 7-aminoheptanoic acid). These results suggested that the amino acids -Phe-D-Trp-Lys-Thr- of this analogue and the corresponding residues 7-10 of somatostatin contain all the elements necessary for the expression of the above biological activities, and that the fragment -Cys-Aha-Cys- serves as a conformational constraint, allowing the tetrapeptide to attain a bioactive conformation. It was concluded that such constraint also results in the observed reduced susceptibility to metabolism by peptidases such as trypsin and has permitted both a long duration of action and oral activity2,3. If the sole purpose of the -Cys-Aha-Cys- sequence is indeed only conformational constraint, then it should be possible to design alternative, simpler constraining moieties. We have used a computer modelling and graphics system⁴ to examine possible alternative molecular fragments and report here the synthesis of a highly active cyclic hexapeptide analogue of somatostatin.

Proton NMR studies have resulted in a precisely defined model for the solution conformation of I (refs 2, 5). Because of the diminished molecular flexibility of this molecule, it has been proposed that this model may also be close to the 'bioactive conformation' (ref. 2). Using these defined coordinates and the Merck computer modelling system, analogue I was divided at the bonds indicated in Fig. 1, eliminating the -Cys-Aha-Cys-portion and leaving the tetrapeptide fragment in the 'bioactive conformation'. Various bridging units were examined as possible choices to tie together the free ends of the tetrapeptide unit. Visualization of this process was aided by the use of a structure superposition program in the Merck Molecular Modelling

Fig. 1 Cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys) I, an analogue of somatostatin which shows high biological potency. For computer studies of alternate bridging groups, the molecule was separated at the bonds marked by the arrows.

Table 1 Characterization of somatostatin analogues (cyclo(A-B-Phe-D-Trp-Lys-Thr))

	Co	mpound			Amino	acid anal	ysis					Inhibition of growth
	A	В	Α	В	Phe	Trp	Lys	Thr	$[\alpha]_{\mathrm{D}}^{22}$	Purity HPLC	δ Lys γ-CH ₂	hormone release (relative potency)
1	Ala	Pro	1.00	1.02	1.03	0.98	1.04	0.92	-107.0	98	0.39, 0.55	0.06 (0.03, 0.12)
2	Pro	Pro		2.02	0.99	0.95	0.99	0.99	-171.6	97	0.33, 0.53	0.008 (0.003, 0.12)
3	D-Ala	D-Pro	0.99	0.99	0.99		1.00	1.02		100	0.2-0.7*	0.006 (0.003, 0.02)
4	D-Ala	Pro	0.99	1.05	1.00	0.98†	0.97	0.99	-61.8	93	0.30, 0.45	<0.002
5	Pro	Ala	1.02	1.00	0.98	0.92†	1.00	1.02	-83.9	97	0.23, 0.45	<0.002
6	Pro	D-Ala	1.03	1.02	1.00	1.06†	1.03	0.96	-12.6	96	0.43, 0.60	<0.002
7	Aib	Pro	1.04	1.01	0.99	0.85†	1.00	0.98	-25.5	88	0.20, 0.43	<0.002
8	Phe	Pro		0.97	2.04	0.97	0.99	1.03	-23.6	98	0.40, 0.55	
9	Phe	Phe		*****	2.98	****	0.99	1.04	-56.9	93	0.23, 0.44	1.74 (1.31, 2.32)
10	Phe	D-Phe	Interior		3.04	0.93	0.99	1.03	-21.5	95	0.59, 0.68	0.27 (0.22, 0.33)
11	D-Phe	Pro	-	1.00	1.99	0.85	1.00	0.99	-80.1±	96	0.39, 0.68	0.22 (0.20, 0.25)
12	Pro	D-Phe	0.97		2.01	0.84†	0.99	1.02	-27.4	99	,	<0.002
13	D-Phe	D-Pro		1.05	1.96	0.87	0.98	1.02	-17.7		0.45, 0.61	0.03 (0.03, 0.03)
I	Cys Aha	-vananay					0.96	1.01	-11.1	93	0.56, 0.71	<0.002
	- 7 - 1 - 1 - 1	- <i>J</i> -	****	···					******		0.32, 0.48	$1.24 (0.81, 1.88)^3$

For optical rotation, c = 1 in methanol. Reverse phase chromatography was used to estimate purity from the area under the curve of the UV trace at either 210 or 280 nm. Amino acids were analysed after hydrolysis for 20 h in 6 M HCl at 110 °C. Inhibition of spontaneous growth hormone release was evaluated by incubation of isolated pituocytes with somatostatin or analogue at graded doses (at least 6 doses per analogue) ranging from 10⁻¹⁰-10⁻⁵ M. Three replicate plates were used at each dose level. After 4 h incubation, growth hormone levels released into the medium were determined by a double antibody radioimmunoassay for rat growth hormone. Potency of the analogues relative to somatostatin (=1) were calculated using a relative potency formula for parallel line bioassays 12 . 95% confidence limits are given in parentheses. This bioassay is essentially as has been described elsewhere 13 . The ED₅₀ for somatostatin in this test is 5×10^{-8} M.

System called 'COMPARE' (ref. 4; Fig. 2). Among the molecular fragments examined for completion of the ring were acetyl dipeptide methylamides in the conformations defined for the various types of β -turn⁶. The resulting structure is a cyclic hexapeptide having two β -turns, a common structural occurrence as indicated by X-ray studies of cyclic hexapeptides 7.8. A least-squares fit of the terminal overlapping atoms (two atoms at one end, three at the other) was carried out without allowing any bond rotations. Fits ranging in average deviation from 0.38 to 0.61 Å were obtained for the six β -turns of types I-III and I'-III'. Better matches were attained (deviation as low as 0.10-0.31 Å) by allowing some rotation about the bond angles

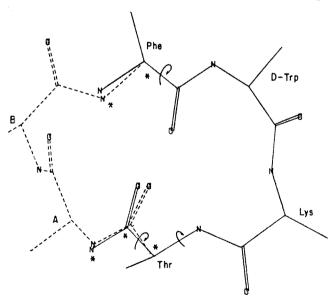


Fig. 2 Computer matching of the termini of a tetrapeptide unit and an acetyl dipeptide-N-methylamide unit having the conformation of a type 3 β -turn. The tetrapeptide unit has the conformation suggested for the -Phe-D-Trp-Lys-Thr- portion of the proposed bioactive conformation of somatostatin analogues² A least-squares fit of the atoms marked by an asterisk was carried out while simultaneously allowing rotation about the bonds marked by arrows. The average deviation of the matched atoms in this case was 0.20 Å.

indicated in Fig. 2, two of which were not defined in the study of the solution conformation of I (ref. 2).

A reasonable match in some cases led us to prepare a number of cyclic hexapeptides having the general structure cyclo(A-B-Phe-D-Trp-Lys-Thr) where A and B were chosen to fit a variety of β -turns either because they have been observed to be part of a β -turn in some X-ray study or because we thought they would be compatible with one or more of the proposed turn types. Alanine, rather than amino acids with more complex side chains, was used in combination with proline in our early analogues, on the assumption that the sole purpose of the A-B unit is conformational constraint.

The cyclic hexapeptides were prepared as outlined below:

$$\begin{array}{c} \text{H-D-Trp-Lys(2ClZ)-Thr(Bzl)-Phe-Pro-Phe-O} \\ \\ \downarrow \text{NH}_2\text{NH}_2 \\ \text{H-D-Trp-Lys(2ClZ)-Thr(Bzl)-Phe-Pro-Phe-NHNH}_2 \\ \\ \downarrow \text{(1)} \text{ i-amyl nitrite} \\ \text{(2)} \text{ $dilute 1 mg ml}^{-1} \\ \text{(3)} \text{ base} \\ \downarrow \text{(4)} \text{ HF} \\ \\ \text{cyclo(D-Trp-Lys-Thr-Phe-Pro-Phe)} \end{array}$$

The linear peptides were prepared by the solid phase method on a Beckman 990 peptide synthesizer using a protocol described elsewhere9. The cyclization procedure also follows basically the procedure described for other cyclic peptide analogues of somatostatin1. The analogues were characterized as shown in Table 1. In addition to a consistent amino acid analysis, each compound showed an appropriate NMR spectrum. The position of the γ-CH₂ of lysine is given for each compound (Table 1) as the unusual upfield shift is indicative of a proximity of the lysine and tryptophan side chains, a feature which has been postulated as important for biological activity⁵. Proximity of the two side chains was achieved in all the cyclic hexapeptides which suggests a similar conformation in this region of the various peptides.

Each analogue was evaluated for inhibition of the release of insulin, glucagon and growth hormone1. The results for inhibition of growth hormone release in vitro are given in Table 1. (For most of the low-activity analogues, all other bioassays showed no activity at the doses tested.) Table 1 shows that cyclic

^{*} Two conformers.

[†] By UV.

 $[\]ddagger c = 2.$

hexapeptides 1-3 have real activity but only low potency. However, even the most active analogue of this group (1) shows only 6% the potency of somatostatin. We considered that this low potency was due to elimination of an important contributor to receptor binding rather than a failure to attain the correct conformation for the bioactive tetrapeptide fragment. The most reasonable possibility was that we had eliminated a hydrophobic binding element. We therefore prepared several analogues incorporating phenylalanine rather than alanine in the A-B dipeptide unit (compounds 8-13 of Table 1) including an analogue (9) which constitutes cyclization of the sequence 6-11 of [D-Trp8] somatostatin. Most striking is the inhibition of growth hormone release in vitro by analogue 8 compared with that of analogue 1 (Table 1); the former is almost twice as potent as somatostatin and 29 times as potent as 1 on a molar basis. This in vitro test comes closest to measuring binding affinity as enzymatic inactivation is likely to be minimized. The fact that circular dichroism spectra of 1 and 8 in the region 190-250 (Fig. 3) are very similar indicates that these two molecules have a similar peptide backbone conformation and suggests that the higher activity of 8 results from interaction of the additional benzene ring with receptors.

The PMR spectrum of 8 shows features indicative of a high degree of molecular rigidity in the backbone and side chains. A solution conformation has been derived which will be reported elsewhere. The molecular rigidity seems to have lowered the susceptibility of this molecule to the action of trypsin, as was observed for I. Analogue 8 is cleaved by trypsin only slowly, at about the same rate as that of I in identical conditions³. The special importance of proline to the high potency of the cyclic peptide 8 is seen by comparison with 9. The increased potency of 8 seems to be due to the presence of the N-substituted cyclic amino acid not present in 9, probably through an influence on the conformation of the cyclic hexapeptide. The altered circular dichroism of 9 in the region of amide bond absorbance (200–250 nm) supports this conclusion (see Fig. 3).

The *in vivo* potency (by weight) of 8, relative to somatostatin, for inhibition of growth hormone release in rats stimulated by pentobarbital is $\sim 20-25$ (see Table 2 legend for method). (Variability in this test precludes evaluation of 95% confidence limits.) Potency (by weight), relative to somatostatin, for inhibition of insulin release is 5.2 (2.4, 11), and for inhibition of glucagon release is 8.0 (1.4, 60.2) as measured in the urethane-anaesthetized rat. (Inhibition of insulin and glucagon release was measured, relative to somatostatin (=1.0), by the ability to decrease the blood levels of glucagon and insulin in urethane-anaesthetized rats as described elsewhere 1. Saline or peptide

P < 0.05; † P < 0.001; ‡ P < 0.01; § P < 0.02.

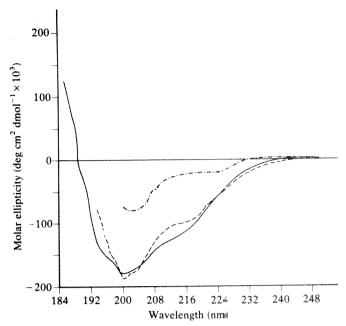


Fig. 3 Circular dichroism spectra of cyclo(Phe-Pro-Phe-D-Trp-Lys-Thr) 8 (——); cyclo(Ala-Pro-Phe-D-Trp-Lys-Thr) 1 (---); and cyclo(Phe-Phe-D-Trp-Lys-Thr) (-·-·-). All spectra taken in phosphate buffer pH 7.4.

were given by the external jugular vein and blood samples were taken 5 min later via the portal vein. Insulin and glucagon levels in the plasma were measured by radioimmunoassay.)

The duration of action of 8 is much longer than that of somatostatin and is comparable with that of the bicyclic peptide I, as shown by the inhibition of stimulated growth hormone release 5 h after subcutaneous (s.c.) injection (Table 2). We have shown that somatostatin does not reduce growth hormone levels 75 min after a 250 μ g per kg s.c. dose in this same protocol, whereas I causes significant reductions 135 min after injection of 125 μ g per kg (ref. 3). In our study, 8 has a duration comparable with that of I when evaluated simultaneously (Table 2). Both I (500 μ g per kg) and 8 (750 μ g per kg) show a statistically significant reduction in growth hormone levels 5 h after s.c. administration; 8 is also effective in inhibiting the release of growth hormone 1 and 3 h after oral administration (25 mg per kg) in this same test system (Table 2). There was no suppression

Table 2 Inhibition of growth hormone release by somatostatin analogues in vivo

Compound	Dose (µg per kg)	Route of administration	Growth hormone (ng mI ⁻¹)	Time (h)
None	· ··········	s.c.	353 ± 130	5
I Cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)	125	s.c.	534 ± 300	5
I Cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)	250	s.c.	221 ± 123	5
I Cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)	500	s.c.	$31 \pm 11^*$	5
8 Cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	250	s.c.	207 ± 49	5
8 Cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	500	s.c.	470 ± 222	5
8 Cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	750	s.c.	9 ± 1 *	5
None	***************************************	p.o.	$1,064 \pm 177$	3
8 Cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	25,000	p.o.	99 ± 55†	3
None	and and	p.o.	$1,124 \pm 341$	9
8 Cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	25,000	p.o.	9 ± 4‡	1
None	-	p.o.	$1,128 \pm 338$	1
I Cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)	25,000	p.o.	140 ± 48 §	1

Inhibition of pentobarbital-stimulated growth hormone release was measured essentially according to a procedure described elsewhere ¹. Male Sprague-Dawley rats (190-200 g) were given saline or compound either subcutaneously (s.c.) or orally (p.o.). After the designated time period, sodium pentobarbital was given i.v. and 15 min later the rats were bled from the orbital sinus and the plasma assayed for growth hormone content by radioimmunoassay. The data are expressed as the mean ±s.e.m. (6 rats per group). The control is given for each experimental run.

when somatostatin was given orally at this dose, even after a shorter time interval (1 h), whereas the bicyclic analogue I showed statistically significant suppression of growth hormone release 1 h after oral administration at this dose, but not after

Thus, a highly active and long-acting cyclic hexapeptide analogue of somatostatin has been designed by replacing 9 of the 14 amino acids of somatostatin with a single proline. The long duration of action of this analogue could render this compound class valuable in tests of the potential application of somatostatin-like compounds in the treatment of juvenile diabetes 10,11

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- Veber, D. F. et al. Proc. natn. Acad. Sci. U.S.A. 75, 2636-2640 (1978).
- Veber, D. F. in Peptides: Proc. 6th Am. Peptide Symp. (eds Gross, E. & Meienhofer, J.) 409-419 (Pierce Chemical Co., Rockford, Illinois, 1979). Veber, D. F. et al. Nature 280, 512-514 (1979).

- Veber, D. F. et al. Nature 280, 512-514 (1979).
 Gund, P., Andose, J. D., Rhodes, J. B. & Smith, G. M. Science 280, 1425-1431 (1980).
 Arison, B. H., Hirschmann, R. & Veber, D. F. Bioorg. Chem. 7, 447-451 (1978).
 Chandrasekaren, R., Lakshminarayanan, A. V., Pandya, U. V. & Ramachandran, G. N. Biochim. biophys. Acta 303, 14-27 (1973).
 Brown, J. N. & Teller, R. G. J. Am. chem. Soc. 98, 7565-7569 (1976).
- Hossain, M. B. & van der Helm, D. J. Am. chem. Soc. 100, 5191-5198 (1978).
 Strachan, R. G. et al. J. med. Chem. 22, 586-588 (1979).
 Gerich, J. E. Metabolism 27, 1283 (1978).

- Veber, D. F. & Saperstein, R. A. Rep. med. Chem. 14, 209 (1979).
 Finney, D. J. Statistical Methods in Biological Assay Ch. 4, 99-138 (Griffin, London. 1964).
 Vaie, W. & Grant, G. Meth. Enzym. 37, 5-93 (1980).

Oestradiol, sexual receptivity and cytosol progestin receptors in rat hypothalamus

Bruce Parsons, Thomas C. Rainbow, Donald W. Pfaff & Bruce S. McEwen

The Rockefeller University, New York, New York 10021, USA

Oestradiol and progesterone regulate feminine reproductive behaviour in rodents. Their administration induces changes of biosynthetic activity in the hypothalamus¹, one of which—the induction of progestin receptors—is correlated with the induction of sexual receptivity²⁻⁴, conveniently indicated (in the rat) by the lordosis reflex of the mounted female. We have attempted to define what might be considered as a minimal regime of oestradiol administration—one which is just 'sufficient' for activating the lordosis reflex—and have used this paradigm to measure associated changes in nuclear oestrogen (NOER) and cytosol progestin (CPRs) receptors in the mediobasal hypothalamus-preoptic area (MBH-POA) of the rat brain. We find that two 1-h exposures to oestradiol are sufficient to induce the lordosis reflex and to increase significantly CPRs in the MBH-POA. An essentially discontinuous pattern of NOERs in MBH-POA can promote these changes.

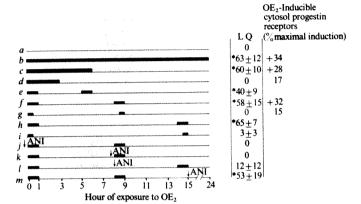
Female rats ovariectomized for 5-7 days were fitted with 5-mm silastic capsules containing oestradiol, which produced physiological levels of the hormone in serum⁴. For behaviour, each female was assigned to a separate mating group of 5-10 animals. Females received 10 mounts by male rats 24 h after initiation of oestradiol treatment. Four h before testing, each female received 500 µg P (subcutaneously (s.c.) in oil). The lordosis quotient (LQ) was calculated as: number lordoses/number of mounts × 100. Results are means ± s.e.m.

In some behavioural experiments, animals received s.c. injections of the protein synthesis inhibitor anisomycin, dissolved 20 mg ml⁻¹ in saline⁵. For all biochemical studies, four to seven samples were used for each observation, each sample consisting of MBH-POA regions from two animals. The amount of oestradiol bound to nuclear receptors in the MBH-POA was determined by using an in vitro exchange assay6. NOERs were measured 0.5, 1 and 6 h after implantation of oestradiol capsules, as well as 3 and 6 h after their removal. Results are means ±s.e.m. CPR levels in the MBH-POA were quantified using ³H-R 5020 as the radioligand for an in vitro assay⁷. CPRs were measured 24 h after insertion of oestradiol capsules, in the absence of exogenous progesterone. CPRs were measured in animals not exposed to oestradiol (controls); this binding represents a class of CPRs which is not influenced by oestradiol administration or withdrawal8. CPRs were also determined in animals which had received s.c. injections of oestradiol benzoate (15 µg daily for 3 days) to induce CPRs to maximal levels. The % maximal induction of CPRs was calculated as: (3H-R 5020 binding in experimental group) minus (3H-R 5020 binding in control group)/(3H-R 5020 binding in oestradiol benzoate) minus (3H-R 5020 binding in control group).

The effects of oestradiol treatment on sexual receptivity as measured by the LQ score indicate that a discontinuous exposure to oestradiol, in two 1-h segments, is sufficient to activate the lordosis reflex. The LQ score of animals which receive oestradiol continuously for 24 h is 63 ± 12 (Fig. 1b) and that of animals which receive oestradiol continuously for 6 h is equivalent at 24 h (Fig. 1c). However, animals which receive oestradiol continuously for 3 h are not sexually receptive at 24 h (Fig. 1d). Figure 1 (and unpublished data) demonstrates that receptivity comparable with 24 or 6 h of continuous oestradiol treatment is seen if animals receive only two 1-h segments of oestradiol, provided that the second treatment is not less than 4 nor more than 13 h after the end of the first period (Fig. 1e, f, h) ('sufficient treatment'). No receptivity is seen in animals which receive two 0.5-h segments of oestradiol (Fig. 1g, i) ('insufficient treatment').

We studied oestrogen receptor translocation following 'sufficient treatment' and found that retention of oestradiol by MBH-POA cells is transient even though this oestradiol treatment promotes delayed changes in sexual receptivity. These results may be summarized as follows: (1) The level of NOERs in MBH-POA after 1 h of oestradiol treatment resembles that seen after 6 h continuous treatment (1 h: 228±9 fmol ³Hoestradiol per mg DNA; 6 h: 197 ± 23 fmol ³H-oestradiol per mg DNA), and represents $\sim 60\%$ of the nuclear oestradiol receptor capacity⁹. (2) A second 1-h period of oestradiol treatment, beginning 7 h after the end of the first, produces NOER levels in the MBH-POA comparable with those seen after the first 1-h oestradiol period (data not shown). (3) One hour of oestradiol treatment produces at least twice the level of NOERs as does 0.5 h of oestradiol treatment $(0.5 \text{ h}: 93 \pm 11 \text{ fmol }^3\text{H}$ oestradiol per mg DNA). (4) Six hours after removal of Silastic capsules, NOER levels in the 1- and 0.5-h treatment groups decrease to ~10% and 7% of receptor capacity, respectively. Thus, an essentially discontinuous pattern of NOERs in MBH-POA is sufficient to activate the lordosis reflex at 24 h. Sufficient to activate lordosis was that NOERs in MBH-POA be elevated to 60% of their capacity, at both an early and late period, as outlined above. Six hours of continuous oestradiol treatment can be regarded as sufficient to activate lordosis because this paradigm contains two such 1-h periods.

Two classes of progestin receptors have been identified in the rat brain—one which is unaffected by oestradiol treatment, the other induced by oestradiol (ref. 8). Although the physiological significance of these two classes of progestin receptors is unclear, note that oestradiol induces progestin receptor synthesis in those areas of the brain known to mediate feminine reproductive behaviour, the MBH and POA. Moreover, a 'threshold level' (25-35% maximal induction) of oestrogen-inducible CPRs in the MBH-POA has been shown to be correlated with the appearance and disappearance of sexual receptivity4. We used several temporal paradigms of oestradiol treatment to study inducible CPRs in the MBH-POA, and found that sufficient treatment significantly elevates inducible CPRs to threshold levels in these brain regions. In animals which receive oestradiol continuously for 24 h, CPRs in MBH-POA increase from an unstimulated level of 8.1 ± 0.3 to an induced level of 12.7 ± 0.7 fmol ³H-R 5020 per mg protein. This increase of approximately 4.6 fmol ³H-R 5020 per mg protein represents a 34% maximal elevation in oestrogen-inducible CPRs, an increase to threshold levels (Fig. 1b). In animals which receive oestradiol continuously for 6 h, CPR levels in MBH-POA increase from an unstimulated level of 8.1 ± 0.3 to an induced level of 11.8 ± 1.2 fmol ³H-R 5020 per mg protein. This increase of ~3.3 fmol ³H-R 5020 per mg protein represents a 28% maximal elevation in oestradiol-inducible CPRs, also an



The effects of oestradiol (OE₂) and anisomycin (ANI) on Fig. 1 the lordosis quotient (LQ) and on the induction of cytosol progestin receptors (CPRs) in the mediobasal hypothalamus-preoptic area (MBH-POA) of the female rat. Biochemical results are expressed as % maximal induction (see text). For behaviour, animals were tested with an experienced male 24 h after the initiation of oestradiol treatment. Four hours before testing, each animal received 500 µg progesterone (s.c. in oil). In some behavioural experiments, animals received s.c. injections of the protein synthesis inhibitor, anisomycin, dissolved 20 mg ml⁻¹ in saline. For CPR measurements, animals were killed 24 h after the insertion of Silastic capsules. No exogenous progesterone was given before death. The temporal paradigms for these experiments are described below. a, No OE2, control group; b, OE2 0-24 h; c, OE2 0-6 h; d, OE₂ 0-3 h; e, OE₂ 0-1 h and 5-6 h; f, OE₂ 0-1 h and 8-9 h; g, $OE_2 0-0.5 \text{ h}$ and 8.5-9 h; h, $OE_2 0-1 \text{ h}$ and 14-15 h; i, OE_2 0-0.5 h and 14.5-15 h; j, OE_2 0-1 h and 8-9 h, ANI at -0.25 h; k, OE₂ 0-1 h and 8-9 h, ANI at 8.75 h; l, OE₂ 0-1 h and 14-15 h, ANI at 8 h; m, OE₂ 0-1 h and 8-9 h, ANI at 15 h. We conclude that a discontinuous exposure to estradiol, in two 1-h segments, is sufficient to activate the lordosis reflex and to increase CPRs in the female rat. ANI experiments demonstrate that protein synthesis essential for the activation of the lordosis reflex occurs during the period bounded by and including these two 1-h segments of OE₂ treatment. CPR levels are (fmol ³H-R 5020 per mg protein): a, 8.1 ± 0.3 ; b, 12.7 ± 0.7 ; c, 11.8 ± 1.2 ; d, 10.3 ± 0.7 ; f, 13.3 ± 1.1 ; g, 10.0 ± 0.6 . CPR levels in animals which receive oestradiol benzoate for 3 days before death (15 µg per day) are 21.1 ± 1.2 fmol ³H-R 5020 per mg protein.

increase to threshold levels (Fig. 1c). However, animals which receive oestradiol continuously for 3 h do not show threshold levels of oestradiol-inducible CPRs at 24 h (Fig. 1d); CPR levels in MBH-POA are 10.3 ± 0.7 fmol ³H-R 5020 per mg protein. CPR elevation comparable with 24 or 6 h of continuous oestradiol treatment is seen in animals which receive only two 1-h segments of oestradiol, as outlined above (sufficient treatment). CPRs in this group increase from an unstimulated level of 8.1 ± 0.3 fmol ³H-R 5020 per mg protein to an induced level of 12.3 ± 1.1 ³H-R 5020 per mg protein. This increase of ~4.2 fmol ³H-R 5020 per mg protein represents a 32% maximal elevation in oestradiol-inducible CPRs, an increase to threshold levels (Fig. 1f). Threshold levels of oestrogen-inducible CPRs are not seen in animals which receive two 0.5-h segments of oestradiol (Fig. 1g); CPRs in these animals are 10.0 ± 0.6 fmol ³H-R 5020 per mg protein at 24 h. We conclude that a discontinuous exposure to oestradiol, in two 1-h segments, is the minimum period of oestradiol treatment used which is sufficient to elevate inducible CPRs to threshold levels in the MBH-POA of the female rat. Recent work using two widely spaced (14 h) intravenous injections of oestradiol to elevate CPRs supports the notion that a discontinuous oestradiol signal can trigger CPR formation in brain and pituitary¹⁰. These data are consistent with, but not proof of, the concept that one of the neurochemical events mediated by oestradiol which accompanies the activation of behavioural receptivity is the induction of CPRs in the MBH-POA.

We also used anisomycin to demonstrate the time course of the oestradiol-induced changes in protein synthesis which are essential for the activation of the lordosis reflex. It has been shown previously that anisomycin (100 mg per kg) substantially inhibits protein synthesis in the MBH and POA of the female rat for 4 h after administration⁵. If anisomycin (100 mg per kg) is given 15 min before the first (Fig. 1j), 15 min before the second (Fig. 1k), or between the two 1-h segments of oestradiol treatment (Fig. 1l), almost no receptivity is seen at 24 h. However, if the inhibitor is given 6 h after the second period of oestradiol, no significant decrease in LQ is observed at 24 h (Fig. 1m). These data demonstrate that at least a large portion of the protein synthesis essential for the activation of the lordosis reflex is completed within 6 h after termination of the second segment of oestradiol.

The kinetics of oestradiol stimulation of uterine development have been studied by other investigators and offer parallels to our present findings. Anderson and co-workers11 found that oestradiol is retained by uterine neclei for many hours, whereas oestriol is cleared rapidly. Harris and Gorski12 showed that although multiple injections of oestriol were as effective in stimulating DNA synthesis at 24 h as a single injection of oestradiol, not all the oestriol injections were required. They suggested that the requirement for oestrogen was not continuous throughout the first 12-15 h, but only at critical phases in the response cycle. Our results suggest that there may also be a discontinuous requirement in brain for pestradiol to activate the lordosis reflex. The appearance of sexual receptivity in the female rat may be determined not only by an initial period of oestrogen-stimulated protein synthesis, but also by a later phase which may be initiated 5-14 h after the prerequisite initial oestradiol exposure.

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^{*} LQ scores in groups a-m were compared with each other using a one-way analysis of variance. A significant treatment effect was seen (F=11.21, P<0.01). Comparisons of individual means using the Newman–Kuels tests revealed that groups b, c, e, f and h were statistically different from the control group (P<0.01).

⁺ Cytosol progestin receptor levels in groups a-d, f and g were compared with each other using a one-way analysis of variance. A significant treatment effect was seen $(F=4.91,\ P<0.05)$. Comparisons of individual means using the Newman-Kuels tests revealed that groups b, c and f were significantly different from the control group (P<0.05).

^{1.} McEwen, B. S., Davis, P. G., Parsons, B. & Pfaff, D. W. A. Rev. Neurosci. 2, 65-112 (1979).

Blaustein, J. D. & Feder, H. H. Brain Res. 177, 489-498 (1979).
 Moguilewsky, M. & Raynaud, J.-P. Endocrinology 165, 516-522 (1979)

Parsons, B., MacLusky, N. J., Krey, L. C., Pfaff, D. W. & McEwen, B. S. Endocrinology 107, 774-779 (1980).

^{5.} Rainbow, T. C., Davis, P. G. & McEwen, B. S. Brain Res. 194, 548-555 (1980).

^{6.} Roy, E. J. & McEwen, B. S. Steroids 30, 657-669 (1977).

MacLusky, N. J. & McEwen, B. S. Endocrinology 106, 192–202 (1980)
 MacLusky, N. J. & McEwen, B. S. Nature 274, 276–278 (1978).

^{8.} MacLusky, N. J. & McEwen, B. S. Nature 274, 276-278 (1978).
9. McGinnis, M. Y., Krey, L. C., MacLusky, N. J. & McEwen, B. S. Neuroendocrinology (in the

McCinnis, M. Y., Krey, L. C., MacLusky, N. J. & McEssen, B. S. Neuroendocrinology (in t press).
 Clark C. R. Marlusky, N. J. & Naftolin, F. Endocr. Soc. (abstr.) 129 (1980).

Clark, C. R., MacLusky, N. J. & Naftolin, F. Endocr...Soc. (abstr.) 129 (1980).
 Anderson, J. N., Peck, E. J. & Clark, J. H. Endocrinslogy 98, 676-684 (1975).

^{12.} Harris, J. & Gorski, J. Endocrinology 103, 240-245 ∉ 978).

Vaccination against autoimmune encephalomyelitis with T-lymphocite line cells reactive against myelin basic protein

Avraham Ben-Nun*, Hartmut Wekerle† & Irun R. Cohen*

Despite differences in initiating events and pathophysiology, the aetiological agents of all autoimmune diseases are lymphocytes specifically reactive against normal constituents of the individual. Recently we have isolated and grown as a cell line rat T lymphocytes reactive against myelin basic protein (BP)1. This T-cell line originated from rats in which we had induced experimental autoimmune encephalomyelitis (EAE) by immunizing them against BP. Inoculation of syngeneic rats with the T-cell line led to the relatively rapid onset of EAE1. We report here that attenuation of this cell line provides an agent for establishing resistance to induction of active EAE. Intravenous (i.v.) inoculation of syngeneic rats with cells of the line attenuated by treatment with irradiation or mitomycin C augmented resistance to EAE caused by an encephalitogenic challenge with BP. Thus, aetiological agents of autoimmune disease, like those of microbial disease, when suitably attenuated can be used as effective vaccines.

EAE can be induced in susceptible animals such as rats. guinea pigs, rabbits, monkeys² or man³ by injecting them with BP emulsified in an adjuvant such as complete Freund's adjuvant (CFA). In Lewis rats the disease is characterized by paralysis that is most marked in the tail and hind limbs and which starts usually ~12 days after a single injection of BP in CFA. Histologically the central nervous system shows perivascular infiltrates of mononuclear cells⁴. Unless the rats are aged or have undergone splenectomy or thymectomy⁵ they recover spontaneously from clinical paralysis after a number of days. To study the pathophysiology of EAE we have isolated and propagated in vitro a line of Lewis rat Tlymphocytes that reacts only against BP, designated Z1a (ref. 1). We found that i.v. inoculation of as few as 105 cells of the Z1a line led to the onset of paralysis in ~4 days. Inoculation of 106 or more cells produced paralysis in ~2-3 days. Most rats recovered from this form of EAE if properly nursed during their paralysis. Table 1 shows the specificity of the proliferative response of the anti-BP Z1a line compared with that of the Z1c line which had been selected for its reactivity against another antigen, the purified protein derivative (PPD) of the mycobacteria present in CFA. The cells of each line responded to its specific antigen, and were also activated by the T-cell mitogen concanavalin A (Con A). Essentially all the cells in both the Z1a and Z1c lines proved to be positive for a T-cell marker using a specific monoclonal antibody (Sera-lab, UK; clone W3/13 HLK) immunofluorescence assay6.

We investigated the effect of attenuating the Z1a line by inhibiting its cell division. Table 2 shows that i.v. injection of 1×10^7 untreated cells of the Z1a line into syngeneic Lewis rats produced EAE in 18 of 20 rats within 2-3 days. Irradiation of the cells with 1,500 rad or treatment with mitomycin C, agents that block cell division, abrogated the ability of these cells to cause EAE. None of 25 rats that received Z1a cells treated in this way developed EAE. Furthermore, inoculation of

Table 1 Anti-BP and anti-PPD T-cell lines are immunospecific

	Proliferative response (c.p.m. $\times 10^{-3} \pm s.d.$)						
T-cell line	No antigen	BP	PPD	Con A			
Anti-BP (Z1a)	1.7 ± 0.3	48.7 ± 6.1	1.9 ± 0.4	71.4 ± 9.2			
Anti-PPD (Z1c)	1.4 ± 0.7	1.2 ± 0.4	77.9 ± 10.4	82.3 ± 12.7			

The Z1a and Z1c cell lines originated from the same draining lymph node cell population obtained from female Lewis rats immunized with BP in CFA as described elsewhere 1. To develop the cell lines, Lewis rats were injected in each footpad with 0.05 ml containing BP ($25 \mu g$, extracted from guinea pig spinal cords¹⁰ emulsified in equal volumes of phosphate-buffered saline and CFA containing 4 mg ml 1 of Mycobacterium tuberculosis H₃₇Ra (Difco). On day 9, the draining lymph nodes were removed and a single-cell suspension prepared. The cells were then selected in vitro for BP or PPD by culturing them with either antigen for 72 h. The lymphoblasts that were generated were separated by a discontinuous Ficoll gradient and propagated and maintained in vitro as a cell line for several months in medium enriched with T-cell growth factor as reported elsewhere¹. The proliferative responses of the T-cell lines were tested *in vitro* as follows¹. Briefly, 2.5×10^4 cells of either Z1a or Z1c cells were cultured in quadruplicates in flat-bottom microtitre wells in 0.2 ml of Eagle's medium supplemented with 1% fresh autologous rat serum, 2-mercaptoethanol (5×10^{-5} M), L-glutamine (2×10^{-3} M) and antibiotics (streptomycin and penicillin) with added irradiated (1,500rad) normal syngeneic lymph node cells as accessory cells $(5 \times 10^6 \text{ cells ml}^{-1})$ and antigens, BP $(50 \mu \text{g ml}^{-1})$, or PPD $(25 \mu \text{g ml}^{-1})$; Staten Serum Institut) or Con A $(2.5 \mu \text{g ml}^{-1})$; Miles-Yeda, Israel). After 24 h the cultures were pulsed with ³H-thymidine $(1 \mu \text{Ci per well, specific})$ activity 10 Ci mmol⁻¹; Nuclear Research Centre, Israel) for 16 h. The cells were then collected on glass fibres using an automatic collector and thymidine incorporation measured in a liquid scintillation counter.

untreated cells of the Z1c line also failed to induce EAE. Thus, induction of EAE is a function of the specific anti-BP Z1a line, a property lost after irradiation or treatment with mitomycin C.

We then tested whether inoculation with cells incapable of inducing EAE could affect the susceptibility of rats to active induction of EAE by later challenge with BP in CFA. Table 3 shows that untreated Lewis rats were highly susceptible to induction of EAE on injection with BP in CFA; 69 of 71 rats developed disease. Intravenous inoculation of cells of the Z1c anti-PPD line, either untreated or irradiated, did not affect this susceptibility and EAE was induced in all 20 rats challenged with BP in CFA. In contrast, a single i.v. injection of 1×10^7 Z1a cells attenuated by treatment with mitomycin C or irradiation led to significantly increased resistance to induction of EAE. Only 14 of a total of 40 rats showed any signs of paralysis and the degree of the paralysis in these rats was judged to be much milder than that appearing in the other groups. Thus it seems that vaccination with attenuated autoimmune T lymphocytes

Table 2 Attenuated T lymphocytes of the Z1a anti-BP line do not produce EAE

	Inoculation of T-cell lines				
Line	Treatment	Incidence of EAE			
Anti-BP (Z1a)	Untreated Irradiated	18/20 0/15			
	Mitomycin C	0/10			
Anti-PPD (Z1c)	Untreated	0/20			

Healthy female Lewis rats (2–3 months old) were injected i.v. with 1×10^7 cells of T-lymphoblast cell lines specifically reactive against BP (Z1a) or PPD (Z1c). Before inoculating the cell lines into normal syngeneic animals, they were re-stimulated in vitro with the relevant antigen, in the presence of irradiated (1,500 rad) syngeneic accessory cells for 72 h (ref. 1). The cells, >80% lymphoblasts, were then collected and injected, either untreated or attenuated by irradiation (1,500 rad) from a ^{60}Co source, or treatment with mitomycin C (50 μg per 10^7 cells per ml; Sigma) at 37°C for 40 min. The treated cells were washed extensively before being inoculated. EAE was diagnosed clinically by overt paralysis of the hind limbs and histologically by perivascular mononuclear cell infiltration of the central nervous system 4 .

^{*}Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

[†]Max-Planck-Institut für Immunbiologie, Freiburg, FRG

Table 3 Attenuated anti-BP T-cell line vaccinates rats against induction of EAE

Vaccination with T-cell line	Incidence of EAE in response to injection of BP in CFA	% Inhibition of EAE
None	69/71	
Anti-PPD (Z1c) Untreated Irradiated	10/10 10/10	0
Anti-BP (Z1a) Irradiated Mitomycin C	8/25* 6/15*	68 60

EAE was induced in naive Lewis rats (2-3 months old) or in animals that had been vaccinated i.v. 3 weeks earlier with 107 cells of the anti-PPD Z1c T-cell line or with cells of the anti-BP Z1a T-cell line that were either irradiated (1,500 rad) or treated with mitomycin C, as described in Table 2 legend. EAE was induced by injecting BP in CFA into the hind footpads of the animals, as described in Table 1 legend. *P < 0.001.

provided protection against active EAE for about 65% of the

We do not know the mechanism by which the attenuated T lymphocytes increased resistance to induction of EAE; however, it seems reasonable to suspect that some process of immunity was involved. The Z1a anti-BP lymphocytes probably differed from the ineffective Z1c anti-PPD lymphocytes in the structure of their antigen receptors (Table 1). Antigen receptors of Tlymphocytes⁷ as well as of Blymphocytes or antibodies⁸ can be immunogenic. Immunity against antigen receptors, antiidiotypic immunity, has been proposed to serve as a mechanism that regulates immune responses by suppressing or activating specific clones of lymphocytes bearing the target receptors. Vaccination with attenuated Z1a cells might have produced an immune response against endogenous clones of lymphocytes with anti-BP receptors. As anti-BP clones are the aetiological agents of EAE, development of the disease would be inhibited. Therefore, our results could be explained by anti-receptor immunity raised against the autoimmune lymphocytes that mediate EAE. However, other explanations are possible and the anti-receptor hypothesis must be tested experimentally.

Whatever the mechanism of protection, the procedure described here can be conceptually related to vaccination against infectious diseases in which inoculation of an attenuated agent of disease induces a degree of protection against the virulent pathogen. In the case of autoimmunity, the aetiological agent of disease is not a microbe, but arises within the immune system of the individual. Our results indicate that an artificially induced autoimmune disease may be mitigated or prevented by vaccination against specific effector lymphocytes. A different but related problem is posed by the need to treat the spontaneous, often chronic processes that characterize the important autoimmune diseases of man.

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- Ben-Nun, A., Wekerle, H. & Cohen, I. R. Eur. J. Immun. 11, 195-199 (1981).
 Paterson, P. Y. in Autoimmunity, Genetic, Immunology, Virology and Clinical Aspects (ed. Talal, N.) 664-692 (Academic, New York, 1977).
- 3. Paterson, P. Y. in Immunological Disease 3rd edn (eds Samter, M. et al.) 1400-1435 (Little, Brown and Co., Boston, 1978)
- 4. Paterson, P. Y., Drobish, D. G., Hanson, M. A. & Jacobs, A. F. Int. Archs Allergy appl. Immun. 37, 26-40 (1970). Ben-Nun, A., Ron, J. & Cohen, I. R. Nature 288, 389-390 (1980).
- Ben-Nun, A., Wekerle, H. & Cohen, I. R. (in preparation). Binz, H. & Wigzell, H. J. exp. Med. 144, 1438-1457 (1976)
- Hämerling, G., Bleck, S. J., Berek, C., Eichmann, K. & Rajewski, K. J. exp. Med. 143, 861-869 (1976).
- Jerne, H. K. Anals Immun. Inst. Pasteur, Paris 125, 373-389 (1974).
 Hirshfeld, H., Teitelbaum, D., Arnon, R. & Sela, M. FEBS Lett. 7, 317-320 (1970).

C-terminal sequence of the secreted form of mouse IgD heavy chain

Renate Dildrop & Konrad Beyreuther

Institut für Genetik der Universität zu Köln, D-5000 Köln, 41, FRG

Immunoglobulins have been identified as membrane-bound molecules on the surface of B lymphocytes and as secreted products of plasma cells. In the case of immunoglobulin M(IgM) the carboxy-terminal sequences of the μ -chains of membranebound and secreted molecules differ from each other and are encoded by different exons of the μ constant region ($C\mu$) gene. The coding sequence for the C-terminus of the secreted μ -chain is contiguous with the 3' end of the Cµ4 exon and separate exons downstream of Cµ4 encode the C-terminus of the membranebound chain¹⁻³. Immunoglobulin D is also found membranebound and as a secreted molecule, and recent data indicate that the exon arrangement of the Cô gene is in part similar to that of the Cµ gene4.3. However, the amino acid sequence analysis presented here demonstrates that in the case of IgD the Cterminus of the secreted δ-chain is encoded by a separate exon (the CoDC exon of Tucker et al.5) and not by the CoAC sequence which corresponds topographically to the sequence expressed at the C-terminus of secreted µ chains.

The cell line B1-8. δ 1 (IgD, λ 1) has been isolated as a switch variant of the cell line B1-8.64.1 (IgM, λ 1) and is of C57BL/6 origin⁶. It secretes a monoclonal IgD antibody with specificities for the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP). Anti-NP antibodies from B1-8.81 ascites fluid were purified by affinity chromatography⁶. The heavy chains of these molecules are linked to each other and to the light chains by disulphide bridges (ref. 6 and unpublished data). After complete reduction and carboxyamidomethylation the heavy and light chains were eluted from Sephadex G-100 with 4.5 M urea, 1 M propionic

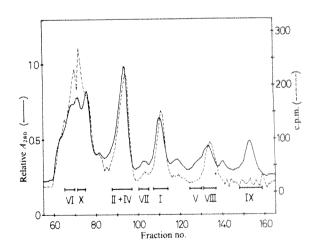
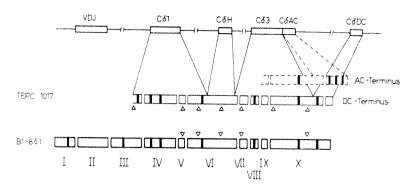


Fig. 1 Gel filtration of cyanogen bromide-cleaved peptides of 14 C-carboxyamidomethylated B1-8. δ 1 heavy chain. Cyanogen bromide cleavage of 50 mg of the completely reduced and alkylated heavy chain was performed in 70% formic acid at a concentration of 10 mg ml⁻¹ with a fivefold excess (w/w) of CNBr for 24 h at 20 °C. The mixture was dried under a stream of nitrogen. The peptides were dissolved in 3.5 ml of 0.1 M formic acid containing 6 M deionized urea and applied to a Sephadex G-50 superfine column (2×200 cm) equilibrated in the same solvent. Fractions of 3.3 ml were collected and aliquots of $20~\mu l$ were used for liquid scintillation counting in 5 ml of Bray's solution 10 . Roman numerals of the pooled fractions refer to the position of the corresponding peptides in the sequence of the B1-8.81 heavy chain as given in Fig. 2. Peptide no.III was insoluble in 6 M urea, 0.1 M formic acid, and could thus be isolated without chromatographic separation.



acid. The isolated heavy chains were treated with cyanogen bromide and peptides were separated by column chromatography on Sephadex G-50 (Fig. 1). Column fractions containing more than one peptide were rechromatographed on either Sephadex G-100 or Sephadex G-50. The purified CNBr fragments of the δ heavy chain were characterized by end group and compositional analysis and carbohydrate and cysteine contents (cysteines were labelled with ¹⁴C-iodoacetamide during reduction and alkylation, carbohydrate was detected as glucosamine in amino acid analysis).

The published IgD heavy-chain DNA sequence⁵ enabled us to identify all constant-region CNBr fragments. They correspond to the C δ 1, C δ H and C δ 3 exons as defined by Tucker et al. (Fig. 2). Five of the seven predicted⁵ carbohydrate attachment sites carry sugar, except the first proposed site in C δ 1 and the second one in C83. The lack of carbohydrate in the first proposed site in C81 can be deduced from the absence of carbohydrate from CNBr fragment III of the B1-8.δ1 chain. It is known that the sequence asparagine-phenylalanine-threonine is not necessarily used as a carbohydrate attachment site7. However, our determination of radioactive iodoacetamide incorporation (data not shown) indicates that the CNBr fragment III also does not contain the cysteine residue expected in position 5 of C δ 1 (Fig. 2). If the 5' intron-exon junction of C δ 1 were defined not by the third RNA splice site proposed by Tucker et al.5 but by the fourth site situated 22 nucleotides further downstream 1 the Cδ1 domain would be shortened by seven amino acids and lack both cysteine and carbohydrate attachment site. Alternatively, the lack of these two structures in the B1-8.81 CNBr fragment III could reflect an allotypic difference between the δ -chains from C57BL/6 and those of BALB/c from which the TEPC 1017 tumour⁵ originates.

The protein sequence of the second carbohydrate attachment site in $C\delta 3$ is conserved in the B1-8. $\delta 1$ heavy chain, but not used for carbohydrate attachment. Neither the predicted C-terminal CNBr fragment of the AC-gene segment nor that of the DC-exon was identified (Fig. 2). This can be explained by a base pair exchange in the corresponding methionine codon, representing a sequence difference between B1-8. $\delta 1$ and TEPC 1017 δ -

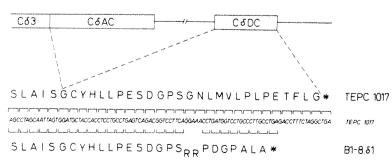
Fig. 3 Sequence of the C-terminal part of secreted B1-8.δ1 heavy chain. The C-terminal sequence of IgD heavy chains from TEPC 1017 and B1-8.δ1 are aligned to the corresponding TEPC 1017 exons and cDNA sequence. The TEPC 1017 sequence was taken from ref. 5. The B1-8.δ1 sequence was obtained by automated Edman degradation of tryptic peptides using an updated Beckman 890 B sequencer. Degradations were performed in the presence of 3 mg of the non-protein carrier Polybren 12 using a 0.2 M Quadral program 13. Phenylthiohydantoin derivatives obtained after conversion using the Sequemat P6 autoconverter were identified by HPLC 14 and TLC 15. The C-terminal nine amino acids of B1-8.δ1 are different from the TEPC 1017 cDNA sequence but not from the sequence deduced from the germ-line DNA 8. The cDNA and germline DNA sequence differ by one inserted and one exchanged nucleotide. A key to the single letter amino acid code is provided by Dayhoff 16.

Fig. 2 Schematic representation of the CNBr-cleaved peptides in the sequence of the secreted B1-8. δ 1 heavy chain. The genomic organization of the C δ gene according to Tucker et al.⁵ is given at the top of the figure. The translated TEPC 1017 cDNA sequence is schematically represented by CNBr-peptides (open boxes). The positions of cysteines (black bars) and of putative carbohydrate attachment sites (open arrow heads) are indicated. Hypothetical CNBr-fragments corresponding to a chain which would terminate with the AC-segment are shown as dashed lines. The alignment of the CNBr-fragments of the B1-8. δ 1 heavy chain is given in the lower part of the figure. Alignment of peptides I-III is according to Bothwell et al.¹¹. No carbohydrate was found in peptide III and in the N-terminal tryptic peptide of X. Peptide III contains only one cysteine.

chains. If the AC-gene segment were expressed in the secreted form of the δ heavy chain the loss of the cyanogen bromide cleavage site would result in a new CNBr fragment containing four cysteines. By comparing the amount of incorporated radioactivity with that of other cysteine-containing peptides, the CNBr fragment X, the only candidate for the C-terminus, was found to contain only two cysteines. This would be expected if the DC-segment is expressed in the secreted B1-8. δ 1 heavy chain.

Tryptic peptides of this CNBr fragment were separated on Sephadex G-50 in 0.5 M formic acid and further analysed by automated Edman degradation. The sequence of the C-terminal 27 amino acids is presented in Fig. 3. The sequence data clearly show that the C-terminus of the secreted B1-8. δ 1 heavy chain is encoded by the C δ DC exon. The sequence of the C-terminus is identical to that predicted by the sequence of the TEPC 1017 cDNA in the C-terminal 5 amino acid positions of C δ 3 and the first 13 positions of C δ DC. The last nine residues of the B1-8. δ 1 heavy chain do not fit the predicted sequence at all. This problem can be resolved, however, by exchanging in the TEPC 1017 DNA a single base pair and inserting another one (note that both changes occur in the upper part of a possible hairpin structure). The resulting frameshift generates the B1-8. δ 1 C-terminal heavy-chain sequence.

It is now clear that the difference between the cDNA-derived TEPC 1017 C-terminal δ -chain sequence and the sequence of the B1-8. δ 1 chain C-terminus is not due to genetic polymorphism. Tucker et al. have very recently isolated the C δ DC exon from BALB/c liver DNA and found that it encodes an amino acid sequence identical to that expressed in the C-terminus of the B1-8. δ 1 chain. Thus, the TEPC 1017 tumour line has either accumulated somatic mutations in the C δ gene or, more likely, the difference between cDNA and germ-line gene sequences is due to errors in DNA-RNA or RNA-DNA transcription. Taken together, our results show that the C-terminus of the secreted δ -chain is encoded by the C δ DC exon and that C δ AC-encoded sequences are not expressed in the secreted δ -chain. B1-8. δ 1 cells also carry IgD on the surface and the surface-bound δ -chains have a higher molecular weight than the



secreted ones⁶. It seems likely that, as in the case of IgM, the C-termini of the two chains differ from each other and an as yet unidentified exon encodes the C-terminus of the surface-bound δ-chain.

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- Singer, P. A., Singer, H. H. & Williamson, A. R. Nature 285, 294-300 (1980).

- Rogers, J. et al. Cell 20, 313-319 (1980).
 Liu, C., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. Science 209, 1348-1352 (1980).
 Tucker, P. W., Liu, C., Mushinski, J. F. & Blattner, F. R. Science 209, 1353-1360 (1980).
 Neuberger, M. S. & Rajewsky, K. Proc. natn. Acad. Sci. U.S.A. 78, 1138-1142 (1981).

- Neuberger, M. S. & Rajewsky, K. Proc. natn. Acad. Sci. U.S.A. 78, 1138-1142 (1981). Kehry, M., Sibley, C., Fuhrman, J., Schilling, J. & Hood, L. E. Proc. natn. Acad. Sci. U.S.A. 76, 2932-2936 (1979). Tucker, P. W. et al. in The Lymphocytes in the Immune Response (eds Mosier, D., Klinman N., Sher, E. & Vitetta, E.) (Elsevier, Amsterdam, in the press). Waxdal, M. J., Konigsberg, W. H., Henley, W. L. & Edelman, G. M. Biochemistry 7, 1959-1966 (1968).
- Bray, G. Analyt. Biochem. 1, 279-285 (1960). Bothwell, A. L. M. et al. Cell (in the press).
- Tarr, G. E., Beecher, J. F., Bell, M. & McKean, D. J. Analys. Biochem. 84, 622-627 (1978)
- 13. Beyreuther, K., Raufuss, H., Schrecker, O. & Hengstenberg, W. Eur. J. Biochem. 75, 75-286 (1977).
- Johnson, N. D., Hunkapiller, M. W. & Hood, L. E. Analyt. Biochem. 100, 335-338 (1979).
- Beyreuther, K. in Solid Phase Methods in Protein Sequence Analysis, 107-119 (Elsevier, Amsterdam, 1977).
- Dayhoff, M. Atlas of Protein Sequence and Structure Vol. 5, D2 (National Biomedical Research Foundation, Washington, 1972).

SV40 T antigen binds specifically to a cellular 53 K protein in vitro

Frank McCormick*, Robin Clark†, Ed Harlow* & Robert Tjian†

* Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK

† Department of Biochemistry, University of California, Berkeley, California 94720, USA

Simian virus 40 (SV40) large T antigen is a multifunctional protein that is essential for virus replication and the establishment and maintenence of cell transformation1. The molecular basis of these functions probably involves specific interactions of T antigen with DNA²⁻⁴ and cellular proteins. An example of such an interaction has been described recently; a significant fraction of the T antigen extracted from cell lysates is tightly bound to a cellular phosphoprotein of molecular weight 53,000 (53K)⁵⁻⁹. Here we demonstrate in vitro the direct interaction of highly purified T antigen (D2 T antigen or SV80 T antigen) with 53K in lysates from a variety of mouse cell lines. All the 53K detected in these lysates was able to bind to added T antigen, and it was the only cellular protein with which T antigen formed a stable complex. We conclude that 53K binds directly to a specific site on T antigen and that binding occurs without previous modification and without involvement of other virus-encoded or induced proteins.

The function of 53K is unknown, but several interesting properties have been attributed to this protein in addition to its association with large T antigen. Transformed cells seem to express significantly higher levels of 53K than normal cells^{6,10}. Similarly, SV40-infected mouse or monkey cells synthesize 53K more rapidly than uninfected cells^{9,11}. Furthermore, lymphocytes freshly prepared from the spleens of young mice express

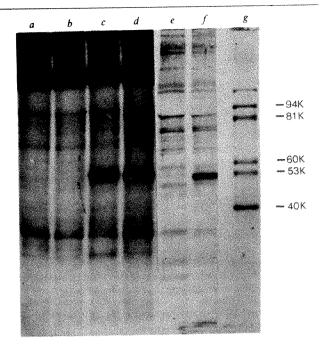


Fig. 1 Binding of purified D2 to 53K in vitro. F9 mouse embryonal carcinoma cells (supplied by P. Goodfellow) were labelled with ³²P-labelled inorganic phosphate (1 mCi ml⁻¹ phosphate-free medium) or ³⁵S-Met (1 mCi ml⁻¹ Met-free medium) as described elsewhere7. Monolayers were washed with phosphate-buffered saline and cells lysed in buffer A (2% NP-40, 10 mM Tris, 140 mM NaCl, pH 8.0; 4 °C for 15 min). These lysates were centrifuged for 2 min in an Eppendorf microfuge and the supernatants used as a source of labelled 53K. D2 protein (1 µg of purified protein) were added to lysates corresponding to ~2×10° F9 cells in 200 µl extraction buffer. After incubation at room temperature for 15 min, 5 µl of preimmune rabbit serum was added and incubated at 4 °C overnight. Rabbit immunoglobulin G (IgG) was removed with 50 μl of 10% fixed Staphylococcus aureus. The remaining solution was immunoprecipitated and immune complexes were washed twice in buffer A and twice in 2 M urea, 0.4 M LiCl, 10 mM Tris, pH 8.0, and subjected to SDS-polyacrylamide gel electrophoresis as described elsewhere 18 . Tracks a-d are from cells labelled with 32 P-phosphate; tracks e-f are from 35 S-labelled cells. Track g is a molecular weight marker mix. a, 5 µl preimmune rabbit serum; b and e, 5 μ l anti-D2 serum; c and f, 5 μ l anti-D2 serum in the presence of 1 μ g D2 protein; d, anti-53K serum F5 (ref. 7).

detectable levels of 53K only after mitogenic stimulation with an agent such as concanavalin A12. Another interesting observation is that some mice bearing SV4D-induced tumours raise autoantibodies against 53K^{5,8}. Because 53K synthesized by SV40-transformed or infected cells becomes complexed with large T we are interested in determining how these proteins interact and whether this interaction relates to the role of large T in promoting DNA synthesis 13.14, negulating early mRNA synthesis 15-17 and in cellular transformation (see discussion in ref. 1).

Here we describe investigations of the mechanism of binding of SV40 large T with 53K in an in vitro system. We have used the adenovirus: SV40 hybrid protein D2 as a convenient source of T antigen and have assayed for the ability of purified D2 protein to bind radiolabelled 53K in detergent lysates from various cells. In early experiments, 3T6 cells were used. However, the F9 line of embryonal carcinoma cells seems to express significantly more 53K and was therefore used here.

Purified D2 protein was added to lysates of labelled F9 cells and incubated at room temperature for 15 min. It was then immunoprecipitated from these lysates using antiserum raised against purified D2 protein or with monoclonal antibodies which recognize a single determinant on the protein. It is clear from Fig. 1 that anti-D2 antibodies immunoprecipitated a 53K phosphoprotein from those lysates to which D2 protein had

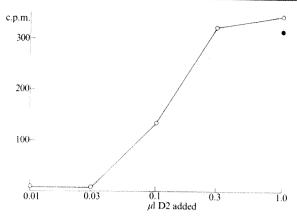


Fig. 2 Titration of D2 against 53K. Various concentrations of D2 protein were added to lysates of ³⁵S-Met-labelled F9 cells. The amount of 53K immunoprecipitated by monoclonal antibodies against D2 was estimated by cutting the 53K bands from a dried gel and counting them in a liquid scintillation system. In these immunoprecipitations, monoclonal antibodies which recognize a determinant on D2 protein were used. The hybridoma cells which produce these antibodies were obtained by fusing spleen cells from BALB/c mice bearing SV40-induced tumours with the myeloma line P-3-NS1-1Ag-4-1. These mice had been given an injection of partially purified T antigen/53K complex 3 days before spleen removal. Details of this and other hybridoma cell lines will be described elsewhere ¹⁹. One of these lines, L21, produces antibodies directed against 53K¹⁹.

been added. In the absence of added D2 protein, no cellular proteins were precipitated specifically. Similar results were obtained with T antigen purified from SV80 cells. These results suggest that the added T antigen binds to labelled 53K in these lysates and thus allows its immunoprecipitation by antisera that have no detectable activity against 53K itself. The 53K protein that seemed to bind to T antigen was not released during stringent washing of immune complexes with buffers containing 0.5% NP-40, 0.4 M LiCl or 2 M urea. In these conditions, no other labelled cellular proteins were immunoprecipitated specifically in association with T antigen. Thus the complex

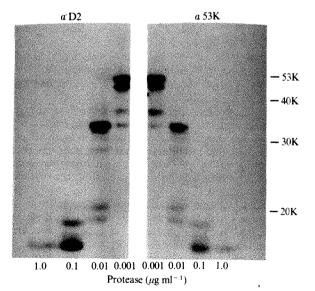


Fig. 3 V8 protease digestion of 53K. 53K was immunoprecipitated directly with monoclonal antibodies against 53K (from hybridoma clone L21) or indirectly by monoclonal antibodies which recognize D2 (clone L7) in the presence of added D2 protein. Immunoprecipitates were treated as described in Fig. 1 legend and the 53K bands removed from gels electrophoretically. Aliquots of the labelled protein were digested with various concentrations of V8 protease and again subjected to SDS-PAGE¹⁸.

formed in vitro resembled that extracted from SV40-transformed 3T3 cells⁷. We can also conclude that the polypeptide sequences which large T shares with small t are not required for binding, as these sequences are not represented on D2 protein, and that the mutation on SV80 T antigen which destroys its ability to initiate SV40 DNA synthesis (Gish and Botchan, personal communication) does not affect its ability to bind 53K.

When increasing concentrations of D2 protein were added to a constant volume of F9 cell lysate, the amount of 53K immunoprecipitated by anti-D2 antibodies was proportional to the amount of D2 T antigen added (Fig. 2). The maximum amount of 53K that could be detected by this indirect immunoprecipitation assay corresponded closely to the maximum amount that could be immunoprecipitated directly by a monoclonal antibody against 53K. Thus, all the immunologically reactive 53K from these cell lysates seemed to be available for binding to added D2.

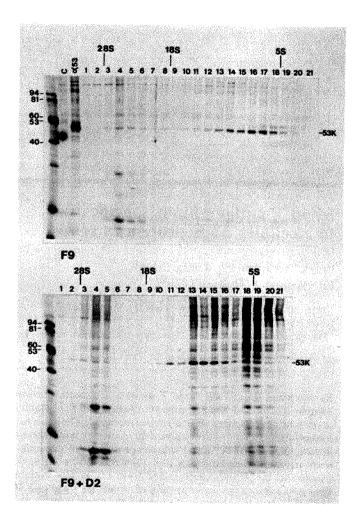


Fig. 4 Sedimentation of 53K in the presence of added D2 protein. F9 cells were labelled with ³⁵S-Met (0.24 mCi ml⁻¹, 2 h) and lysates from these cells were incubated at 22 °C in the presence (lower panel) or absence (upper panel) of D2 protein (2 μg per ml lysate). Each lysate was centrifuged at 25,000 r.p.m. through sucrose gradients as described elsewhere and each fraction was immunoprecipitated with 10 μl L21 anti-53K hybridoma supernatant. Immune complexes were treated as in Fig. 1 legend. Tract C in the top panel represents an immunoprecipitation of unfractionated, untreated F9 lysate with anti-alkaline phosphate monoclonal antibodies. Track 53K represents immunoprecipitation of the same lysate with anti-53K antibodies.

The 53K species immunoprecipitated directly by antibodies was compared with the 53K species that formed a stable complex with D2 protein. Figure 3 shows that the products of partial proteolysis generated by addition of V8 protease were identical for the two 53K samples, which suggests that these are closely related if not identical, proteins.

We have done similar in vitro binding assays using lysates from a variety of cell lines: these include 3T3 cells transformed by polyoma virus⁵, 3T6 cells infected with wild-type polyoma virus, mouse L-cells and 3T6 cells. In each case, a single polypeptide of molecular weight (MW) 53,000 was immunoprecipitated by anti-D2 sera after addition of T antigen to the respective lysates (data not shown). The availability of 53K for binding in these cell lysates suggests that other proteins in the lysates, for example, polyoma virus T antigen, do not compete effectively for binding with SV40 large T.

The data described here provides direct evidence that purified antigen added to lysates of cells expressing 53K is able to form a complex with this protein. We have further demonstrated this association by determining the sedimentation properties of 53K in the presence and absence of D2 protein. Figure 4 shows that most of the 53K extracted from F9 cells sediments at ~8S, corresponding to a molecular weight of the order of 200,000. This could represent a tetrameric structure, or an association of 53K with other macromolecules. A small fraction of the 53K from these cells sediments at 20S, which suggests a native molecular weight of up to 1 million. It is unclear whether this represents homopolymers of 53K or a high-molecular-weight complex which contains other components. After addition of D2 protein to lysates of F9 cells, the lower-molecular-weight form shifted to a sedimentation value of 12S, which corresponds to a native molecular weight of ~300,000. Similar results were obtained when either anti-53K or anti-D2 antibodies were used to immunoprecipitate the complex. These results suggest that one molecule of D2 protein binds to a 200,000-MW form of 53K or these conditions. It should be stressed, however, that these sedimentation values cannot be used to determine molecular weights with a high degree of reliability, as the sedimentation properties of proteins are affected by their configuration and gradients of the type described here do not provide high resolution. Nevertheless, it is clear that the addition of D2 protein to lysates of F9 results in a significant increase in the molecular weight of 53K in its native state which indicates a physical association of the two protein species.

We have been able to demonstrate the binding of SV40 T antigen to a cellular 53K protein in vitro, by two independent methods. The development of this system should allow us to assess whether binding of 53K affects the biochemical activity of T antigen and thus modifies its biological functions.

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1. Tooze, J. in Molecular Biology of Tumor Viruses, Part 2 (ed. Tooze, J.) (Cold Spring Harbor Laboratory, New York, 1980). Tjian, R. Cell 13, 165-179 (1978)

- 3. Shortle, D. R., Margolskee, R. F. & Nathans, D. Proc. natn. Acad. Sci. U.S.A. 76, 6128-6131 (1979)
- Myers, R. M. & Tjian, R. Proc. natn. Acad. Sci. U.S.A. 77, 6491-6495 (1980).
 Lane, D. P. & Crawford, L. V. Nature 278, 261-263 (1979).
 Linzer, D. I. H. & Levine, A. J. Cell 17, 43-52 (1979).

- Linzer, D. I. H. & Levine, A. J. Cell 17, 43-52 (1979).
 McCormick, F. & Harlow, E. J. Virol. 34, 213-224 (1980).
 Gurney, E. G., Harrison, R. O. & Fenno, J. J. Virol. 34, 752-763 (1980).
 Harlow, E., Pim, D. C. & Crawford, L. V. J. Virol. 37, 564-573 (1981).
 Crawford, L. V., Pim, D. C., Gurney, E. G., Goodfellow, P. & Taylor-Papadimitrou, J. Proc. natn. Acad. Sci. U.S.A. 78, 41-45 (1981).
 Linzer, D. I. H., Maltzman, W. & Levine, A. J. Virology 48, 303-318 (1979).
 Milner, J. & McCormick, F. Cell Biol. int. Rep. 4, 663-669 (1980).
 Tegtmeyer, P. J. Virol. 10, 591-598 (1972).
 Tight P. Fey, G. & Graessman A. Proc. natn. Acad. Sci. U.S.A. 75, 1279-1283 (1978).

- Tjan, R., Fey, G. & Graessman, A. Proc. natn. Acad. Sci. U.S.A. 75, 1279–1283 (1978).
 Tegtmeyer, P., Schwartz, M., Collins, J. K. & Rundell, K. J. Virol. 16, 168–178 (1975).
- 16. Reed, S. I., Stark, G. R. & Alwine, J. C. Proc. natn. Acad. Sci. U.S.A. 72, 1605-1609
- 17. Rio, D., Robbins, A., Myers, R. & Tjian, Proc. natn. Acad. Sci. U.S.A. 77, 5706-5710
- Smith, A. E., Smith, R. & Paucha, E. Virol. 28, 140-149 (1978).
- 19. Harlow, E., Crawford, L., Pim, D. & Williamson, N. J. Virol. (in the press).

Persistence and expression of histone genes injected into Xenopus eggs in early development

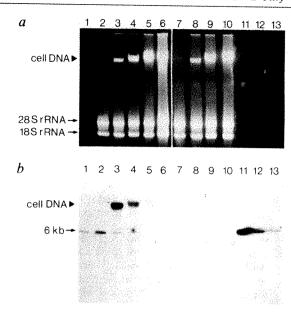
Mary M. Bendig

Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland

Microinjection of amphibian oocytes with purified cloned DNA has proven to be a valuable assay system for studying eukaryotic gene expression¹⁻¹³. The oocyte's ability to express microinjected genes has already allowed a functional analysis of several genes and their sequence manipulated counterparts 14-20. Although such studies are now possible with several other expression systems, the oocyte system is unique in that it represents a cell destined, on fertilization, to divide and differentiate into an embryo. Thus, it was envisaged that the technique of microinjecting genes and their mutant analogues into fertilized eggs might eventually be used as an assay system for studying the transcriptional control processes that occur during cell differentiation and development. To this end, fertilized eggs of Xenopus laevis were injected with a cloned repeat of sea urchin histone genes and the fate and expression of the injected genes examined during the early stages of Xenopus development. After replication during the early cleavage stages. the injected sea urchin histone DNA sequences persisted to at least the swimming tadpole stage. The injected genes were also at least in part faithfully transcribed into RNA species with the correct histone mRNA 5' and 3' termini. Thus, these results, reported here, meet the prerequisites for an assay system capable of being used to investigate the factors involved in the developmental control of gene activity.

Fertilized eggs were injected with sea urchin histone DNA in one of two forms—as a linear 6-kilobase (kb) HindIII fragment recovered from the recombinant h22 (ref. 21) or as supercoiled, circular pBR322 recombinant plasmid DNA containing two copies of the 6-kb HindIII fragment tandemly integrated head to tail. The h22 repeat contains all five histone genes and transcription of all five proceeds in the same direction²². Nucleic acid was isolated from the developing embryos at various stages post-injection and the persistence and physical state of the injected DNA sequences were analysed by blot hybridization. In Figs 1 and 2, each gel slot was loaded with a sample representing one embryo equivalent of nucleic acid isolated at the indicated stage of development. The hybridization probe was 32P-labelled pBR322 recombinant plasmid DNA containing the coding region of the sea urchin H1 histone gene. This H1 probe was chosen because, in contrast to the entire repeat, it gave no background hybridization to nucleic acid samples prepared from uninjected embryos (Fig. 1, lanes 7-10). Clear hybridization is seen in developing embryos after injection of either linear (Fig. 1) or circular DNA (Fig. 2). Moreover, the injected DNA sequences were replicated in the early stages of development, consistent with, and extending, the recent report that DNA injected into unfertilized eggs supports semiconservative replication of the injected DNA²³. Thus, by comparing lanes 2 and 3 in Figs 1 and 2 with known amounts of DNA (Fig. 1, lanes 11-13; 2,500, 250 and 25 pg, respectively), clearly there is at least a 10-fold increase in the amount of hybridizable sequences in the embryo at late blastula as compared with the amount in the egg immediately following injection. As the embryos developed further, the amount of hybridizable DNA per embryo steadily decreased, presumably reflecting selective degradation of the injected DNA (Figs 1, 2, lanes 3-6). However, faint amounts of injected DNA sequences were detectable as late as the swimming tadpole stage following injection of linear DNA (Fig. 1, lane 6) and as late as the early

Fig. 1 Analysis by DNA blotting of the persistence and physical state of injected DNA sequences in developing embryos of X. laevis following the injection of fertilized eggs with linear molecules of sea urchin histone DNA. Artificially fertilized eggs of X. laevis were prepared as described by Billet and Wild²⁹. Mature male and female frogs received two priming doses of Pregnyl (Organon). Eggs were squeezed out through the cloaca and fertilized immediately with a freshly prepared suspension of sperm. After chemical decapsulation with papain, healthy, fertilized eggs were injected within 1 h, before the first cleavage event occurred. The microinjection technique was essentially the same as used for oocytes^{1,2}, except that in the fertilized egg the germinal vesicle has disappeared and direct nuclear injection is not necessary. A linear 6-kb HindIII fragment containing the histone gene repeat of P. miliaris was recovered from the recombinant λ h22 (ref. 21) and purified by actinomycin/CsCl gradient centrifugation. In most experiments, 250 pg of DNA in a volume of 10 nl was injected per egg. Eggs injected with 500 pg of DNA did not develop beyond the late blastula stage. According to previous reports, however, even after injection of 2-4 ng of DNA, 15% of the embryos reached the feeding tadpole stage³⁰. About 2 h after injection, embryos, usually now at the four-cell cleavage stage, were transferred from Barth's medium to sterile 'aged' water plus antibotics (Gibco) and the developing embryos incubated at 18 °C. Samples of 5-10 embryos were collected immediately following injection and at the indicated stages of development³¹ up to and including the swimming tadpole. Embryos were homogenized at 0 °C in 10 mM EDTA, 100 mM Tris-HCl (pH 7.5), 300 mM NaCl, 1 mg ml⁻¹ proteinase K and 2% w/v SDS. After a 30-min incubation at room temperature, the homogenate was phenol-extracted three times, chloroform-extracted twice and the nucleic acids precipitated with two volumes of ethanol. The equivalent of one egg or embryo's worth of nucleic acid isolated from progressive stages of development was electrophoresed



through a 0.8% agarose gel. The gel was stained with ethidium bromide and photographed (panel a). To obtain efficient blotting of supercoiled, circular DNAs and large molecular weight DNAs, the gel was treated for 10 min with 0.25 M HCl before denaturation with 0.5 M NaOH, neutralization and transfer to nitrocellulose filters $^{32.33}$. The transferred DNA was hybridized to a 32 P-nick-translated probe of a pBR322 recombinant plasmid containing the ~ 500 -bp coding region of the H1 histone gene of the h22 repeat (as constructed and provided by M. Busslinger). Autoradiography was performed with an intensifier screen at ~ 70 °C (panel b). Lane 1 is a control sample taken from the loaded injection needle before egg injection and represents the amount of DNA injected per egg. Lanes 2-6 represent nucleic acid samples prepared from egg/embryos at various developmental stages following DNA injection, and lanes 7-10 represent similarly prepared samples from uninjected embryos: lanes 2, 2, stage 2 (late blastula); lanes 2, 2, stages 2 (early tail-bud); lanes 2, 2, stages 2 (late blastula); lane 2, stages 2

tail-bud stage following injection of circular DNA (Fig. 2, lane 5).

The results of these experiments also show that the physical form of the injected DNA sequences changes dramatically during the early stages of development, extending the previous observation that linear DNA molecules rapidly ligate and concatemerize after injection into unfertilized eggs²⁴. This same ligation phenomenon seems to be occurring after the injection of the 6-kb linear sea urchin histone DNA into fertilized eggs.

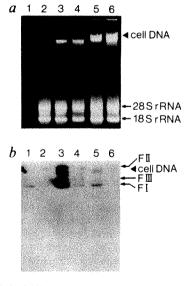


Fig. 2 Analysis by DNA blotting of the persistence and physical state of injected DNA sequences in developing embryos of X. laevis after the injection of fertilized eggs with supercoiled circles of sea urchin histone plus plasmid DNA. Circular, supercoiled pBR322 plasmid DNA containing two tandem copies of the h22 6-kb HindIII fragment was purified by CsCl gradient centrifugation and used to injected fertilized eggs. The injection procedure and subsequent analysis of embryonic nucleic acids were as described for injection of lines. DNA molecules (Fig. 1). Again, a is the ethidium-stained gel and b the corresponding autoradiograph. As in Fig. 1, lane 1 is a control sample representing the amount of DNA injected per egg (250 pg). Lanes 2-6 represent one embryo equivalent of nucleic acid extracted at stage 1 (egg), stage 8 (blastula), stages 10-12 (gastrula), stage 22 (early tail-bud) and stage 40 (swimming tadpole), respectively.

Thus, by late blastula, most of the hybridizable sequences co-migrate with high-molecular-weight cell DNA rather than as 6-kb linear DNA molecules (Fig. 1, lane 3). Together, these results suggest that after injection into fertilized eggs, linear DNA molecules ligate and then replicate in the developing embryos as high-molecular-weight concatemers.

However, supercoiled, circular (FI) DNA injected into eggs is processed differently. Previous work has shown that when SV40 FI DNA is injected into oocyte nuclei, the FI DNA is first relaxed but within 10 h re-acquires a supercoiled structure²⁵. Figure 2, lane 2, illustrates that immediately following injection of FI DNA into fertilized eggs at least half of the injected FI DNA is relaxed. By the blastula stage, there has been a large increase in the total amount of injected DNA sequences per embryo, and most appear as FI DNA (Fig. 2, lane 3). Thus, relaxed DNA seems to be converted back to FI DNA and replicates as such. After injection of either linear or circular sea urchin DNA, at least some of the injected DNA sequences persist as DNA co-migrating with high-molecular-weight cell DNA (Fig. 1, lanes 5, 6; Fig. 2, lane 5). Although there is no direct evidence that any of the injected DNA sequences have become integrated into the Xenopus genome, this notion is supported, at least for a minor fraction of the injected DNA sequences, by the persistence of some of the sequences to later stages in the high-molecular-weight range.

Having determined that the microinjected sea urchin histone genes persist in the Xenopus embryos, at least during early development, the next question was whether they were also faithfully transcribed, as judged by S₁ mapping of mRNA termini²⁶. As the injected gene copy number is highest at about the late blastula stage, the initial experiments examined RNA from this stage. S₁ mapping experiments were performed using a multiple probe capable of detecting simultaneously all five sea urchin histone mRNA 5' termini. As shown in Fig. 3, lanes 2-4, three bands co-migrating with the 5'-terminal sequences of the authentic H2B, H2A and H3 mRNAs are clearly visible. There is a faint band corresponding to the 5'-terminal sequence of H1 mRNA, but no band corresponding to the protected 5'-terminal sequence of H4 mRNA was detected. Controls with RNA from uninjected Xenopus embryos show no bands of protected sequences (Fig. 3, lane 5). This pattern of S₁ protection is

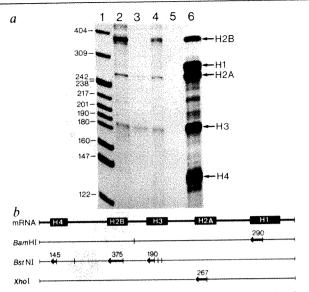


Fig. 3 Analysis by S₁ mapping of 5-14S RNA from Xenopus blastulae injected as eggs with sea urchin histone DNA. Artificially fertilized Xenopus eggs were injected with sea urchin histone DNA in either the linear or circular form as described in Figs 1 and 2 legends. Sucrose gradient-purified 5-14S RNA from the equivalent of 3.5 embryos (stage 9, late blastulae) was hybridized to a 32P-5'-end-labelled probe of triple-digested (BamHI-BstNI-Xhol) 6-kb h22 DNA. As illustrated in panel b, this multiple probe is capable of protecting the 5' termini of all five sea urchin histone mRNAs from S₁ digestion'. S₁ mapping was performed as previously described^{7,26,34}. Lane 1 is a size marker of ³²P-5'-end-labelled *Hpa*II fragments of pBR322. Lanes 2-4 are RNA samples from injected embryos; lanes 2 and 4 were injected with linear DNA molecules, lane 3 with the circular form. Lane 5 is RNA prepared in parallel from uninjected embryos. Lane 6 is 5 µg of total RNA from P. miliaris embryos at the 128-cell stage of development.

identical to that previously established for transcription in the oocyte7, suggesting that the same factors are governing the production and accumulation of the mRNAs and that no developmental change has occurred. Interestingly, both the injection of circular and linear DNA templates gave this pattern although, as discussed above, the actual templates in the blastulae were probably either circles or high-molecular-weight concatemers; moreover, the circles contained DNA sequences from the plasmid vector pBR322. These results suggest that the transcriptional pattern is a fundamental reflection of the relative efficiency of the individual sea urchin histone promoter sequences within the Xenopus transcriptional machinery.

RNA from injected blastulae was also analysed for transcripts with the correct 3' termini. The 3'-terminal sequences of the Psammechinus miliaris H2B, H3, H2A and H1 histone mRNAs can be detected using an S₁ mapping multiple probe of ³²P-3'end-labelled HpaII-XhoI restriction fragments of h22 (ref. 7). Bands of protected fragments co-migrating with the protected 3'-terminal sequences of authentic H2B and H2A mRNAs were visible (not shown), but the correct 3'-terminal sequences of the H3 and H1 mRNAs were not detected. With either the 5'- or 3'-multiple probe, RNA from the injected embryos gave numerous faint bands of unknown protected sequences, indicating that although at least some of the correct 5' and 3'termini are synthesized, there is also some incorrect transcription.

RNA samples from progressive stages of development (from immediately following injection of the fertilized egg to the swimming tadpole stage) were also examined. The transcriptional expression pattern was found to be quite similar at all stages, with the overall level of sea urchin histone gene transcription apparently governed only by the abundance of the injected gene sequences rather than by the developmental stage of the Xenopus embryo. This result is not unexpected because genes from a heterologous source would probably not be expected to be brought under Xenopus developmental control. For example, adult rabbit β -globin gene was also found to be expressed in Xenopus embryos using the same microinjection protocol²⁷. Also, as has recently been shown for the 5S rRNA genes²⁸, it is questionable whether naked DNA, even in a homologous system, respects developmental signals. Nevertheless, the approach described here paves the way for an assay capable of investigating what the units responding to developmental signals actually are and whether, for example, integration of genes into the correct chromosomal location is necessary before such signals are effective.

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- 1. Kressmann, A., Clarkson, S. G., Telford, J. L. & Birnstel, M. L. Cold Spring Harb. Symp. quant. Biol. 42, 1077 (1977).
- Kressmann, A., Clarkson, S. G., Pirrotta, V. & Birnstiel, M. L. Proc. natn. Acad. Sci. U.S.A. 75, 1176 (1978).
- 75, 11 / 0 (1978).
 Cortese, R., Melton, D., Tranquilla, T. & Smith, J. O. Nucleic Acids Res. 5, 4593 (1978).
 De Robertis, E. M. & Olson, M. V. Nature 278, 137 (1979).
 Brown, D. D. & Gurdon, J. B. Proc. nam. Acad. Sci. U.S.A. 75, 2849 (1978).
 Probst, E., Kressmann, A. & Birnstiel, M. L. J. molec. Biol. 135, 709 (1979).

- Hentschel, C., Probst, E. & Birnstiel, M. L. Nature 288, 100 (1980).
 Trendelenburg, M. F. & Gurdon, J. B. Nature 276, 292 (1978).
- Trendelenburg, M. F., Mathis, D. & Oudet, P. Proc. natn. Acad. Sci. U.S.A. 77, 5984
- Etkin, L. D. & Maxson, R. E. Devl Biol. 75, 13 (1980).
- Wickens, M. P., Woo, S., O'Malley, B. W. & Gurdon,
- DeRobertis, E. M. & Mertz, J. E. Cell 12, 175 (1977).
- Rungger, D. & Türler, H. Proc. natn. Acad. Sci. U.S.A. 75, 6073 (1978)
- 14. Kressmann, A., Hofstetter, H., Di Capua, E., Grosschedl, R. & Birnstiel, M. L. Nucleic Acids Res 7, 1749 (1979).
- Hofstetter, H., Kressmann, A. & Birnstiel, M. L. Cell 24, 573 (1981).
 DeFranco, D., Schmit, O. & Söll, D. Proc. natn. Acad. Sci. U.S.A. 77, 3365 (1980).
- Sakonju, S., Bogenhagen, D. F. & Brown, D. D. Cell 19, 13 (1980).
 Bogenhagen, D. F., Sakonju, S. & Brown, D. D. Cell 19, 27 (1980).
- Grosschedl, R. & Birnstiel, M. L. Proc. natn. Acad. Sci. U.S.A. 77, 1432 (1980).
 Grosschedl, R. & Birnstiel, M. L. Proc. natn. Acad. Sci. U.S.A. 77, 7102 (1980).
- 21. Clarkson, S. G., Smith, H. O., Schaffner, W., Gross, K. W. & Birnstiel, M. L. Nucleic Acids Res. 3, 2617 (1976)
- Gross, K., Schaffner, W., Telford, J. & Birnstiel, M. L. Cell 8, 479 (1976).
- 23. Harland, R. M. & Laskey, R. A. Cell 21, 761 (1980).
- Clerc, R. thesis, Univ. Zürich (1980).
- Clerc, R. thesis, Univ. Zurich (1980).

 Wyllie, A. H., Laskey, R. A., Finch, J. & Gurdon, J. E. Devl Biol. 64, 178 (1978).

 Hentschel, C., Irminger, J. C., Bucher, P. & Birnstiel, M. L. Nature 285, 147 (1980).

 Rusconi, S. & Schaffner, W. Proc. natn. Acad. Sci. U.S.A. (in the press).

 Korn, L. J. & Gurdon, J. B. Nature 289, 461 (1981).

- Billett, F. S. & Wild, A. E. Practical Studies of Animal Develop Hall, London, 1975).
- Gurdon, J. B. & Brown, D. D. in The Molecular Biology of the Mammalian Gene Apparatus (ed. Tso, P.) 11-123 (Elsevier, Amsterdam, 1977).

 31. Nieuwkoop, P. D. & Farber, J. Normal Table of Xenopus laevis (Daudin) (North-Holland,
- Amsterdam, 1967).
 32. Wahi, G. M., Stern, M. & Stark, G. R. Proc. nam. Acad. Sci. U.S.A. 76, 3683 (1979).
- outhern, E. M. J. molec. Biol. 98, 503 (1975).
- 34. Berk, A. J. & Sharp, P. A. Proc. natn. Acad. Sci. U.S.A. 75, 1274 (1978).

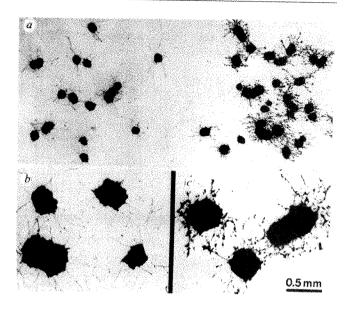
Preferential adhesion of tectal membranes to anterior embryonic chick retina neurites

Willi Halfter, Michael Claviez & Uli Schwarz

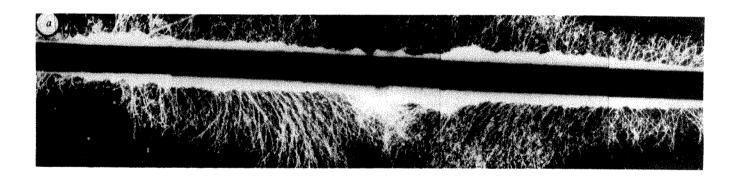
Max-Planck-Institut für Virusforschung, Abt. Biochemie, D-7400 Tübingen, FRG

Recognition between cells during embryogenesis is essential for the formation of precisely patterned neuronal networks such as the projection of axons from the retina on to the tectum in the avian optical system. A gradient of adhesion between retinal and tectal cells has been detected, with cells from the dorsal area of the neural retina adhering preferentially to cells of the ventral half of the visual tecta and vice versa (for review see ref. 1). This has supported the idea that topological relationships between cells are established through markers on the cell surface2. Such findings are based on adhesion between cell bodies, whereas it is only the axon tip of the retina ganglion cell which makes contact with and presumably recognizes tectal cells. Here, we have used a system in which outgrowing neurites from the retina were exposed to tectal cell surfaces in vitro and found a striking antero-posterior polarity in the chick retina.

Fig. 1 Adhesion of tectal membranes to neurites from anterior and posterior retina explants. A retina from a 6-day White Leghorn chick embryo was isolated. Squares (150×150 μ m) from the anterior and posterior region were explanted on a collagen-coated dish (Falcon 30001 F) and incubated at 37 °C in 4% CO2 for 2 days (2 ml of Eagle's minimal essential medium with 2.4% NaHCO₃ and 5 mM HEPES, 10% fetal calf serum, 2% chick serum, 1% beef embryo extract, 2 mM glutamine, penicillin and streptomycin; all from Gibco). Finally, 30 µl of a suspension of tectal membranes (see below; 150-200 µg protein)^{10,11} were added and incubation continued for another 2 h with gentle shaking at 30-min intervals. The medium was sucked off, the dish washed six times with prewarmed medium and the cultures were stained with Sudan black by a modification of the method of Wood¹² (after fixation with 2.5% glutaraldehyde in phosphatebuffered saline, impregnation in 0.19% OsO₄ and 0.5% K₂Cr₂O₇). Coating of the dish with collagen: native collagen was prepared essentially according to Elsdale and Bard¹³ (final concentration 350 μg ml⁻¹). At the onset of gelling, the dish was turned into a vertical position leaving a thin layer of collagen. The membranes were prepared by a modification of the procedure of Brunette and Till¹⁴. Tecta from 20 9-day-old chick embryos were homogenized in 4 ml of 10 mM Tris, 1.5 mM CaCl₂ and 1 mM spermidine (pH 7.4) in a Dounce homogenizer. After centrifugation (10 min at 1,000g) membranes were enriched in a biphasic system: a solution of dextran (13.1g dextran T-500, Pharmacia, in 67.8 ml H₂O bidest.) and a solution of polyethylene glycol 6000 (Carbowax, 8.95g, in 67.8 ml $\rm H_2O$ bidest.) were heated for 20 min at 80 °C, cooled and added to a solution of NaCl (15 mg NaCl in 113.4 ml 0.22 M Na-phosphate buffer, pH 7.0). After shaking and standing for 2 days at 4 °C, two phases had formed. The upper phase (polyethylene glycol) and lower (dextran) phases were separated and sterilized by membrane filtration. The membranes were homogenized in 5 ml of the polyethylene glycol solution and 5 ml of the



dextran phase was added. The two phases were mixed and centrifuged in a swing-out rotor for 10 min at 1,000g. Membranes from the interphase were resuspended in 4 ml polyethylene glycol. After addition of 4 ml dextran phase and mixing, separation was repeated by centrifugation for 10 min at 23,500g in a Sorvall HB4 rotor. The membranes were again collected at the interphase and washed twice in culture medium. Based on the specific activity of marker enzymes $^3 \text{ (Mg}^{2+}\text{-ATPase, NA}^+ - \text{K}^+\text{-ATPase}$ and alkaline phosphatase), the membranes were enriched by a factor of four over the homogenate. The recovery of alkaline phosphatase in the membrane was 30% of the enzyme activity found in unfractionated homogenate. a, Explants from the anterior half of the retina (right) and from the posterior half of the retina (left) grown on the same culture dish; b, c, explants at higher magnification.



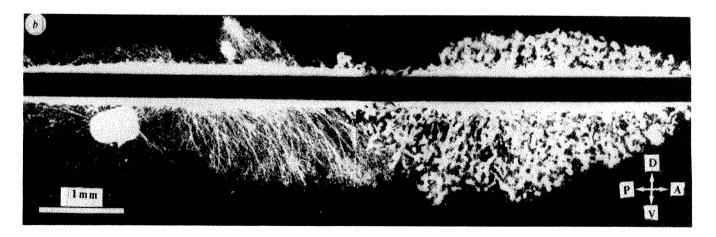


Fig. 2 Adhesion of tectal membranes to antero-posterior strips from the dorsal retina. A retina from a 6-day-old White Leghorn chick embryo was isolated intact and spread on a membrane filter (SM 13006, black; Sartorious); the ganglion cell layer was oriented upwards. The retina and membrane filter were cut into 0.3-mm strips in the antero-posterior direction, inverted on to collagen-coated dishes, and cultured as before, the ganglion cell layer now facing the collagen (Fig. 1), with the exception that a thicker collagen layer was used (0.7 ml collagen solution was allowed to form a gel in Petriperm dishes; Heraeus). After incubation for 30 h, a suspension of tectal membranes (see Fig. 1) was added. After incubation for 2 the unbound membranes were washed off (see Fig. 1) and the specimen observed directly by dark-field illumination. a Is a control without membranes added, showing the oriented outgrowth of fibres towards the original choroid fissure which is oriented vertically through the centre of the strip; b shows the specific adhesion of tectal membranes to the neurites sprouting from the anterior half of the retina. The original orientation of the retina in the embryo is indicated by the windrose (D, dorsal; A, anterior, V, ventral; P, posterior).

Retina from 6-day chick embryos, when explanted and incubated on collagen, shows vigorous fibre growth. Using the choroid fissure as a topographical marker, small pieces of retina from defined regions were explanted. After 2 days in culture, a suspension of tectal cells, isolated by mechanical dissociation, was added and the incubation continued for a further 2 h with gentle shaking at 30-min intervals. Finally, the culture was washed and observed by dark-field illumination. In these experiments, preferential adhesion of tectal cells to neurites growing out from anterior retinal explants was observed. However, clarity of the results was impaired by the fact that the cells adhered to collagen and also to each other (data not shown). A much sharper distinction was obtained using membrane preparations from tectal cells, instead of whole cells. A striking preferential adhesion of tectal membranes to neurites from explants of anterior retina, as opposed to neurites growing out from posterior sections, was observed (Fig. 1). Identical results were obtained with membranes prepared by the elaborate procedure of Merrell and Glaser³

The specific adhesion of tectal membranes to anterior neurites could reflect a continuous gradient or a discontinuous step of adhesiveness in the antero-posterior dimension of the retina. To decide on this alternative the retina was spread on a membrane filter and cut into narrow strips with a tissue chopper. The retinal strips were transferred on to collagen with the filter as a mechanical support. Using the choroid fissure for orientation, strips were cut in antero-posterior and in dorso-ventral directions. Such explants produced neurites in vitro. In agreement with results reported by Bonhoeffer and Huf4, their orientation is similar to that of axons in vivo5, that is, neurites grew centripetally towards the original position of the choroid fissure (Fig. 2a). Antero-posterior strips were explanted and incubated for 30 h and the adhesion of tectal membranes to the neurites was then tested as before. Microscopic examination showed very clearly two distinct areas with a sharp boundary; neurites from the anterior part of the retina were heavily labelled with

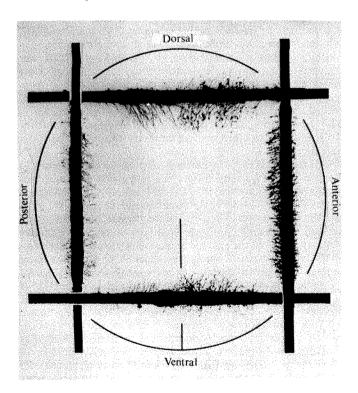


Fig. 3 Composite of four photographs taken from strips of the retina cut as indicated, explanted, incubated and treated with tectal membranes as described (Fig. 2). The antero-posterior sections from the dorsal and ventral parts of the retina came from one retina; the other two sections from another retina of a 6-day-old chick embryo. The samples were stained with Sudan black (Fig. 1) before photography. Over 200 experiments yielded identical results. The vertical line indicates the position of the choroid fissure; the strips are 0.3 mm wide.

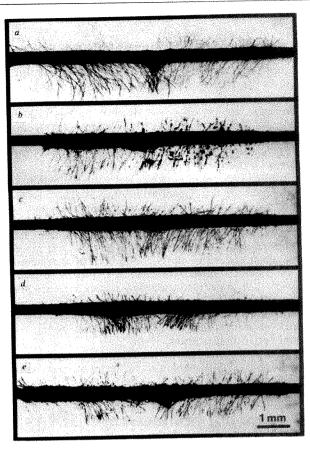


Fig. 4 Adhesion of membranes of different origin to neurites sprouting from antero-posterior sections through chick retina. The retina sections were prepared and incubated as described. After incubation for 30 h, membrane preparations from different parts of the central nervous system were prepared and the samples processed as described in Fig. 1 legend. a, Untreated control; the remaining preparations were treated with: b, membranes from optic tectum; c, membranes from brain prepared after removal of the optic tectum; d, membranes from retina and e, from forebrain. The membranes had all been prepared from 9-day-old White Leghorn chick embryos.

membranes, in contrast to the posterior fibres (Fig. 2b). The neurites from dorso-ventral strips through the anterior part of the retina were covered with large amounts of membrane material whereas on dorso-ventral strips through the posterior part of the retina much less membrane material was bound. Only by scanning electron micrography could membrane vesicles be detected on posterior neurites. A composite of photographs taken from four sections through the anterior, ventral, posterior and dorsal regions of the retina indicates the distinctive adhesion of tectal membranes to neurites originating from the anterior half of the retina, independently of the dorso-ventral coordinate (Fig. 3). All results indicate that neurites from the anterior and posterior part of the retina differ strikingly in binding tectal membranes. A similar antero-posterior polarity in retina can also be seen after a modification of the test system4 in which neurites growing out on cell monolayers can choose between retina and tectum cells; anterior neurites prefer tectum cells, posterior neurites do not (F. Bonhoeffer, personal com-

The sprouting of neurites from retina explants reflects the time course of axon outgrowth in vivo⁵, which occurs between days 5 and 8. All stages within this range showed the specific binding of tectal membranes to anterior neurites. The age of the tectum used for membrane preparation was not relevant for adhesion; membranes from 6-13-day-old embryos all showed adhesion to anterior neurites. Retinal axons do not reach the tectum until day 6 of development⁶⁻⁸, indicating that the neurites do not recognize retina-derived components on the tectum. It remains to be studied whether the neurites in vitro are true axons which originate mainly from ganglion cells, and whether they grow out de novo or are due to

regeneration. However, fibre orientation, time of outgrowth and the agreement between the number of ganglion cells in our explants and the number of fibres (M.C., in preparation), are consistent with a ganglion cell origin.

The origin of the membranes is crucial for the recognition by neurites. In contrast to the membranes from the optic tectum. membranes from fore-brain were not bound to anterior neurites in a selective manner (Fig. 4). However, a slight general adhesiveness of these membrane preparations to the neurites was observed (Fig. 4c-e). Membranes from non-neural tissue such as liver also showed some adhesion to all neurites with no preference for the anterior part.

The adhesion of membranes was not inhibited by blocking protein synthesis in the explant with cycloheximide (5 µg ml⁻¹) before and during incubation with the membranes. Similarly, the addition of concanavalin A (2.5 µg ml⁻¹) and colchicine (12.5 ng ml⁻¹) showed no effect at concentrations which interfered with further neurite elongation. However, fixation with glutaraldehyde and, interestingly, the addition of cytochalasin B (5 µg ml⁻¹) blocked membrane-binding. Cytochalasin B has a specific effect on growth cone activity9, causing a retraction and wilting of the microspikes, by which the axonal environment is explored. Intact function of growth cones, therefore, might be essential for the recognition and/or adhesion of the tectal membranes to retinal neurites.

The antero-posterior polarity as we found it in vitro might be involved in the establishment of retino-tectal connections dur-

ing in vivo development; this assumption, however, requires further study. In any case, the findings clearly show that the retina consists of subareas distinguishable by their interactions with tectal membranes; they provide a relatively simple assay for further studies of this interaction. For example, we can test whether the antero-posterior polarity in the retina has a counterpart in the tectum. Furthermore, a biochemical characterization of the components involved in the interaction of the neurites with the tectal membranes should be possible.

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- Gottlieb, D. I. & Glaser, L. A. Rev. Neurosci. 3, 303-318 (1980). Sperry, R. W. Proc. natn. Acad. Sci. U.S.A. 50, 703-710 (1963). Merrell, R. & Glaser, L. Proc. natn. Acad. Sci. U.S.A. 70, 2794-2798. Bonboeffer, F. & Huf, J. Nature, 288, 162-164 (1980).

- Goldberg, S. & Coulombre, A. J. Comp. Neurol. 146, 507-517. Goldberg, S. Devl Biol. 36, 24-43 (1974).

- Crossland, W. J., Cowan, W. M. & Rogers, L. A. Brain Res. 91, 1-23 (1975).
 Rager, G. & von Oeynhausen, B. Expl Brain Res. 35, 213-227 (1979).
 Yamada, K. M., Spooner, B. S. & Wessels N. K. J. Cell Biol. 49, 614-635 (1971).
 Kalb, V. F. & Bernlohr, R. W. Analyt. Biochem. 82, 362-371 (1977).
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265-275 (1951).
- Wood, P. M. Brain Res. 115, 361-375 (1976).
 Elsdale, T. & Bard, J. J. Cell Biol. 54, 626-637 (1972).
- 14. Brunette, D. M. & Till, J. E. J. Membrane Biol. 5, 215-224 (1971).

One gene, but two messenger RNAs encode liver L and red cell L' pyruvate kinase subunits

Joelle Marie, Marie-Pierre Simon, Jean-Claude Dreyfus & Axel Kahn

Institut de Pathologie Moléculaire, INSERM U 129, Chu Cochin 75674 Paris Cedex 14, France

Pyruvate kinase subunits of red cells and liver differ in their molecular weights. Peptide mapping of the rat enzymes has shown that this difference is due to a single exon peptide present in the red cell enzyme¹⁻³. There is strong genetic evidence in man that both enzymes are encoded by the same structural gene (refs 4-8 and G. E. J. Staal et al., personal communication). Here we describe in vitro protein synthesis experiments using RNA extracted from rat red cells and liver which demonstrate that the difference is reflected in tissue-specific mRNAs. Thus the difference is not due to post-translational processing and presumably involves either gene rearrangement or differential processing of a common nuclear RNA precursor.

In our laboratory, we have demonstrated that pyruvate kinase is synthesized in red cells as L' subunits $(M_r 63,000)$ and in liver as L subunits (M_r 60,000). In vitro, the L'₄ tetramers can be converted by mild tryptic attack into a form with molecular weight and properties similar to liver L_4 pyruvate kinase⁹⁻¹². As both trypsin-treated red cell enzyme and liver pyruvate kinase exhibit improved regulatory properties with respect to native L'4 (refs 9, 12), it was assumed that, in liver, L-type pyruvate kinase undergoes a proteolytic processing. To test this model we have characterized pyruvate kinase neosynthesized in a rabbit reticulocyte lysate system under the direction of human liver RNA, rat liver RNA or rat reticulocyte RNA. If the L and L' subunits differ due to a proteolytic processing, then reticulocyte and liver RNAs should direct the synthesis of identical subunits with an identical molecular weight to L'.

Anti-human red cell pyruvate kinase antiserum was raised in rabbits and specific antibodies purified by chromatography on a pyruvate kinase-linked agarose bead column¹³. The purified

antibodies were then coupled to agarose beads. By exploiting the cross-reactivity between human and rat enzymes, it was possible, using this immunoabsorbent, to purify pyruvate kinase from human liver, rat liver and rat red cells in only one step (Fig. 1). Rat L' and L subunits were slightly heavier than their human counterparts (Mrs 64,000 and 61,000 respectively). Reticulocytosis was induced in rats by treatment with acetylphenylhydrazine¹⁴. Total cellular high-molecular-weight RNA was purified by ethanol precipitation in 7 M guanidine/HCl solution followed by chloroform/isoamyl alcohol extraction and washing in 3 M sodium acetate 13,15. Reticulocyte RNA was fractionated by two successive centrifugations on denaturing methylmercury/formamide gradients. The fractions corresponding to sedimentation coefficients 18S-~24S were pooled and, after ethanol precipitation, RNA was used for in vitro translation. Total cellular high-molecular-weight liver RNA was used to direct protein synthesis without further fractionation.

Cell-free synthesis was performed, in some experiments, in the presence of various antiproteolytic agents: 0.01 mM leupeptin, 0.1 mM chymostatin, 0.1 mM antipain, 0.1 mM pepstatin, 0.1 mM diisopropylphosphofluoridate and 1% (v/v)aprotinin. Neosynthesized pyruvate kinase subunits were purified by immunoaffinity chromatography on microcolumns containing 5 µl of the specific immunoabsorbents¹³. Specificity of the neosynthesized bands was checked by immunological competition with unlabelled L-type pyruvate kinase¹³. Figure 2 shows that rat liver RNA directed synthesis of a polypeptide of M_r 61,000—the same molecular weight as rat liver L-type pyruvate kinase. This molecular weight was the same whether cell-free translation was performed with or without antiproteolytic agents. This polypeptide could be firmly identified as neosynthesized pyruvate kinase because it did not bind to an antipyruvate kinase-agarose column previously saturated with excess unlabelled L-type enzyme but was specifically retained by a nonsaturated column (Fig. 3). The same results were obtained with human liver RNA (not shown).

Figure 4 shows that, by contrast, rat reticulocyte RNA directed synthesis of a $64,000-M_r$ polypeptide (thus similar to rat L') which immunologically competed with unlabelled L-type enzyme for binding to the antibody column. When a mixture of reticulocyte and liver RNAs was translated together, both the

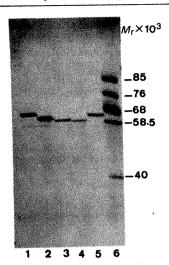


Fig. 1 Purification of red cell and liver pyruvate kinase from man and rat by immunoaffinity microchromatography. Cells or tissues were lysed in a 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl, 0.1 mM β-mercaptoethanol, 10 mM EDTA, 10 mM ε-aminocaproic acid, 2% (v/v) aprotinin, 1 mM diisopropylphosphofluoridate, 0.1 mM pepstatin, 0.1 mM chymostatin, 0.1 mM antipain, 0.01 mM leupeptin, 1% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate (= lysis buffer). After elimination of the insoluble cell debris by centrifugation (at 36,000 g for 20 min), the extracts were applied to microcolumns made with Eppendorf blue tips and containing 10 µl of immunoabsorbent (4 mg of specific anti-L-type pyruvate kinase immunoglobulins per ml of resin). About 3 IU of pyruvate kinase were bound per column. The columns were washed successively with 5 ml of lysis buffer, 5 ml of 0.1 M sodium phosphate buffer (pH 7.4) containing 1 M KCl and 5 ml of 0.1 M sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl. They were eluted with 800 µl of 0.1% (w/v) SDS in 10% (v/v) acetic acid. The eluates were lyophilized and the lyophilisates dissolved in 40 µl of 0.0685 mM Tris/HCl buffer (pH 6.8) containing 10% glycerol (v/v)5% β -mercaptoethanol (v/v) and 0.0 01% bromophenol blue (w/v); dodecylsulphate concentration of these samples was therefore 2% (w/v), such that they could be directly applied, after dissociation (2 min in boiling water), to dodecylsulphate/polyacrylamide gradient gel electrophoresis according to Laemmli²⁷. Proteins were stained with Coomassie brilliant blue. 1, rat red cell; 2, rat liver; 3, human liver; 4, trypsin-treated human red cell enzyme⁹; 5, untreated human red cell enzyme; 6, protein markers: urease (Mr 85,000), transferrin (76,000), bovine serum albumin (68,000), glucose phosphate isomerase (58,500) and half urease (40,000). The faint band corresponding to a M_r of $\sim 57,500$ observed in the pyruvate kinase preparations corresponds to Lc, a partially proteolysed form

61,000 and 64,000 bands could be detected (not shown). This result, and the fact that antiproteolytic agents did not modify the molecular weight of neosynthesized liver pyruvate kinase, exclude the possibility that neosynthesized chains are cleaved by proteases present in the reticulocyte lysate or by neosynthesized proteases. Clearly, therefore, in rat, L and L' pyruvate kinase subunits are synthesized under the direction of two different species of mRNA. Although it was not possible to study human reticulocyte RNA, the same conclusion can probably be drawn in man because human liver RNA directs synthesis of L, and not L' as predicted by the hypothesis of the 'proteolytic processing'.

As studies of inherited red cell pyruvate kinase deficiency indicate unambiguously that L and L' subunits are encoded by the same gene, our results strongly suggest that this gene is associated with two types of mRNA present in the different cells—erythroid and liver. Two basic mechanisms could explain this finding: a rearrangement of the L-type pyruvate kinase gene during erythroid or liver differentiation, or a differential processing of a common nuclear RNA precursor.

In lower eukaryotes, a gene rearrangement mechanism has been implicated, such as in the yeast mating system 16,17 and modification of trypanosome surface antigens 18.

In vertebrates, gene rearrangement has been described only for immunoglobulin genes during B-cell differentiation19 Differential processing of nuclear RNA has been described for immunoglobulin μ chains²²⁻²⁴, where both types of μ chain, and therefore both types of mRNA, are produced in the same cells, whereas in the case of pyruvate kinase, L' is the only form produced in the reticulocytes and L the only form in the liver. Differential splicing of nuclear RNA in salivary glands and liver 25,26 has also been shown for mouse α -amylase; in this case the two species of mRNA encode the same protein. These results suggest that differentiation can be associated with changes of protein form that are not directly related to the usual phenomena of gene activation and inactivation, but rather to modifications of gene arrangement or of processing of nuclear RNA into cytoplas mic translatable mRNA. The study of pyruvate kinase genomic DNA in different cells should elucidate this problem, but requires a cDNA probe which may be difficult to obtain because of the very low amount of specific mRNA:

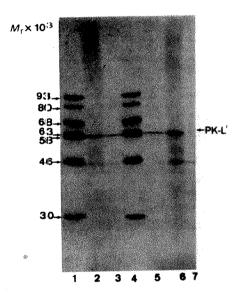


Fig. 2 Characterization of neosynthesized liver L-type pyruvate kinase subunits. Rabbit reticulocyte lysate was deprived of endogenous RNA by micrococcal nuclease treatment, according to ref. 28. Translation mixture (250 µl) contained, in final concentration, 20 μM essential amino acids except methionine, 0.5 mCi ³⁵Smethionine (specific activity 1,000 Ci mmol-1), 1 mM ATP, 0.2 mM GTP, 0.3 mM spermidine, 1 mM dithiothreitol, 10 µM haemin, 10 mM creatine phosphate, 60 µg ml⁻¹ creatine phosphokinase, 20 mM HEPES buffer, pH 7.4, 120 mM K acetate, 1.20 mM Mg a cetate and 40 µg liver RNA. Translation was carried out for 1 h at 37 °C, then the reaction was diluted with 2 vol of the 'lysis buffer' described in Fig. 1 legend and centrifuged at 36,000 g for 30 min. The diluted translation mixture was applied to two combined microcolumns (inserted into each other), containing 10 μ l nonimmune γ -globulin-linked resin at the top and 5 μ l anti-pyruvate: kinase resin at the bottom. After about six passages through this system, the microcolumns were separated, individually washed and eluted as described in Fig. 1 legend. Lanes 1 and 4, C-labelled protein markers: rabbit muscle phosphorylase (M, 93,000), human muscle phosphofructokinase (M_r 80,000), bovine serum albumin (M, 68,000), human L'4 red cell pyruvate kinase $(M_r 63,000)$, human glucose phosphate isomerase $(M_r 58,500)$, hen egg ovalbumin (M, 46,000) and bovine carbonic anhydrase (M_r 30,000). Lanes 2 and 6, eluates from anti-pyruvate kinase columns, synthesis without addition of antiproteolytic agents. 3 and 5, eluates from anti-pyruvate kinase columns, synthesis in the presence of 0.01 mM leupeptin, 0.1 mM pepstatin, 0.1 mM chymostatin, 0.1 mM antipaīn, 0.1 mM diisopropylphospho-fluoridate, 1% (v/v) aprotinin. 7, eluate from a nonimmune column. The $43,000-M_r$ band, observed in the eluates from nonimmune systems as well as from some specific immuno-absorbent columns, can be identified as contaminant actin 13. Fluorography was performed as in refs 29 and 30.

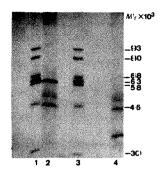


Fig. 3 Characterization of neosynthesized liver L-type pyruvate kinase by immunological competition. Cell-free translation was carried out as indicated in Fig. 2 legend, but the reaction was passed, successively, through saturated and nonsaturated antipyruvate kinase resins. The immunoabsorbent was saturated with 100 µg cold human L-type enzyme, then the res in was extensively washed before being used with the neosynthesized products. Lanes 1 and 3, ¹⁴C-labelled protein markers (as in Fig. 2 legend). 2, eluate ⁴C-labelled protein markers (as in Fig. 2 legend). 2, eluate from a 5-µl nonsaturated anti-pyruvate kinase column. 4, eluate from 5-µl saturated anti-pyruvate kinase column. Detection of the radioactive bands by fluorograph v.

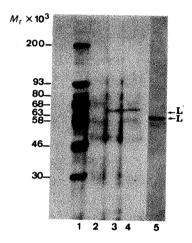


Fig. 4 Characterization of neosynthesized L' red cell pyruvate kinase by immunological competition. Comparison with neosynthesized L enzyme. Cell-free synthesis was directed by fractionated 18S-24S reticulocyte RNA (30 µg per 250 µl of translation medium) (lanes 2-4) or by rat liver RNA, as indicated in Fig. 2 legend (lane 5).

Fractionation of reticulocyte RNA was carried out by centrifugation in denaturing methylmercury formamide/sucrose gradient. 400 µg RNA were first dissolved in 20 µl 10 mM methylmercury, incubated at room temperature for 5 min, then mixed with 200 µl of 90% deionized formamide (v/v) in 10 mM Tris-HCl, pH 7.5, 10 mM iodoacetate, 5 mM EDTA and 0.5% lauroyl sarcosine. After a 15-min incubation at room temperature, this RNA solution was diluted with 250 µl of the Tris-HCl buffer described above, but without formamide, and applied to an 11-ml gradient sucrose: 10-25% (w/v) sucrose in 10 mM Trisi-HCl, pH 7, containing 50% (v/v) deionized formamide, 10 mM iodoacetate, 5 mM EDTA and 0.5% (w/v) lauroyl sarcosine. Centrifugation was carried out in a SW 41 Beckman rotor, at 40,000 r.p.m. for 14 h. The 18S-24S RNA was pooled and precipitated by ethanol, and then applied to a second gradient. The 18S-2:4S fraction obtained from the second round was used for directing cell-free

translation.

Lane 1, 14 C-labelled protein markers: myosin (M_r 200,000), phosphorylase, muscle phosphofructokinase, bovine serum albumin, L' human pyruvate kinase, glucose phosphate isomerase, ovalbumin, carbonic anhydrase and lactoglobulin A $(M_r 18,500)$. Lane 2, synthesis directed by reticulocyte RNA, eluate from a 5-µl saturated anti-pyruvate kinase column. Lanes 3 and 4, synthesis directed by reticulocyte RNA; eluates from 5-µl nonsaturated anti-pyruvate kinase columns. Lane 5, synthesis directed by liver RNA, eluate from 5-µl nonsaturated anti-pyruvate kinase column. Detection by fluorography.

pyruvate kinase represents < 0.05% of the proteins synthesized under the direction of liver RNA in cell-free conditions and this proportion is even less for reticulocyte RNA.

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Note added in proof: We recently succeeded in translating in a cell-free system both L and L' messenger RNA from fetal human liver, which confirms that in man as well as in rat L and L' pyruvate kinase subunits are encoded by two different species of messenger RNAs.

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- 1. Cleveland, D. W., Fisher, S. G., Kirschner, M. W. & Laemmli, U. K. J. biol. Chem. 252,
- 2. Harada, K., Saheki, S., Wada, K. & Tanaka, T. Biochim. biophys. Acta 524, 327-339
- Saheki, S., Saheki, K. & Tanaka, T. FEBS Lett. 93, 25-28 (1978)
- Sailett, S., Sailett, K. & Tanaka, I. FEBS Lett. 93, 25-28 (19/8). Bigley, R. H. & Koler, R. D. Ann. hum. Genet. 31, 383-390 (1965). Kahn, A., Marie, J., Galand, C. & Boivin, P. Scand. J. Haemat. 16, 250-257 (1976). Nakashima, K. et al. Blood 43, 537-548 (1974).

- Nakashima, K. et al. J. Lab. clin. Med. 90, 1012-1020 (1977). Shinohara, K. et al. Am. J. hum. Genet. 28, 474-481 (1976).
- Kahn, A., Marie, J., Garreau, H. & Sprengers, E. D. Biochim. biophys. Acta 523, 59-74
- 10. Marie, J., Garreau, H. & Kahn, A. FEBS Lett. 78, 91-94 (1977)

- Marie, J. & Kahn, A. Biochem. biophys. Res. Commun. 91, 123-129 (1979).

 Sprengers, E. D. & Staal, G. E. J. Biochim. biophys. Acta 570, 259-270 (1979).

 Kahn, A., Cottreau, D., Daegelen, D. & Dreyfus, J. C. Eur. J. Biochem. 116, 7-12 (1981). Hunt, T. & Jackson, R. J. in Modern Trends in Hunnan Leukaemia (eds Neth. R., Gallo, R. C., Spiegelman, S. E. & Stohlman, F.) 300-307 (Lehmans, Munich, 1974).
 Cox, R. A. Meth. Enzym. 12, 120-129 (1968).
 Hicks, J., Strathern, J. & Klar, A. J. S. Nature 282, 478-482 (1979).
 Nasmyth, K. A. & Tatchell, K. Cell 19, 753-764 (1980).
 Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P. & Cross, G. A. M. Nature 284, 78-80 (1980).

- 284, 78-80 (1980).
- 19. Davis, M. M. et al. Nature 283, 733-739 (1980).
- 20. Early, P. W., Davis, M. M., Kaback, D. B., Davidson, N. & Hood, L. Proc. natn. Acad. Sci. U.S.A. 76, 857-861 (1979)
- Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. Cell 19, 981-992 (1980).
 Alt, F. W. et al. Cell 20, 293-301 (1980).
 Early, P. et al. Cell 20, 313-319 (1980).

- Singer, P. A., Singer, H. H. & Williamson, A. R. Nature 285, 294-300 (1980).
- Hagenbüchle, O. et al. Nature 289, 643-646 (1981). Young, R. A., Hagenbüchle, O. & Schibler, U. Cell 23, 451-458 (1981). Laemmli, U. K. Nature 227, 680-685 (1970).
- 28. Pehlam, H. P. B. & Jackson, R. J. Eur. J. Biochem. 67, 247-256 (1976).
- Bonner, W. M. & Laskey, A. R. Eur. J. Biochem. 46, 83-88 (1974)
 Laskey, R. A. & Mills, A. D. Eur. J. Biochem. 56, 335-341 (1975).

Nucleotide sequence of the

haemagglutinin gene of a human influenza virus H1 subtype

Greg Winter*, Stan Fields* & George G. Brownlee†

*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

†Sir William Dunn School of Pathology, University of Oxford. South Parks Road, Oxford OX1 3RE, UK

Influenza remains a serious cause of disease in man and of death in the aged. Vaccination is only transiently effective in providing immunity1, because the surface haemagglutinin molecule of the virus can evolve rapidly, allowing the virus to escape neutralization by immunizing antibody. Of the three influenza A subtypes² known to cause infection in man, the H1 subtype is of particular interest as it has proved to be the dominant human subtype this century and is now co-circulating with variants of the H3 subtype. Here we present the sequence of the haemagglutinin gene of an early H1 subtype (strain A/PR/8/34) as determined by recombinant DNA methods and dideoxy sequencing. From the deduced amino acid sequence of the haemagglutinin we define an antigenic site at amino acid residue 160. Comparison with haemagglutinin molecules³⁻⁹ shows that the H1 and H2 subtypes are more homologous to one another than to the other subtypes, which suggests that they have diverged only recently from one to another.

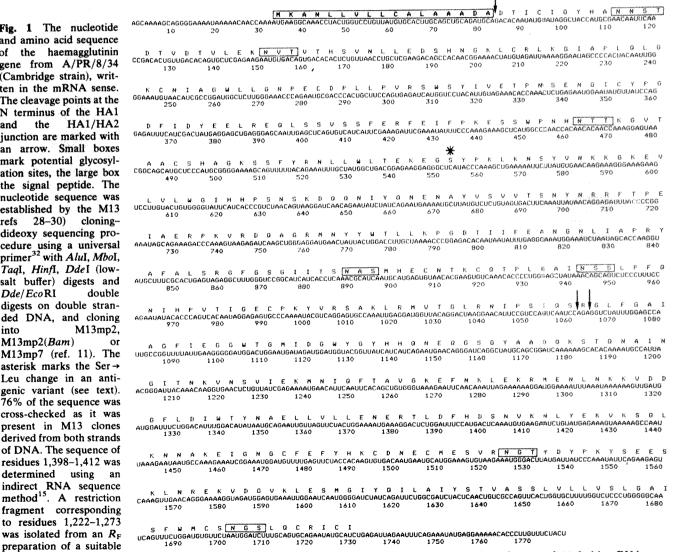
Influenza is a negative-stranded virus with eight RNA genes coding for 10 or more proteins, of which band 4 codes for the haemagglutinin gene. The main strategy for sequencing this gene was similar to that used in a parallel study of the neuraminidase gene 10-12. Briefly we prepared restriction fragments derived from double-stranded DNA, itself prepared by in vitro copying of the RNA gene 4 by reverse transcriptase. These restriction fragments were cloned into the bacteriophage M13 vector and sequenced by the Sanger method^{13,14}. By sequencing clones which were isolated at random and derived from several different restriction enzyme digests, we hoped to overlap these clones and deduce a unique sequence for the haemagglutinin gene. This objective was only 99% achieved; for the remaining 1% of the sequence we had to use an indirect RNA sequencing method¹⁵. We also used a direct RNA sequencing method to confirm the sequence of one end of the gene 16.

Figure 1 shows the deduced nucleotide sequence, written in the mRNA sense. Its organization is similar to haemagglutinin molecules previously studied in other subtypes^{3-9,17}. We can identify a 17-residue hydrophobic signal peptide, an HA1 subunit (326 residues long) and an HA2 subunit (222 residues long). These, as in the other human subtypes, are separated by a single arginine residue (327) which is presumably recognized during processing by a trypsin-like enzyme. The HA2 subunit contains the characteristic conserved 14-amino acid long hydrophobic N-terminal sequence as well as a hydrophobic tail, believed to anchor the molecule to the phospholipid bilayer of the cell18.

Our studies provided no direct information on the secondary structure of the haemagglutinin, but we can deduce from the conservation of cysteine residues (Fig. 2) that the pattern of disulphide bonds is probably similar to that of the H2 and H3 subtypes, where most cysteines are involved in S-S bridges within a subunit, except for C(14) and C(466) (see Fig. 2) which interconnect the separate subunits19. There is no information on the three conserved cysteine residues close to the C terminus of the HA2 subunit. The haemagglutinin is a glycoprotein in which we observe seven potential N-X-S or N-X-T sequences (Fig. 1) where carbohydrate could be attached²⁰. There have been no direct studies of the location of the carbohydrate in A/PR/8/34, but studies of related strains (A/WSN/33)21 and other subtypes (A/Japan/57)4 suggest that the majority are glycosylated. Presumably the most N-terminal glycosylation site in the HA1 subunit (N-N-S-T-a sequence also conserved in the H2 subtype) is glycosylated at one but not both of the asparagine residues4.

Monoclonal antibodies have been previously used to select A/PR/8/34 variants which escape antibody neutralization because of their altered antigenicity²². A common variant contained a leucine residue replacing a serine residue in the parental tryptic peptide (SEPGY)K, although this peptide could not be further localized within the sequence. From the amino acid sequence predicted here (Figs 1, 2), we identify this variant as serine → leucine (160) caused by a C → U transition at base 553. Residue 160 (for numbering of amino acids, see Fig. 2

Fig. 1 The nucleotide and amino acid sequence of the haemagglutinin gene from A/PR/8/34 (Cambridge strain), written in the mRNA sense. The cleavage points at the N terminus of the HA1 the HA1/HA2 junction are marked with an arrow. Small boxes mark potential glycosylation sites, the large box the signal peptide. The nucleotide sequence was established by the M13 refs 28-30) cloningdideoxy sequencing procedure using a universal primer³² with AluI, MboI, TaqI, HinfI, DdeI (lowsalt buffer) digests and Dde/EcoRI double digests on double stranded DNA, and cloning M13mp2, into M13mp2(Bam)M13mp7 (ref. 11). The asterisk marks the Ser→ Leu change in an antigenic variant (see text). 76% of the sequence was cross-checked as it was present in M13 clones derived from both strands of DNA. The sequence of residues 1,398-1,412 was determined using an indirect RNA sequence method¹⁵. A restriction fragment corresponding to residues 1.222-1.273 was isolated from an R_F



recombinant haemagglutinin clone and was used as a primer for dideoxy sequencing using reverse transcriptase and total virion RNA as template ¹⁰. The 5' end of band 4 virian RNA was checked by direct RNA sequencing ¹⁶. The computer programs of Staden ³¹ were used to assist data handling and overlapping of fragments.

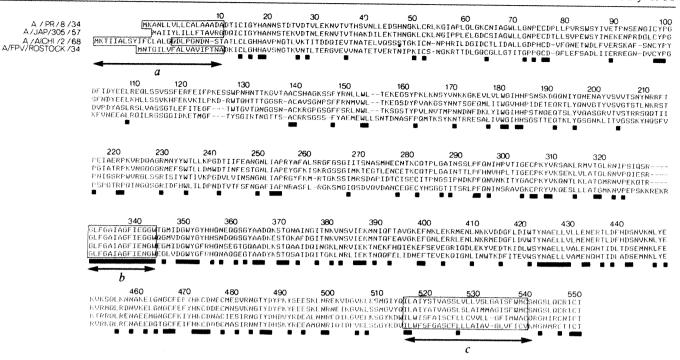


Fig. 2 The amino acid sequences of haemagglutinin from A/PR/8/34 (H1 subtype), A/Jap/305/57 (H2 subtype), A/Aichi/2/68 (H3 subtype) and A/FPV/Rostock/34 (H7 subtype) are listed with gaps inserted generally to maximize amino acid homology and particularly the alignment of cysteine, tryptophan and proline residues. The precise position of gaps is rather subjective but was finally decided after inspection of the three-dimensional structure of the H3 subtype. Saps were then introduced into regions which, by analogy with the H3 subtype, might be expected to be in surface loops. The numbering system is based on consecutive amino acid residues (ignoring gaps) starting at the terminus of the H3 subtype (A/Aichi/2/68). Thus residue 160 of A/PR/8/34 is actually residue 157 from its N terminus, but is homologous to 160 of A/Aichi/2/68. The boxed areas a, b and c mark, respectively, the signal peptide region, the conserved N terminus of the HA1 subunit and the membrane attachment C-terminal tail.

legend) has not previously been implicated as an antigenic site in the better studied H3 subtype, despite the fact that the homologous residue threonine (160) seems to be on the surface of the haemagglutinin in the proposed site B in the recently available three-dimensional model of the H3 subtype²³. Perhaps the presence of the nearby carbohydrate side chain at residue 165 masks this region in the H3 subtypes. Further studies of other laboratory variants as well as field strains with altered antigenicity are required to define the antigenic regions of the H1 subtype in more detail.

Figure 2 shows a comparison of the amino acid sequence of the A/PR/8/34 haemagglutinin with representative strains of three other subtypes. The fact that only occasional gaps were required in one or other of the sequences to maintain alignment confirms the basic homology of all haemagglutinin subtypes. Furthermore, the conservation of cysteine and many proline residues predicts a common overall shape. Table 1 gives a quantitative measure of the extent of amino acid and nucleotide similarity between the different subtypes. This clearly shows that the H1 and H2 subtypes are more closely related to one another in both their HA1 and HA2 subunits than either is to the H3 or

Table 1 Conservation of amino acid and nucleotide sequences in strains of different H subtypes

		% Amino acid conservation			
	H1	H2	Н3	H7	
H1		58(79)	35(53)	33(51)	
H2	61(72)		36(50)	35(53)	
H3	45(58)	45(57)		36(65)	
H7	44(58)	46(59)	45(66)		
	%	Nucleotide con	servation		

The conservation in the HA1 subunit is given first, with the conservation in the HA2 subunit shown in parentheses. Amino acid conservation is listed above the diagonal and nucleotide conservation below. See Fig. 1 legend for the specific strains used in this comparison.

H7 subtypes. The 58% amino acid conservation in the HA1 subunit of the H1 and H2 subtypes is far greater than the typical 33-36% similarity of the other possible comparisons. The fact that H1 and H2 subtypes are related is also apparent from the nucleotide sequence comparisons (Table 1).

The demonstrable homology of all haemagglutinin subtypes shows that they must have evolved from a common ancestral gene by a process of point mutation and the occasional deletion and addition. From the extent of antigenic drift in the human H3 subtypes, we know that change can occur quickly at a rate in the order of 1% amino acid change per year for the HA1 subunit (a value estimated from the extent of drift in the H3 series A/NT/60/68, A/Memphis/102/72 and A/Victoria/2/75)^{24,8} Thus, in theory, if this rate of drift were maintained and were also applicable to the H1 subtype, the 42% difference in the amino acid sequence of the HA1 subunit of the H1 and H2 subtypes could have arisen on the assumption that H2 evolved from the H1 subtype in a 42-yr period. But surveillance of human influenza strains since their isolation in 1933 (ref. 25) suggests that the H1 subtype did not undergo such an extensive 'drift', at least in man. No intermediate linking strains spanning the H1 and H2 subtypes were isolated; the H2 subtype appeared suddenly in 1957 (along with a new neuraminidase subtype) presumably from a mammal or bird reservoir in which influenza is common²⁶. There remains the possibility that drift of the H1 subtype giving rise to the H2 subtype occurred in a mammalian or avian reservoir. Indeed, H1 strains do infect pigs25 but again no evidence of intermediate strains between the H1 and H2 subtype have been observed in the last 48 yr.

Thus, the H1 and H2 subtypes are evolutionary more closely related to one another than either is to the H3 and H7 subtypes but the evolutionary time scale cannot yet be estimated. It will be interesting to sequence completely some of the eight other known mammalian or avian haemagglutinin molecules because distant relationships between subtypes could have remained undetected in the traditional immunological screening methods used to classify haemagglutinin subtypes². An independent

sequence analysis of the haemagglutinin of another H1 subtype, A/WSN/33, has recently been completed²⁷. This strain also shows homology to the H2 subtype.

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Note added in proof: We recently learnt of a Chinese report³³ providing serological evidence of shared antigenic determinants between late variants of the H1 subtype and early variants of the H2 subtype; this correlates well with their molecular homology.

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- Tyrrell, D. A. J. & Smith, J. W. G. Br. med. Bull. 35, 77-85 (1979).
- Tyrrell, D. A. J. & Smith, J. W. G. Br. med. Bull. 35, 77-85 (1979). Schild, G. C. et al. in Structure and Variation in Influenza Virus (eds Laver, W. G. & Air, G. M.) 373-383 (Elsevier, New York, 1980). Porter, A. G. et al. Nature 282, 471-477 (1979). Gething, M. J. et al. Nature 287, 301-306 (1980). Both, G. W. & Sleigh, M. J. Nucleic Acids Res. 8, 2561-2575 (1980). Min Jou, M. et al. Cell 19, 683-696 (1980). Sleigh M. J. et al. in Structure and Variation in Influenza Virus (eds Laver, W. G. & Air. 1980).

- Min Jou, M. et al. Cell 19, 683-696 (1980).
 Sleigh, M. J. et al. in Structure and Variation in Influenza Virus (eds Laver, W. G. & Air, G. M.) 69-78 (Elsevier, New York, 1980).
 Verhoeyen, M. et al. Nature 286, 771-776 (1980).
 Ward, C. W. & Dopheide, T. Virology 103, 37-53 (1980).
 Winter, G. & Fields, S. Nucleic Acids Res. 8, 1965-1974 (1980).
 Fields, S., Winter, G. & Brownlee, G. G. Nature 290, 213-217 (1981).
 Winter, G. et al. Nucleic Acids Res. 9, 237-245 (1981).
 Sanger, F., Nicklen, S. & Coulson, A. R. Proc. natn. Acad. Sci. U.S.A. 74, 5463-5467 (1977).

- Sanger, F. et al. J. molec. Biol. 143, 161-178 (1980).
 Hamlyn, P. H. et al. Cell 15, 1067-1075 (1978).
 Simoncsitz, A. et al. Nature 269, 833-836 (1977).
- 17. Brownlee, G.G. in Structure and Variation in influenza Virus (eds Laver, W. G. & Air, G. M.) 385-390 (Elsevier, New York, 1980). Waterfield, M. D. et al. Br. med. Bull. 35, 57-63 (1979).

- Waterfield, M. D. et al. Br. med. Bull. 35, 57-03 (1979).
 Waterfield, M., Scrace, G. & Skehel, J. Nature 289, 422-424 (1981).
 Ward, C. W. & Dopheide, T. A. Br. med. Bull. 35, 51-56 (1979).
 Nakamura, K., Bhown, A. S. & Compans, R. W. Virology 107, 208-221 (1980).
 Laver, W. G., Gerhard, W., Webster, R. G., Frankel, M. E. & Air, G. M. Proc. natn. Acad. Sci. U.S.A. 76, 1425-1429 (1979).
 Wiley, D. C., Wilson, I. A. & Skehel, J. J. Nature 289, 373-378 (1981).
- 24. Brownlee, G. G. in Expression of Eucaryotic, Viral and Cellular Genes (Academic, London, Brownlee, G. G. in Expression of Eucaryotic, viral and Centular Genes (Academic in the press).

 Pereira, M. S. Br. med. Bull. 35, 9-14 (1979).

 Laver, W. G. & Webster, R. G. Br. med. Bull. 35, 29-33 (1979).

 Hiti, A. L., Davis, A. R. & Nayak, D. P. Virology (in the press).

 Gronenborn, B. & Messing, J. Nature 272, 375-377 (1978).

 Rothstein, R. J. et al. Meth. Enzym. 68, 101-110 (1979).

 Messing, J., Crea, R. & Seeburg, P. H. Nucleic Actist Res. 9, 309-321 (1981).

 Staden, R. Nucleic Acids Res. 8, 3673-3694 (1980).

 Duckworth, M. L. et al. Nucleic Acids Res. 9, 1691-1706 (1981).

 Gengai J. et al. Scientis Sinica 23, 1063-1069 (1979).

- 33. Gengqi, L. et al. Scientia Sinica 23, 1063-1069 (1979).

The origins of replication of the yeast mitochondrial genome and the phenomenon of suppressivity

Miklos de Zamaroczy, Renzo Marotta, Godeleine Faugeron-Fonty, Regina Goursot, Marguerite Mangin, Giuseppe Baldacci & Giorgio Bernardi

Laboratoire de Génétique de Moléculaire, Institut de Recherche en Biologie Moléculaire, 2, Place Jussieu, 75005 Paris, France

The 'petite colonie' mutation of Saccharomyces cerevisiae1,2 is characterized by an irreversible loss of respiration and by an extraordinarily high spontaneous mutation rate^{3,4}. Crosses of wild-type cells with petite mutants exhibit a non-mendelian segregation of the mutation, yielding either wild-type progeny , or both wild-type and petite mutants in proportions essentially dependent on the particular petite used3. In the first case, the petites entering the cross are called neutral, in the second one suppressive³. While the molecular basis of the spontaneous petite mutation is now understood⁴⁻¹², suppressivity has remained an elusive phenomenon for the past 25 yr. We report here that the mitochondrial genome of most spontaneous petites (which is exclusively made up by the tandem repetition of a DNA segment excised from the genome of parental wild-type cells -8) carries at least one of the ori

sequences of the parental wild-type genome. These are long homologous DNA stretches showing striking similarities with the origins of replication of mitochondrial DNAs from mammalian cells. The properties (intact or altered primary structure, high or low number) of the ori sequences of petite genomes seem to determine suppressivity—the level of transmission of petite genomes to the progeny of crosses with wild-type cells. These results indicate that ori sequences are indeed origins of DNA replication and that suppressivity depends on the relative replication efficiencies of petite and wild-type genomes.

We previously proposed that the mitochondrial genome of wild-type yeast cells contains several origins of replication and that at least one of them is carried by the excised segment which will become the repeat unit of a spontaneous petite¹³. We tentatively localized such an ori sequence within the common central stretch of the genome of two spontaneous petites excised from the same region of the wild-type genome^{6,7}. This stretch is characterized (refs 7, 12 and Figs 1, 2, 4) by two short GC clusters, A and B, flanking a 23-nucleotide AT palindrome, p, and a short sequence, s; and by one long GC cluster, C, separated from B by long AT sequence, l. We then found a common 80-base pair sequence centred on cluster B, and more recently, the whole *ori* sequence in petite genomes derived from five regions of the wild-type genome 10-12,14,15.

Screening of the mitochondrial genomes of ~400 spontaneous petites derived from wild-type strain A⁵ revealed that all except two contained an ori sequence, as judged from the restriction sites located on clusters A, B and C (Fig. 1; Table 1). Restriction mapping showed that the eri sequences of different petite genomes were surrounded by different flanking regions and indicated the existence of at least seven ori sequences, ori 1-7 (Fig. 1), on the wild-type genome. This conclusion was confirmed by hybridization experiments in which labelled mitochondrial DNAs from petite genomes carrying different ori sequences showed seven common hybridization bands on HaeIII fragments from the parental wild-type genome; these fragments had the same sizes as HaeIII fragments carrying the different ori sequences on the petite genomes of Fig. 1. An eighth hybridization band might indicate the existence of yet another origin.

Figure 1 also shows that (1) three petite genomes, a-3/1/5/B1, b17 and a-3/1, contained two ori sequences each; (2) two petite genomes, a-3/1/5 and a-3/1/33, had an ori sequence lacking cluster C, and two petite genomes, a-1/1R/14 and a-1/1R/1/26, had an ori sequence lacking cluster A (ori petites, having a partially deleted ori sequence); (3) petite a-15/4/1 contained two ori sequences in opposite orientations—the only rearranged repeat unit found so far among spontaneous petites (this sort of petite will be indicated as ori'); (4) petite a-15/4/1/10/3, derived by subcloning from the previous one, lacked an ori sequence altogether; another ori petite, a-3/1/B4, was a diploid petite issued from a cross of a-3/1 with wild-type strain B and derived from the 15S-oxi 3 region of the B genome; these ori⁰ petite genomes will be discussed in detail elsewhere.

Figure 2 presents ori sequences 1-5 as determined on the repeat units of 11 different spontaneous petites (see Table 1). The ori 6 sequence and the right end of ori 7 were found in the published sequences of petites DS-400/A12 and DS-14 (refs 16, 17). Homology in the primary structure of different petite genomes carrying ori 1 or 3 was nearly perfect, the very rare differences being indicated by asterisks in Fig. 2. Comparison of different ori sequences indicated perfect homology in clusters A, B and C (some differences in clusters A and B of ori 6 being probably due to sequence uncertainties16 and several differences in region l between clusters B and C.

The stretch shared by all ori sequences is 265 base pairs long, its left border at nucleotide 69, and its right border at nucleotide 334 of Fig. 2. Note, however, that at the left of cluster A ori 6 presents a very large GC cluster, α , absent in at least ori 1-5; (2) ori 4 and 6 contain two additional GC clusters, β and γ , identical in both sequence and location; β is located between clusters B

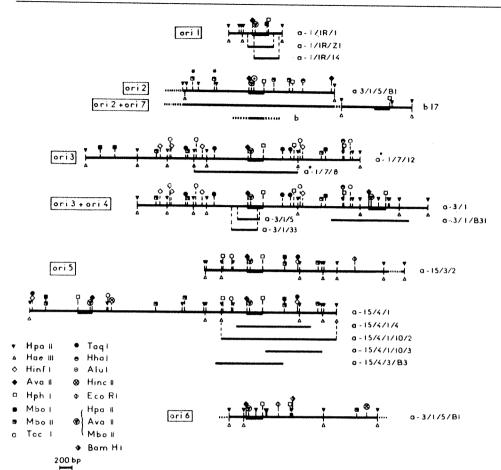


Fig. 1 Restriction enzyme maps of the repeat units of mitochondrial genomes of spontaneous petite mutants. These arose from wild-type strain A, except for a-3/1/5/B1, b17 and b, which derived from a region (oli 1-2) of wild-type strain B in which this genome has the same map as A. Some maps present additions and corrections compared with ref. 5. The map of b17 is a preliminary one; that of b contains an internal deletion in its repeat unit (in preparation). Restriction sites are indicated on some repeat units only and not all AvaII sites are shown. Asterisks indicate restriction sites whose locations need to be confirmed. Ori sequences are underlined: cluster A corresponds to an AvaII site, cluster B to HpaII, AvaII, MboII site clusters, cluster C to a HphI and a MnlI site. All maps are centred on cluster B and oriented with clusters A, B, C, from left to right. Vertical broken lines indicate excision sites where precisely known, bp, Base pairs.

and C, γ at the right of cluster C; at least cluster γ is also shared by ori 7; (3) ori 4-7 are homologous for 30-40 nucleotides at the right of the position of cluster γ ; (4) some sequence homology is present on the left of the ori sequences, particularly in ori 1 and 2; (5) the overall homology between ori 4, 6 and possibly 7 may comprise 370 nucleotides; (6) ori 1 and 2 show homologies for at least 30 base pairs on the right of the ori sequence; and (7) the full sequence of the repeat units of ori petites (ref. 12 and paper in preparation) indicated that these are the result of excisions involving two direct repeats, one of which is within the ori sequence.

Hybridization of labelled petite mitochondrial DNAs containing different ori sequences on restriction fragments from wild-type DNAs has been performed and restriction maps of both sets of DNAs have been compared with maps from other laboratories ¹⁶⁻²⁶. Using these results, it was possible to localize and orient (Fig. 3) ori 1-7 on the mitochondrial genome of wild-type cells and show that the inverted repetition of ori 5 on a-15/4/1 was not present on the wild-type genome. The distribution of the ori sequences is very uneven with five sequences on one-third of the map, the region most often represented among the genomes of spontaneous petites²⁷. Note that ori

Fig. 2 Primary structure of the ori sequences and their flanking regions, as determined by the method of Maxam and Gilbert⁴³ on the repeat units of the mitochondrial genomes from petites a-1/1R/1 (ori 1; position 1 corresponds to position 301 on the repeat unit of the reference sequence of a-1/1R/1)⁷, b (ori 2), a-3/1 (ori 3), a-3/1/B31 (ori 4), a-15/4/1/3/B3 (ori 5), DS-400/A12 (ori 6; from ref. 16) and DS-14 (ori 7; from ref. 17). The sequence on the left of ori 5 is from HS 1948 (ref. 31). Ori sequences were also determined on the repeat units of petites a-1/1R/Z1, a-1/1R/14 (ori 1) a-1/1R/26, a*-1/7/12, a*-1/7/8, a-3/1/5, a-3/1/33 (ori 3). Asterisks indicate the positions of modified nucleotides (indicated as N) or of differences in the same ori sequence as determined on different petites. Regions of ori sequences are indicated by thick lines for GC clusters A, B and C and thin lines for AT regions p, s and l. The positions of clusters β and γ in ori 4, 6 and 7 are given as well as the sequences of these clusters (bottom lines). Restriction sites are indicated by the symbols shown. The sequence of ori 3 corrects some mistakes¹⁴ in the 10 nucleotides at the left of cluster A, and in one nucleotide at the right of cluster B.

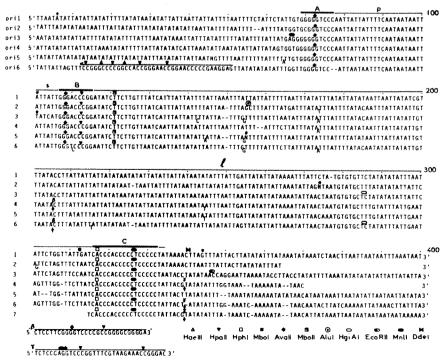


Table 1 The mitochondrial genomes of spontaneous petites: properties of ori sequences and suppressivity

Petite strain	Repeat unit length (base pairs)	Primary structure	Ori sequence	%Suppressivity	Transmission of petite genomes
a-1/1R/1	884	w	ori 1	>95	8:4
a-1/1R/Z1	416	w	ori 1	>95	
a-1/1R/40	606	m	ori 1	>95	
a-1/1R/14	380	w	ori 1"(A")	80	11:0
a-1/1R/1/26	392	w	ori 1-(A-)	80	5:0
b	875	o	ori 2	>95	10:0
a*-1/7/12	4,500	0	ori 3	60-80	36:0
a*-1/7/8	1,760	o	ori 3	85	14:0
a-3/1	4,700	o	ori 3+4	60-80	28:4
a-3/1/5	345	w	ori 3 ⁻ (C ⁻)	<5	5:1
a-3/1/33	400	w	ori 3-(C-)	<5	4F L 4
a-3/1/B31*	1,360	o	ori 4		and the second s
a-15/3/2	4,300	m	ori 5	50-60	12:0
a-15/4/1	4,800	m	ori 5+5	<5	3:12
a-15/4/1/1	1,560	m	ori 5	85	12:0
a-15/4/1/4	1,220	m	ori 5	90	12:0
a-15/4/1/10/1	1,780	m	ori 5	90	12:0
a-15/4/1/10/2	1,830	m	ori 5	85	12:0
a-15/4/1/10/3	970	m	ori ^o	~1	5:13
a-15/4/1/B3*	1,170	0	ori 5	***************************************	J 1 kJ
a-3/1/B/B1*	16,500	m	ori 2+6	******	******
ь17	~7,800		ori 2+7	80	***************************************

w and o indicate that the primary structure is known for the whole repeat unit or for the ori sequence and the flanking sequences, respectively. All other repeat units were physically mapped (m). The transmission of petite genomes concerns ~200 diploid petites from crosses of sponta neous petites with wild-typestrain B (A in the case of petite b). Mitochondrial DNAs were mapped with restriction enzymes. The ratio presented is that of diploids having the repeat units of the parental petite to diploids having modified repeat units (see text).

* Diploid petites from crosses of spontaneous petites with wild-type cells. The repeat units of a-3/1/B31, a-15/4/1/B3 and a-3/1/5/B1 originated from the genomes of a-3/1, a-15/4/1 and B, respectively. They were used to sequence ori 4, ori 5 and to map ori 6, respectively.

sequences do not have the same orientation (Fig. 3) and that ori 3 and 4, and ori 2 and 7, share a very similar tandem arrangement.

Three independent lines of evidence indicate that the ori sequences are indeed origins of replication. (1) The region between clusters A and B inclusive can be folded in a way (Fig. 4) very similar to that reported for a region of the replication origin of mammalian mitochondrial DNA²⁸⁻³⁰; all base pair changes found in this region in different genomes are localized in the looped-out sequence s. Cluster C is almost identical (Fig. 4) in sequence to a GC cluster, similarly located relative to the A-B region, in mammalian mitochondrial DNAs²⁸⁻³⁰. (2) Ori sequences are present in the repeat units of most spontaneous petites. (3) A clear correlation exists between the properties of the ori sequences and suppressivity, as indicated by the following.

We reported previously that crosses of two spontaneous petites (a-1/1R/1 and b) with wild-type cells produced diploids only harbouring the unaltered mitochondrial genome of the parental petite used in the $\cos^{10-12,14,15}$. Both petites were very highly suppressive (that is, the percentage of petites in the progeny was >95%) and had mitochondrial genomes characterized by very short repeat units (~1% of the wild-type genome). We called these petites, which have also been reported from other laboratories 31,32 , supersuppressive, and suggested that their mitochondrial genomes were only ones found in the progeny because they contained multiple copies of the origin of replication due to tandem amplification of their repeat units and they replicated more efficiently than the wild-type genome, which they could compete out $^{10-12,14,15}$. We have now investigated the general case of petites having any degree of suppressivity. The results are presented in Table 1 and lead to the following conclusions.

(1) Most of the 200 petite diploids had restriction maps identical to those of the parental petites, regardless of the suppressivity of the latter. The few cases where a different situation was found (Table 1) corresponded either to a genome resulting from a new excision process which had occurred in the parental petite genome or, more rarely, in the parental wild-type genome.

(2) Partial or total deletions or rearrangements of ori sequences were always accompanied by drastic changes in suppressivity: the total deletion of ori 5 in a-15/4/1/10/3 resulted in a suppressivity of $\sim 1\%$ as opposed to 90% for

a-15/4/1/4; the deletion of cluster C plus about 100 base pairs on its left in ori 3 led to a suppressivity <5% in a-3/1/5 and a-3/1/33, whereas a*-1/7/8 (although having a much larger repeat unit) had a suppressivity of 85%; the deletion of cluster A in ori 1 caused a decrease in suppressivity, from >95% in a-1/1R/Z1 to 80% in a-1/1R/14 and a-1/1R/1/26; and the presence of a duplicated inverted ori 5 sequence in a-15/4/1 was associated with a suppressivity of $\sim5\%$, whereas a-15/3/2, having a comparable repeat length, but only one ori 5 sequence, had a suppressivity of 50-60%.

(3) The repeat unit length affects replication efficiency as seen by comparing the suppressivities of $a^*-1/7/8$ and $a^*-1/7/12$, two petites carrying the same ori 3 sequence, or of a-15/3/2 and the series a-15/4/1, all containing ori 5. In all cases a shorter repeat length, and thus a higher density of ori sequences, is accompanied by a higher suppressivity. Similar observations have been reported previously⁵.

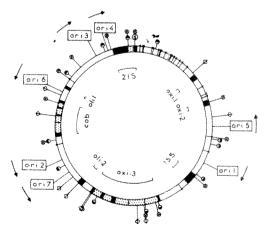


Fig. 3 Localization of ori sequences on the physical map of the mitochondrial genome of wild-type strain A. (This map is almost identical to that of strain KL-14-4 A^{18} .) Positions indicated correspond to cluster B. Restriction sites are indicated by the symbols used in Fig. 1; (§) corresponds to the SaII site, used as the map origin. The orientation of ori sequences is given by arrows pointing in the direction of cluster C. Black and hatched areas correspond to exons and introns, respectively, of mitochondrial genes. Their localization is based on refs 16-26. The localization of tRNA genes is shown by thin radial lines.

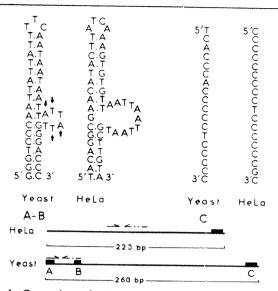


Fig. 4 Comparison of *ori* sequences of mitochondrial genomes from yeast (present work) and HeLa cells²⁸. Homology of potential secondary structure is found for the inverted repeats in the A-B region (arrows indicate the base changes found in this region in different petite genomes). Homology of primary structure is found for cluster C. The bottom compares the two ori sequences; the arrows indicate the inverted repeats of the A-E region, the broken line corresponding to the looped-out sequence. bp, Base pairs.

Two explanations have been put forward to account for suppressivity. The first one proposes a replicative advantage of the mitochondrial genome of suppressive petites over that of wild-type cells³³⁻³⁶. It was directly inspired by the work of Mills et al.³⁷ on the replication of Q β DNA but was not accompanied by any molecular model. The second one proposes a destructive recombination of the petite genome with the wild-type genome³⁸⁻⁴², and predicts that a number of different petite genomes are formed as the consequence of the increased parental genome instability due to the insertion of the petite genome. The present results contradict this latter explanation because most of the diploid petites studied here had genomes identical to those of the parental petites. Indeed, they provide for the first time a precise molecular basis for the former explanation of the replicative competition.

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- 1. Ephrussi, B. in Unités Biologiques Douées de Continuité Génétique, 165-180 (Editions du CNRS, Paris, 1949).
- 2. Ephrussi, B. in Nucleocytoplasmic Relations in Microorganisms, 13-47 (Clarendon, Oxford,
- Ephrussi, B., de Margerie-Hottinguer, H. & Roman, H. Proc. natn. Acad. Sci. U.S.A. 41, 1065-1071 (1955).

- Bernardi, G. Trends biochem. Sci. 4, 197-201 (1979).
 Faugeron-Fonty, G. et al. J. molec. Biol. 134, 493-537 (1979).
 Gaillard, C. & Bernardi, G. Molec. gen. Genet. 174, 335-337 (1979).
 Gaillard, C., Strauss, F. & Bernardi, G. Nature 283, 218-220 (1980).
 Baldacci, G., de Zamaroczy, M. & Bernardi, G. FEBS Lett. 114, 234-236 (1980).
 Bernardi G. & Bernardi, G. FEBS Lett. 115, 159-162 (1980).
- Bernardi, G. et al. in DNA Recombination, Interactions and Repair (eds Zadrazil, S. & Sponar, J.) 77-84 (Pergamon, Oxford, 1980).
- Bernardi, G. et al. in Mobilization and Ressembly of Genetic Information (eds Scott, W. A., Werner, R., Joseph, D. R. & Schultz, J.) 119-132 (Academic, New York, 1980).
- Werner, R., Joseph, D. N. & Schultz, J. J. 119-132 (Academic, New York, 1980).
 Bernardi, G. et al. in The Organization and Expression of the Mitochondrial Genome (eds Kroon, A. M. & Saccone, C.) 21-31 (Elsevier, Amsterdam, 1980).
 Prunell, A. & Bernardi, G. J. molec. Biol. 110, 53-74 (1977).
 de Zamaroczy, M., Baldacci, G. & Bernardi, G. FEBS Lett. 108, 429-432 (1979).
- 15. Goursot, R., de Zamaroczy, M., Baldacci, G. & Bernvardi, G. Curr. Genet. 1, 173-176

- Nobrega, F. G. & Tzagoloff, A. J. biol. Chem. 255, 9828-9837 (1980).
 Macino, G. & Tzagoloff, A. Cell 20, 507-517 (1980).
 Sanders, J. P. M., Heyting, C., Verbeet, M. Ph., Meijlink, F. C. P. W. & Borst, P. Molec. gen. Genet. 187, 239-261 (1977).

 19. Wesolowski, M., Monnerot, M. & Fukuhara, H. Curr. Genet. 2, 121-129 (1980).

 20. Tabak, H. F., Hecht, N. B., Menke, M. M. & Hollenberg, C. P. Curr. Gienet. 1, 33-43 (1979).

- Coruzzi, G. & Tzagoloff, A. J. biol. Chem. 254, 9324-9330 (1979).
 Heyting, C. et al. Molec. gen. Genet. 168, 231-250 (1979).
- Thalenfeld, B. E. & Tzagoloff, A. J. biol. Chem. 285, 6173-6180 (1980).
 Bonitz, S. G., Coruzzi, G., Thalenfeld, B. E. & Tzagoloff, A. J. biol. Chem. (in the press).
- 25. Tzagoloff, A., Nobrega, M., Akai, A. & Macino, G. Curr. Genet. 2, 149-157 (1980).

- 26. Berlani, R. E., Bonitz, S. G., Coruzzi, G., Nobrega, M. & Tzagoloff, A. Nucleic Acids Res. 8, 5017-5030 (1980)
- 27. Mathews, S., Schweyen, R. J. & Kaudewitz, F. in Mitochondria 1977 (eds Bandlow, W. et
- Mainews, S., Schweyen, R. J. & Kaudewitz, F. in Milochonaria 1977 (eds Bandiow, w. et al.) 133-138 (de Gruyter, Berlin, 1977).
 Crews, S., Ojala, D., Posakony, J., Nishiguchi, J. & Attardi, G. Nature 277, 192-198 (1979).
 Gillum, A. M. & Clayton, D. A. J. molec. Biol. 135, 353-368 (1979).
 Kobayashi, M., Yagimuma, K., Seki, T. & Koike, K. in The Organization and Expression of the Mitochondrial Genome (eds Kroon, A. M. & Saccone, C.) 221-229 (Elsevier, Americadam, 1990). Amsterdam, 1980).
- 31. Blanc, H. & Dujon, B. Proc. natn. Acad. Sci. U.S.A. 77, 3942-3946 (1980).
- Gingold, E. B. 10th Int. Conf. of Yeast Genetics and Molecular Biology, Abstr. no 333, 143
- Carnevali, F., Morpurgo, G. & Tecce, G. Science 163, 1331-1333 (1969).
- Rank, G. H. Can. J. Genet. Cytol. 12, 129-136 (1970). Rank, G. H. Can. J. Genet. Cytol. 12, 340-346 (1970).
- Rank, G. H. & Bech-Hansen, N. T. Can. J. Microbiol. 18, 1-7 (1972).
 Mills, D. R., Peterson, R. L. & Spiegelman, S. Proc. natn. Acad. Sci. U.S.A. 58, 217-224 (1967)
- Coen, D., Deutsch, J., Netter, P., Petrochilo, E. & Slonimski, P. P. in Control of Organelle Development, 24th Symp. Soc. exp. Biol., 449-496 (Cambridge University Press, London,

- Deutsch, J. et al. Genetics 76, 195-219 (1973).
 Michaelis, G., Petrochilo, E. & Slonimski, P. P. Molec. gen. Genet. 123, 51-65 (1973).
 Perlman, P. S. & Birky, C. W. Proc. natn. Acad. Sci. U.S.A. 71, 4612-4616 (1974).
 Slonimski, P. P. & Lazowska, J. in Mitochondria 1977 (eds Bandlow, W., Schweyen, R. J., Wolf, K. & Kaudewitz, F.) 39-52 (de Gruyter, Berlin, 1977).

 43. Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560-564 (1977).

Structure of C-terminal half of two H-2 antigens from cloned mRNA

F. Brégégère*, J. P. Abastado*, S. Kvist†, L. Rask*§, J. L. Lalanne*, H. Garoff†, B. Cami*, K. Wiman‡, D. Larhammar‡, P. A. Peterson‡, G. Gachelin*, P. Kourilsky* & B. Dobberstein†

*Unité de Biologie Moléculaire du Gène, ER CNRS no. 201 and SCN INSERM no. 20, Institut Pasteur, 28 rue du Dr Roux. 75724 Paris Cédex 15, France

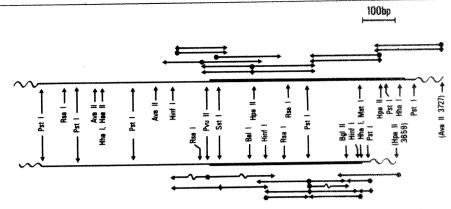
†European Molecular Biology Laboratory, Meyerhofstrasse 1, Postfach 102 209, D-6900 Heidelberg, FRG Department of Cell Research, The Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden

The classical cell-surface histocompatibility antigens (H-2 antigens in the mouse), known to have key roles in cell-to-cell recognition1, are encoded by at least three highly polymorphic genes $(H-2D, K \text{ and } L)^2$. Like their human (HLA) counterparts3, H-2 heavy chains span the cell membrane with a short C-terminal cytoplasmic region and an N-terminal extracellular stretch of about 280 amino acids. HLA antigens seem to be organized in three domains containing β -pleated sheets, with disulphide loops within the second and third domains, but the relative scarcity of material has hampered biochemical studies of the H-2 antigens⁴⁻⁶. We now report the sequencing of plasmids carrying H-2 cDNA as a means of inferring the amino acid sequence of the antigens, and especially of their previously poorly described C-terminal half.

The isolation of recombinant plasmids pH-2^d-1 and pH-2^d-3 is described in Fig. 1 legend and elsewhere7. Restriction maps of the cDNA inserts, 1,150 and 980 base pairs (bp) long, respectively, are different, but can be tentatively aligned on PvuII, SstI and PstI sites (Fig. 1). Both inserts contain a noncoding stretch of about 480 bp next to the poly(A) sequence. The 627- and 479-bp long coding sequences and their corresponding amino acid sequences are given in Fig. 2. They show extensive homologies with available sequences of H-2 and HLA molecules (82% with H-2Kb, 73% with HLA B7) (Fig. 3)^{3-6,8-11} allowing unequivocal alignment in the third domain. With reference to HLA3, we assigned nucleotide 133 to the first tryptophan residue in pH-2^d-1 and nucleotide 181 to the first arginine in pH-2^d-3. Both clones should, accordingly, code for the entire third domain, the membrane spanning region and the cytoplasmic segment.

§Present address: Department of Cell Research, The Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden

Fig. 1 Restriction maps of pH-2^d-1 and pH-2^d-3 inserts and strategies to sequence them. The cDNA library from which pH-2^d-1 (ref. 7) was selected had been constructed using mRNA from SL2 lymphoma cells grown as ascites in DBA/2 mice (H-2^d haplotype). The 400 independent bacterial clones of this library were further screened by in situ hybridization²⁹, using a fragment of the insert of the first H-2 clone as a probe. The DNAs of the positive responders were then tested for the specific binding of H-2 mRNA as already described⁷. At least two of them, pH-2^d-2 and pH-2^d-3, were found positive in this test. The cDNA insert of pH-2^d-2 was found identical to part of that of pH-2^d-1, and was not analysed further.



that of pH-2 -1, and was not analysed further. Plasmid DNA was prepared from cleared lysates ³⁰, partially purified by centrifugation in a CsCl/ethidium bromide gradient, and further purified by fractionation through a 5-40% sucrose gradient ³¹. Digestion with restriction endonucleases (Biolabs, Boehringer or BRL) were carried out in standard conditions. Restriction maps were constructed from the size of the DNA fragments, estimated from electropheretic patterns on agarose or acrylamide gels ^{32,33}. As indicated, each cDNA insert is bordered by two reconstituted *PstI* sites ³⁴. Both inserts have the same orientation with respect to pBR322 map. pBR322 sequences are presented here in their usual orientation, so that the parts of the cDNA sequences corresponding to the 3' ends of the messengers are on the left-hand side of the inserts. The coding regions are represented by thick lines. The restriction fragments sequenced are represented by arrows. They were labelled at their 5' (♠) or 3' (♠) ends, and cleaved secondarily to generate subfragments with only one labelled end ³⁵. Sequencing techniques used were those of Maxam and Gilbert ³⁵ (——) or Maat and Smith ³⁶ (———).

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Fig. 2 Coding sequences of pH-2^d-1 and pH-2^d-3. Both sequences have been aligned as described in the text, the 5' terminus is to the left, the 3' terminus to the right. The putative glycosylation (◆) and phosphorylation (♦) sites are labelled. The positions at which a difference is found between the two nucleotide sequences are labelled (●).

A continuous stretch of 26 uncharged amino acids, mostly hydrophobic, extends from amino acids 282 to 307, displaying at the expected position the characteristic features of a membrane-spanning segment. Noticeably, it contains a repetition (Val-Val-Leu-Gly-Ala-Val, followed by Val-Ile-Leu-Gly-Ala-Val) in pH-2^d-1, also seen at the nucleotide level in pH-2^d-3. The amino acid residues differ in 10 out of the 26 positions, suggest-

ing that the major constraint is the sole maintenance of hydrophobicity. No homology with membrane-spanning segments of other membrane proteins was found.

Amino acids 308-338 correspond to intra-cytoplasmic sequences which have been reported to be phosphorylated ¹² and associated with components of the cytoskeleton ¹³. A possible phosphorylation site (Arg-Asn-Thr) ¹⁴ is found at position 313 in

both H-2 clones. At the border with the membrane, a cluster of four basic residues (Lys-Arg-Arg) is found in both clones. As clusters of basic amino acids in similar positions have been found in HLA-A2 and HLA-B7 (ref. 15), membrane-bound IgM¹⁶, human glycophorin¹⁷ and several viral glycoproteins¹⁸, we propose that they might be involved in the positioning of transmembrane proteins.

The amino acid sequence located at the external membrane border shows many variations. The conserved proline residues at positions 276-278 indicate breakage of the α -helical structure, suggesting that this segment can form a flexible link, in agreement with the accessibility of this region to papain⁶.

pH-2^d-1 codes for the third domain and half of the second, whereas pH-2^d-3 codes for the third domain only. Cysteinyl residues at positions 164, 203 and 269 are likely to be those involved in intrachain disulphide bridges, as they are in H-2K^b (refs 4, 5, 19). Possible glycosylation sites (Asn-Tyr-Thr)²⁰ are found at positions 176 and 256 in pH-2^d-1 and pH-2^d-3, respectively. The two amino acid sequences are extremely similar. Divergences are found mainly as clusters (positions 193-198, 225-227, 255-268, 275-303) also seen in comparisons with HLA with additional variations.

The third domain of HLA shares significant homologies with the constant domains of immunoglobulin heavy chains^{21,22} Using the alignment frame designed for HLA²¹, we found that the third domains of the H-2 molecules encoded by the two plasmids display the same type of homology (in preparation). At 20 out of the 23 aligned positions corresponding to hydrophobic amino acids involved in the β -pleated sheet structure in immunoglobulins, hydrophobic residues are also found in H-2 sequences. These results suggest strongly that the third domain of H-2 antigens, like that of HLA, is folded in an immunoglobulin-like three-dimensional structure. When the aforementioned clusters of amino acid differences between pH-2^d-1 and pH-2^d-3 are placed in the three-dimensional immunoglobulin model, they fall in loop areas (in 8 differences out of 10), while β -pleated sheets correspond to highly conserved regions. This suggests that the three-dimensional structure of the third domain imposes constraints on divergences. This could be true for other parts of the molecule as well and be important in the understanding of the basis of alloantigenicity.

Comparisons with available data on H-2D^d, K^d and L^d (Fig. 3) show that pH-2^d-1 differs from H-2L^d at positions 155, 156, 169 and 262. It has a methionine at position 138, whereas the cyanogen bromide cleavage map of H-2K^d indicates that there is no such residue in the molecule²³. At 57 out of 58 assigned positions the pH-2^d-1 amino acid sequence is identical to that of H-2D^d, the only difference being at amino acid 255, denoted as 'tentatively assigned'¹⁰. Therefore, pH-2^d-1 cannot code for H-2L^d or H-2K^d, but could well code for H-2D^d. The pH-2^d-3

Table 1 Analysis of the nucleotide changes between pH-2^d-1 and pH-2^d-3 sequences

	Replacements	Silent substitutions	Total substitutions
Codons 183-284 (third domains)	17/704 = 0.024	5/214 = 0.023	22/918 = 0.024
Codons 285–308 (membrane spanning regions)	7/148.5 = 0.047	2/58.5 = 0.034	9/207 = 0.043
Codons 309-339 (cytoplasmic fragments)	3/221.5 = 0.014	1/66.5 = 0.015	4/288 = 0.014
Codons 183-339	27/1,074 = 0.025	8/339 = 0.024	35/1,413 = 0.025

pH-2^d-1 and pH-2^d-3 were compared over their aligned sequences (Fig. 2). The rate of 'silent substitutions' (see text) was determined by a computation similar to that described by Lomedico *et al.*²⁸: all possible single-step mutations (that is, three possible changes for each base) were totalled over the 157 aligned codons, and classified as replacements if they involved an amino acid change, and as silent substitutions if they did not. The numbers were then averaged for the two genes. The fractions displayed in the table indicate the number of replacements (or silent or total substitutions) actually recorded over the total number of possible replacements (or silent or total substitutions).

sequence differs from H-2D^d at position 262 (ref. 11) and is compatible with the 15 assigned positions reported for H-2L^d in the corresponding area¹¹. It has a possible glycosylation site²⁰ at positions 252-258 as would be expected for H-2K^d (P. Robinson, personal communication). Protein sequence data are thus too limited to allow conclusive assignments, especially as the cloned sequences could also specify Tla, Qa1, Qa2 or other H-2-like antigens²⁴, in line with the finding that the mouse genome contains multiple H-2-related sequences²⁵.

The nucleotide sequences of pH-2^d-1 and pH-2^d-3 diverge in only 47 (9.7%) of the 485 bases aligned for comparison (including 12 aligned with empty positions, Fig. 2). The third domain shows remarkable conservation with only one base change in a stretch of 156 nucleotides (positions 204-255). Surprisingly, silent changes (with no corresponding amino acid change) are unusually rare, compared with replacement changes (Table 1), whereas in other genes the former arise more often than the latter^{26,27}. This raises several hypotheses: a special conservative constraint might exist on the nucleotide sequences themselves, making the silent changes not neutral. Alternatively, the two proteins may have diverged too rapidly to allow the accumulation of neutral mutations in their genes²⁶. Whether this feature is related to a mechanism involved in the generation of the natural polymorphism of these genes or not remains to be investigated.

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					CHO			
рн-2 ^d -1 рн-2 ^d -3	140 WTAADMAA	150 QITRRKWEQA	160 GAAERDRAYL	170 EGECVEWLRR	180 YLKNGNATLL	190 RTDPPKAHVT	200 HHRRPEGDVT P-SE	210 LRCWALGFYP
H-2 Dd H-2 Ld H-2 Kb HLA-A2 HLA-B7		LKHA-		H-	EQNREE		SDDK AVSDHEA- PISDHEA-	
pH-2 ^d -1 pH-2 ^d -3	220 ADITLTWQLN	230 GEELTQEMEL	240 VETRPAGDGT	250 FQKWASVVVP	260 LGKELKYTCH QNR	270 VEHEG PEPL -YL	GKE 280 TLRWEPPSST	pap 290 KTNTVIIAVP DSYMVL
H-2 Dd H-2 Ld H-2 Kb HLA-A2 HLA-B7	RD	I-D DQDT	R-	-EA	S-Q-QR	- A- - A- - A- - A- - A-	DE-P	VS-M
рн-2 ^d -1 рн-2 ^d -3	300 VVLGAVVILG GMA-I-	310 AVMAFVMKRR V	PO ₄ 320 RNTGGKGGDY	330 ALAPGSQSSD	338 MSLPDCKV -C			TM
HLA-A2 PHLA-1	AV	V-AC		SQ-AS-D-AZ SQ-AC-D-AQ	G-BVSLTA -C			

Fig. 3 Comparison of the amino acid sequences encoded by pH-2^d-1 and pH-2^d-3 with sequences of murine and human histocompati-bility antigens 3-6.8-11,15.23. pHLA-1 is a recombinant plasmid carrying an HLA cDNA sequence37 deduced amino acid sequence shows no difference from the published data on HLA-B7 COOHterminal fragment¹⁵. The amino acids are indicated according to the one-letter code³⁸. pH-2^d-1 insert has been taken as a reference to align the other sequences. Three residues have been taken out of the alignment between positions 274 and 275, as indicated, to keep to numbering conventions already used for H-2 and HLA sequences³. The dashes indicate identity with pH-2^d-1 sequence. X indicates an undetermined amino acid, different from tyrosine.

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Zinkernagel, R. M. & Doherty, P. C. Adv. Immun. 27, 51-177 (1979). Klein, J. Science 203, 516-521 (1979). Strominger, J. L. Prog. Immun. 4, 541-554 (1980). Uehara, H. et al. Biochemistry 19, 306-315 (1980).

- Uehara, H. et al. Biochemistry 19, 6182–6188 (1980). Martinko, J. A. et al. Biochemistry 19, 6188–6193 (1980). Kvist, S. et al. Proc. nam. Acad. Sci. U.S.A. 78, 2272–2276 (1981).
- NVISI, 3. et al., Froz. nam. Actau. 3c. 0.3.7, 18, 2212-2210 (1761).

 Orr, H. T., Lopez De Castro, J. A., Lancet, D. & Strominger, J. L. Biochemistry 18, 5711-5720 (1979).

 Orr, H. T., Lopez De Castro, J. A., Parham, P., Ploegh, H. L. & Strominger, J. L. Proc. natn.
- Acad. Sci. U.S.A. 76, 4395-4399 (1979).

 Nairn, R., Nathenson, S. G. & Coligan, J. E. Eur. J. Immun. 10, 495-503 (1980).
- Coligan, J. E. et al. Proc. natn. Acad. Sci. U.S.A. 77, 1134-1138 (1980).
 Pober, J. S., Guild, B. C. & Strominger, J. L. Proc. natn. Acad. Sci. U.S.A. 75, 6002-6006
- 13. Geiger, B. & Singer, S. J. Cell 16, 213-222 (1979)
- 14. Kemp, D. E., Graves, D. J., Benjamini, E. & Krebs, E. G. J. biol. Chem. 252, 4888-4894
- (1977). Robb, R. J., Terhorst, C. & Strominger, J. L. J. biol. Chem. 253, 5319-5324 (1978). Rogers, J. et al. Cell 20, 303-312 (1980). Tomita, M. & Marchesi, V. T. Proc. nam. Acad. Sci. U.S.A. 72, 2964-2968 (1975). Wickner, W. Science 210, 861-868 (1980).

- HEARIEL, M. SEERIE AAN, 601-606 (1700). Ewenstein, B. M. et al. Proc. natn. Acad. Sci. U.S.A. 75, 2909-2913 (1978). Marshall, R. D. A. Rev. Biochem. 41, 673-709 (1972).
- 21. Orr, H. T., Lancet, D., Robb, R. J., Lopez De Castro, J. A. & Strominger, J. L. Nature 282, 266-270 (1979).
- Tragardh, L., Rask, L., Wiman, K., Fohlman, J. & Peterson, P. A. Proc. natn. Acad. Sci. U.S.A. 77, 1129-1133 (1980).
- Kimball, E. S., Maloy, E. S., Martinko, J. M., Nathenson, S. G. & Coligan, J. E. Molec. Immun. 17, 1283-1291 (1980).

- Immun. 17, 1283-1291 (1980).
 Vitetta, E. S. & Capra, J. D. Adv. Immun. 26, 147-193 (1978).
 Cami, B., Bregegere, F. & Kourilsky, P. Nature (in the press).
 Perler, F. et al. Cell 18, 545-558 (1979).
 Miyata, T., Yasunaga, T. & Nishida, T. Proc. nam. Acad. Sci. U.S.A. 77, 7328-7332 (1980).
 Lomedico, P. et al. Cell 18, 545-558 (1979).
 Grunstein, M. & Hogness, D. S. Proc. natn. Acad. Sci. U.S.A. 72, 3961-3966 (1975).
 Bastia, D. J. molec. Biol. 124, 601-639 (1978).
 Rougeon, F., Kourilsky, P. & Mach, B. Nucleic Acids Res. 2, 2365-2378.
 Smith, H. O. & Birnstiel, M. L. Nucleic Acids Res. 3, 2387-2398 (1975).
 Garoff, H. Frischauf, A. M. Simons, K. Lehrach, H. & Delius, H. Nature 288, 236-241.

- 33. Garoff, H., Frischauf, A. M., Simons, K., Lehrach, H. & Delius, H. Nature 288, 236-241
- Villa-Komaroff, L. et al. Proc. nam. Acad. Sci. U.S.A. 75, 3721–3727 (1978).
 Maxam, J. & Gilbert, M. Meth. Enzym. 65, 499–560 (1980).
 Maat, J. & Smith, A. J. H. Nucleic Acids Res. 5, 4537–4545 (1978).

- 37. Ploegh, H. L., Orr, H. T. & Strominger, J. L. Proc. natn. Acad. Sci. U.S.A. 77, 6081-6085
- 38. IUPAC-IUB Commission on Biochemical Nomenclature J. biol. Chem. 243, 3557-3559 (1968).

Neutron diffraction reveals oxygen-histidine hydrogen bond in oxymyoglobin

Simon E. V. Phillips*† & Benno P. Schoenborn†

* MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

† Department of Biology, Brookhaven National Laboratory, Upton, New York 11973, USA

Myoglobin (Mb) reversibly binds molecular oxygen in vertebrate muscle and consists of a polypeptide chain of 153 residues and one haem, which closely resembles one subunit of a haemoglobin (Hb) tetramer. In oxygenated myglobin (oxyMb) the iron atom is coordinated by four porphyrin nitrogen atoms, Nº of the invariant 'proximal' histidine (F8), and molecular oxygen1. The oxygen molecule lies in a tight pocket, bounded by two hydrophobic groups (Phe CD1 Val E11) and the side chain of the 'distal' histidine (E7). This histidine is present in Hb and Mb of many different organisms, with substitution by glutamine or leucine found in only a few cases. The function of the residue is not clear, although it does present steric hindrance to linear ligands such as carbon monoxide and favours 'bent' ones, such as O2. We report here that the imidazole stabilizes bound molecular oxygen with a hydrogen bond, as revealed by neutron diffraction analysis.

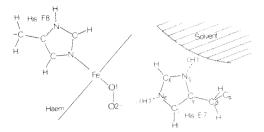


Fig. 1 Arrangement of proximal (F8) and distal (E7) histidines in oxyMb. At pH 8.4, nitrogen-bound hydrogen on E7 imidazole can be bonded to either Nº (the naturally predominant form), with a hydrogen bond to O-2 (dotted line), or to N⁶, where it projects into the solvent surrounding the molecule.

Pauling² first proposed that the imidazole may form a hydrogen bond to the terminal oxygen atom (O-2 in Fig. 1), which carries a formal negative charge in his view of the electronic structure of the FeO2 complex. Evidence suggesting such a bond comes from electron paramagnetic resonance and oxygen affinity data on cobalt-substituted Hb and Mb3-5. The pK of the distal histidine is ~ 5.5 (ref. 4). At physiological pH (and the pD of the crystals used here) the histidine may have hydrogenbonded to either N^{ϵ} or N^{δ} (see Fig. 1), and interaction with O-2 may therefore be by a hydrogen bond, or a simple van der Waals contact. X-ray crystallography of protein crystals cannot distinguish between these alternatives as hydrogen atoms scatter X rays only weakly, and are not normally visible in electron density maps. Neutrons, however, are scattered as strongly by hydrogen and deuterium as C, N, O, S and Fe atoms, and well-ordered H and D atoms may be observed in neutron density maps of proteins^{6,7}.

Crystals of oxyMb were prepared from frozen sperm-whale skeletal muscle¹. Large crystals (8 mm³) were transferred to deuterated mother liquor (pD 8.4) at 20 °C 3 months before data collection, because hydrogen gives strong incoherent scattering of neutrons which increases the background level in diffraction data collection. Replacement of H2O solvent in crystals with D2O, and subsequent replacement of exchangeable H atoms with D in the protein, alleviates this problem and improves the signal-to-noise ratio of the data. It also allows exchangeable H atoms to be identified in the density map, as H scatters out of phase with respect to D.

Neutron diffraction data were collected using the protein crystallography station of the High Flux Beam Reactor at Brookhaven National Laboratory. The diffractometer was equipped with a two-dimensional multiwire proportional counter8 and a cooling device to maintain the crystals at -5 °C and retard oxidation of the haem iron. Two crystals were used for data collection, each being exposed to the neutron beam for 21 days. No radiation damage or oxidation was observed; 88% of available data to 2 Å resolution was collected, together with further data between 2 and 1.5 Å, giving 14,411 independent reflections. The merging R factor between crystals was 14.1% on intensities.

Calculated phases and amplitudes for the neutron data were computed from the coordinates of all C, N, O, S and Fe atoms in the refined X-ray structure1, including 60 ordered H2O molecules. A difference density map (coefficients $|F_0| - |F_c|$: crystallographic R factor 35% for 10,152 reflections with $I > 1.5\sigma(I)$) showed clear peaks for 40% of the missing H and D atoms. Small peaks were visible at both N° and N° of His E7. H and D atoms observed in the map were added to the model, together with unobserved ones whose positions were known from stereochemistry (for example, most C-H groups), but ring nitrogen-bound H or D atoms for histidines were omitted—this reduced R to 33%. A second difference map failed to resolve the ambiguity at His E7, and combined crystallographic and conformational energy refinement was initiated, using methods described for X-ray refinement of oxyMb, but modified for use with neutron data. Seven cycles of coordinate refinement were carried out, with three cycles on individual atomic thermal

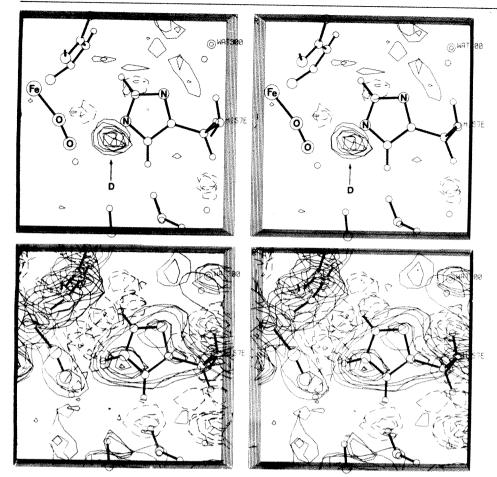


Fig. 2 Stereo view of $|F_0| - |F_c|$ neutron difference density map in a slab 4 Å-thick centred on the plane of E7 imidazole ring. The refined model is superimposed, showing His E7, FeO2 and part of the haem, in a similar orientation to that in Fig. 1. Contours are ± 0.35 , 0.55, 0.75 Fermi per Å, with negative ones shown as broken lines. A strong, positive peak indicates the presence of deuterium bonded to Ne.

Fig. 3 Stereo view of $2|F_0| - |F_c|$ neutron density map in identical orientation to that in Fig. 2. The deuterium has been added to the superimposed model with calculated stereochemistry. The fit to the map is good, although adjustment of the imidazole in later refinement may improve it. Note that whereas D, C, N, O, S and Fe atoms give positive peaks, H scatters out of phase and gives negative peaks. Contours are -1.0, -0.5, 1.0, 2.0, 3.0, 4.0 Fermi per Å³.

parameters, reducing R to 18.8%. A difference map calculated at this stage (Fig. 2) shows a strong peak adjacent to Nº of His E7, indicating the N-bound deuterium. An observed density map (coefficients $2|F_0|-|F_c|$) is shown in Fig. 3, and the high concentration of scattering density at N^s compared with N⁸ confirms the presence of the D atom. As this atom had not been included in the model for refinement or phase calculation, the peaks result from information present in the observed data alone, and are not biased by assumptions about the structure.

The geometry, summarized in Table 1, indicates a medium strength hydrogen bond, although the coordinates may change a little with further refinement when D is included in the model. Such a bond could contribute several kcal per mol to the enthalpy of oxygen binding, and the position of the E7 imidazole might therefore act as a means of control of oxygen affinity in Hb and Mb.

However, direct observation of the hydrogen bond cannot resolve the controversy between proponents of the Fe(III)-superoxide model for oxygen binding^{9,10}, and those favouring a spin-paired model^{2,11-13} with varying degrees of charge transfer between Fe and O2, but it does does suggest that molecular orbital calculations attributing no partial negative charge to O-2 should be regarded with caution, as no hydrogen bond was found between N^e and bound CO, where charge transfer is absent14.

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Table 1 Geometry of histidine-oxygen hydrogen bond

	Distance (Å)*		Angle (degrees)
N°-D	1.04	N^e -DO-2	157
$O-2 \dots D$ $N^{\varepsilon} \dots O-2$	1.98 2.97	O-1-O-2D	96

Deviation of O-2 from plane of E7 imidazole ring is 0.61 Å. Estimated standard deviations for distances, 0.18 Å.

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- 1. Phillips, S. E. V. J. molec. Biol. 142, 531-554 (1980)
- Faming, J. L. V. J. morec. Biol. 174, 331-334 (1960).
 Pauling, L. Nature 203, 182-183 (1964).
 Yonetani, T., Yamamoto, H. & Iizuka, T. J. biol. Chem. 249, 2168-2174 (1974).
- 4. Ikeda-Saito, M., Iizuka, T., Yamamoto, H., Kayne, F. J. & Yonetani, T. J. biol. Chem. 252, 4882-4887 (1977).
- Ikeda-Saito, M., Brunori, M. & Yonetani, T. Biochim. biophys. Acta 533, 173-180 (1978). Schoenborn, B. P. Nature 224, 143-146 (1969).
- Norvell, J. C. & Schoenborn, B. P. Brookhaven Symp. Biol. 27, II-12-23 (1975)
- 8. Alberi, J., Fischer, J., Radeka, L. C. & Schoenborn, B. P. Nucl. Instrum. Meth. 127, 507-523 (1975).
- Weiss, J. J. Nature 202, 83 (1964).
- Need, C. A. & Cheung, S. K. Proc. nam. Acad. Sci. U.S.A. 74, 1780-1784 (1977).
 Olafson, B. D. & Goddard, W. A. Proc. nam. Acad. Sci. U.S.A. 74, 1315-1319 (1977).
 Case, D. A., Huynh, B. H. & Karplus, M. J. Am. chem. Soc. 101, 4433-4453 (1979).
 Drago, S. D. & Corden, B. B. Acc. chem. Res. 13, 353-360 (1980).
- 14. Hanson, J. C. & Schoenborn, B. P. J. molec. Biol. (submitted)

Modulation of platelet shape and membrane receptor binding by Ca²⁺-calmodulin complex

Kuo-Jang Kao, Joachim R. Sommer & Salvatore V. Pizzo

Departments of Pathology and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA

Interaction between factor VIII/von Willebrand factor, a plasma glycoprotein, and platelets is essential for platelet adhesion and subsequent platelet plug formation at sites of vascular injury during primary phase haemostasis^{1,2}. We now report that binding of ¹²⁵I-labelled factor VIII/von Willebrand factor to its platelet membrane receptors is inhibited by preincubation of washed human platelets with inhibitors of Ca2+calmodulin complex (for example, 50 µM trifluoperazine or

chlorpromazine) at 37 °C for 2 min. Scatchard analysis of the binding data indicates that the total number of accessible binding sites on each pretreated platelet is significantly reduced to ~50% of the original value. There is also negative cooperativity of binding or reduced binding affinity in a major portion of binding sites. Platelet shape changes from diskoid to spherical after treatment. The results suggest that Ca²⁺-calmodulin complex modulates platelet shape and membrane receptor behaviour.

Recent studies suggest that calmodulin, an intracellular calcium receptor, is important in mediating Ca2+-regulated cellular secretion, motility and enzyme activities (see refs 3, 4 for reviews). Trifluoperazine (TFP) and other phenothiazines are known to bind to Ca2+-calmodulin complexes and block their action⁵. It has been demonstrated^{6,7} in human platelets that calmodulin is essential for the activity of platelet myosin light chain kinase, which is responsible for phosphorylation of myosin light chain and subsequent actomyosin contraction. Moreover, platelet aggregation induced by ADP, thrombin, adrenaline, collagen or factor VIII/von Willebrand factor (FVIII/vWF) is inhibited by inhibitors of Ca2+-calmodulin complex, such as TFP⁸⁻¹⁰. The exact mechanism by which inhibition of Ca² calmodulin complex function results in failure of platelet aggregation is unknown. Recently, Gerrard et al.11 reported that platelet membrane glycoprotein III immunologically crossreacts with a-actinin and may anchor actin filaments. Phillips et ² reported a close association between actomyosin filaments and platelet membrane glycoproteins. In view of these findings and regulation of distribution of membrane proteins by cytoskeletal elements in other cellular systems¹³, we have examined the possibility that inhibitors of Ca2+-calmodulin complex inhibit the binding of platelet aggregating agents to their membrane receptors. As we have previously demonstrated that binding of FVIII/vWF to its platelet receptors is responsible for FVIII/vWF-induced platelet aggregation¹⁴, the effects of the Ca²⁺-calmodulin inhibitors, TFP and chlorpromazine (CP)⁵, on platelet shape, and the binding of FVIII/vWF to its platelet membrane receptors were studied.

Human platelets used in these experiments were prepared from citrated venous blood of healthy donors who had received no drugs for at least 2 weeks before the study. Fresh platelets were collected by differential centrifugation¹⁵ and washed first with Tyrode's buffer, pH 7.4, containing HEPES (2 mM), EDTA (5 mM), apyrase (75 µg ml⁻¹) but no Ca²⁺ or Mg²⁺. The platelets were washed again in the same buffer (without apyrase) and resuspended in Tyrode's buffer, pH 7.4, containing 2 mM HEPES. The concentration of washed platelets was determined

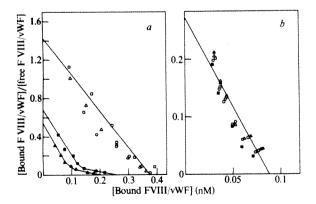
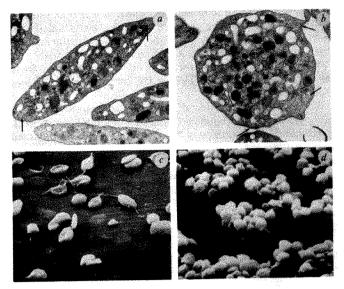


Fig. 1 Scatchard analysis of 125 I-FVIII/vWF binding to platelets pretreated with different phenothiazines. The binding data used for the analysis were obtained by incubating platelets with increasing concentrations of 125 I-FVIII/vWF in the presence of ristocetin (1 mg ml $^{-1}$) as described elsewhere 14 . In a, washed fresh platelets (5×10^6 per incubation) were used. In b, paraformaldehyde-fixed platelets (1.2×10^6 per incubation) were used. \bigcirc , Control; \triangle , TFP pretreated; \bigcirc , CP sulphoxide pretreated. \bigcirc , CP pretreated; \bigcirc , CP sulphoxide pretreated.



Transmission electron micrographs of platelets pretreated with TFP; a shows the control platelets (×24,500). The platelet is diskoid shaped. Electron-dense granules, a granules, and the open canalicular system are visible. Cross-sections of peripheral circular microtubules (arrows) are visible at both ends of the platelet. Similar findings are observed in platelets pretreated with DMSO (0.5%) or TFP sulphoxide (50 µM). b, Platelets pretreated with TFP (50 µM) at 37 °C for 5 min (×24,500); the platelets have become spheroid in shape. Redistribution of microtubules is indicated by arrows. The cross-sections of peripheral microtubules (arrows) suggest that the platelet in b has the same orientation as that in a. The change in shape of platelets after TFP treatment was confirmed by scanning electron microscopy (c, d). c, Control platelets which appear as disks (×33,000). Similar morphology was observed in DMSO (0.5%) or TFP sulphoxide (50 µM)-treated platelets. d, TFP (50 µM)-treated platelets (37 °C, 2.5 min). The platelets appear as spheres (×3,000). Platelets were fixed as described in the text and prepared for scanning electron microscopy as described elsewhere²¹. Platelets were sputter-coated with platinum and examined using an SEM-Phillips 501.

in a haemacytometer by phase-contrast microscopy. The washed platelets were used for all studies of platelet aggregation, FVIII/vWF receptor binding and shape changes. The binding studies were carried out as described in detail elsewhere¹⁴.

To demonstrate that the washed platelets were still responsive to treatment with Ca^{2^+} -calmodulin inhibitors, the effect of TFP or CP on platelet aggregation induced by FVIII/vWF or thrombin was studied by turbidimetry ¹⁴ in a Chronolog model-440 aggregometer at 37 °C. The washed platelets $(5\times10^7\text{ platelets ml}^{-1})$ were aggregated by thrombin (0.5 U ml^{-1}) or by FVIII/vWF $(2\,\mu\text{g ml}^{-1})$ in the presence of ristocetin $(1\,\text{mg ml}^{-1})$. Platelet aggregation was markedly inhibited by either TFP or CP $(50\,\mu\text{M})$ each) but not by the same concentrations of their inactive sulphoxide derivatives (TFP sulphoxide or CP sulphoxide). This result demonstrates not only that platelets are responsive to TFP or CP after our washing procedures, but also confirms the notion that platelet Ca^{2^+} -calmodulin complex plays an essential part in platelet aggregation induced by FVIII/vWF, thrombin or other agents ⁸⁻¹⁰.

The effect of TFP or CP on 125 I-labelled FVIII/vWF binding to platelet receptors was then studied. The specific binding of 125 I-FVIII/vWF to platelets was markedly inhibited when the washed platelets were preincubated with TFP (50 μ M) at 37 °C for 2 min (Table 1). No inhibition of receptor binding was observed when platelets were preincubated with TFP sulphoxide (50 μ M), CP sulphoxide (50 μ M) or dimethyl sulphoxide (DMSO: 0.5%, v/v). Recently, we have demonstrated that platelet membrane FVIII/vWF receptors can be fully preserved after fixation of platelets with 2% paraformaldehyde 14 . To exclude the possible direct interfering effect of TFP or CP on

FVIII/vWF receptor binding, the effect of TFP or CP on 125 I-FVIII/vWF binding to fixed platelets was studied. No inhibitory effect was observed (Table 1). These results demonstrate that neither TFP nor CP directly interferes with 125 I-FVIII/vWF binding to the receptors and that responsive fresh platelets are required for TFP or CP to have any inhibitory effect.

We also studied the effect of different concentrations of TFP on 125I-FVIII/vWF binding (Table 2); an inhibitory effect, apparent after the addition of 25 µM of TFP, reached a plateau after addition of 100 µM of TFP. This dose response is consistent with the observations of White and Raynor¹⁰ who studied the effect of TFP concentrations on thrombin-induced platelet aggregation and serotonin release. The fact that TFP inhibition of platelet-aggregating agents thrombin and FVIII/vWF is concentration dependent suggests that TFP may inhibit platelet function by acting on Ca2+-calmodulin complex.

Kinetic studies were made to determine whether the inhibitory effect of TFP or CP on receptor binding results from reduced receptor binding affinity or a decreased number of accessible binding sites on each platelet. Scatchard analysis of the binding data revealed that the total number of accessible binding sites on each platelet was significantly reduced after platelets were preincubated with TFP or CP (Fig. 1a). Curvilinear Scatchard plots were also obtained. These results indicate either that there is negative cooperativity of binding or that a portion of binding sites shift towards low-affinity binding after pretreatment of platelets with inhibitors of Ca²⁺-calmodulin complex. Again, these effects were not observed in the fixed platelets (Fig. 1b). Our observations suggest that treatment of platelets with Ca2+-calmodulin inhibitors may affect the distribution or the aggregation state of the receptor in the plasma membrane. Thus the accessibility of the receptors for the ligand or the receptor binding behaviour is affected.

We therefore studied the ultrastructural changes of platelets pretreated with TFP or CP using transmission electron microscopy and standard freeze-fracture techniques. Washed platelets were pretreated with different derivatives of the phenothiazines (50 µM) or DMSO (0.5%, v/v) at 37 °C for different time periods. The treated platelets were fixed by adding an equal volume of 1.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, containing 5% sucrose, for 1 h at room temperature. The fixed platelets were sedimented and processed for freeze-fracture using a method described elsewhere 16. Fixed platelets were

Table 1 Effect of trifluoperazine and chlorpromazine on 125I-FVIII/vWF binding to washed fresh platelets and paraformaldehydefixed platelets

	Specific binding (c.p.m.)			
Pretreatment	Washed fresh platelets	Paraformaldehyde- fixed platelets		
Control	37,625 (100.0)	38,624 (100.0)		
DMSO	36,272 (96.4)	38,243 (99.0)		
TFP	17,902 (46.3)	38,204 (98.9)		
TFP sulphoxide	36,068 (95.9)	37,434 (96.9)		
CP	28,594 (75.9)	36,052 (93,3)		
CP sulphoxide	36,220 (96.3)	37,140 (96.2)		

The specific binding of 125 I-FVIII/vWF to 5×106 platelets was determined as described elsewhere 14. The specific radioactivity of the ⁵I-FVIII/vWF protein was 7.3×10⁵ c.p.m. per μg. TFP, CP, TFP sulphoxide and CP sulphoxide were dissolved in DMSO. The platelet suspensions were preincubated with TFP, CP, TFP sulphoxide or CP sulphoxide at 37 °C for 2 min; the final concentrations of all these reagents was 50 μ M. The final concentration of DMSO was 0.5% (v/v). All studies were carried out in duplicate; variation between duplicates was <5%. The experiments were repeated three times, with similar results each time. Only one representative experiment is shown here. TFP sulphoxide and CP sulphoxide are derivatives which inhibit Ca calmodulin complex to an extent which is only 2-3% of that of TFP or CP5. It is unknown whether these derivatives enter platelets. % Binding of control is given in parentheses.

Table 2 Effect of different concentrations of trifluoperazine on 125 I-FVIII/vWF binding to washed fresh platelets

	TFP (uM)					
	0	10	25	50	100	200
Specific binding (c.p.m.)	47,911	48,875	47,111	40,317	20,907	19,400
% of control	100	102.0	98.3	84.1	43.6	40.5

Specific binding was determined as described in Table 1 legend. The specific radioactivity of 125 I-FVIII/vWF was 9.5×10^5 c.p.m. per μg .

also sedimented, fixed by 2% osmium tetroxide, dehydrated and embedded in Epon. Thin sections of sample were cut, stained with uranyl acetate and lead acetate, then examined using a Zeiss EM-10 electron microscope. Results of the study showed that distribution of membrane particles in both the E and P face of the platelet plasma membrane was not affected by pretreatment with TFP (50 µM) or CP (50 µM) at 37 °C for different time periods (2-45 min, data not shown). Nevertheless, thin sections of platelets showed the redistribution of microtubules and that platelets changed from their original diskoid shape into spheres after pretreatment with TFP or CP at 37 °C for 20 min (Fig. 2a, b). This finding was further confirmed by examination of platelets with a scanning electron microscope (Fig. 2c, d). This change in platelet shape was not observed after pretreatment with TFP sulphoxide or CP sulphoxide. Inhibitors of Ca2+calmodulin may effect the change in platelet shape by inhibiting the normal function of actomyosin and microtubules, as it is known that both these cytoskeletal elements are essential for maintaining platelet shape 11,17 and the Ca2+-calmodulin complex regulates microtubule assembly/disassembly and platelet myosin light-chain kinase^{6,7}. In addition, we observed that platelets became spherical after pretreatment with 25 μM TFP at 37 °C for 2 min. The degree of change in shape was not significantly different between platelets treated with 25 μM and 50 µM TFP. However, there was a significant difference in inhibition of 125I-FVIII/vWF binding (Table 2). Thus, it is unlikely that the reduced binding of FVIII/vWF resulted from the change in platelet shape.

Our results are substantiated by recent observations that intra-platelet cyclic AMP inhibits FVIII/vWF binding to platelets 18,19. It is known that cyclic AMP acts as a Ca2+ antagonist by enhancing Ca2+ uptake into the platelet Ca2+ storage sites in the dense tubular system²⁰. The results of this study demonstrate that Ca2+-calmodulin complex may have an important role in regulating platelet membrane receptor behaviour and maintaining the diskoid shape of platelets in the resting state.

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- Sakariassen, K. J., Bolhuis, P. A. & Sixma, J. I. Nature 279, 636-638 (1979)
- Hovig, T. & Stormaken, H. Acta path. microbiol. scand. 248, 105-122 (1974). Means, A. R. & Dedman, J. R. Nature 285, 73-77 (1980).
- Cheung, W. Y. Science 207, 19-27 (1980).
- Weiss, B., Prozialeck, W., Cimino, M., Barnette, M. S. & Wallace, T. L. Ann. N.Y. Acad. Sci. 356, 319-345 (1980) Hathway, D. R. & Adelstein, R. S. Proc. natn. Acad. Sci. U.S.A. 76, 1653-1657 (1979).
- Adelstein, R. S., Conti, M. A. & Pato, M. D. Ann. N.Y. Acad. Sci. 356, 142-150 (1980). Kindness, G., Williamson, F. B. & Long, W. F. Thromb. Res. 17, 549-554 (1980). Kruckberg, W. C., Lowery, M. S. & Brewer, G. J. Clin. Res. 27, 744A (1979). White, G. C. & Raynor, S. T. Thromb. Res. 18, 279-284 (1980).

- 11. Gerrard, J. M., Schollmeyer, J. V., Phillips, D. R. & White, J. G. Am. J. Path. 94, 509-528 Phillips, D. R., Jennings, L. K. & Edwards, H. H. J. Cell Biol. 86, 77-86 (1980).
 Korn, E. D. in Cell Motility. Cold Spring Harb. Conf. Vol. 3B (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 623 (Cold Spring Harbor Laboratory, New York, 1976).
 Kao, K. J., Pizzo, S. V. & McKee, P. A. J. clin. Invest. 63, 656-664 (1979); Proc. natn. Acad. Sci. U.S.A. 76, 5317-5320 (1979); Blood 57, 579-585 (1981).

- Kinlough-Rathbone, R. L. et al. Thromb. Haemostasis 37, 291-308 (1977).
 Sommer, J. R., Wallace, N. R. & Junker, J. J. ultrastruct. Res. 71, 126-142 (1980).
 Barnhart, M. I. Molec. cell. Biochem. 22, 113-137 (1978).
- Tang, S. S., Moake, J. L., Troll, J. H. & Olson, J. D. Clin. Res. 28, 325A (1980).
 Coller, B. S. Clin. Res. 28, 307A (1980).
- 20. Kaser-Glanzmann, E., Gerber, E. & Luscher, E. F. Biochim. biophys. Acta 558, 344-347
- 21. White, J. G. & Krumwiede, M. Blood 41, 823-832 (1973).

Organic solvents modify the calcium control of flagellar movement in sea urchin sperm

Barbara H. Gibbons* & I. R. Gibbons

Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96822, USA

The form of flagellar and ciliary beating in various organisms is regulated by the intracellular concentration of free Ca2+ (refs 1-5). In demembranated sea urchin sperm reactivated with 1 mM Mg-ATP2-, Ca2+ regulates the asymmetry of the flagellar waveform⁶⁻⁸. On reactivation at a free Ca²⁺ concentration below 10-8 M. the flagella beat with almost symmetric bending waves, and the sperm swim in nearly straight paths with an average turning rate of ~ 0.04 radians per beat, whereas in the presence of 10^{-3} M free Ca^{2+} , the bending waves are highly asymmetric and the sperm describe circular paths with an average turning rate between 0.2-0.35 radians per beat. Here we show that low concentrations of various organic solvents interact with the mechanism by which Ca²⁺ induces waveform asymmetry. The solvents studied fall into two groups of which the first, consisting of methanol, 2-propanol and ethylene glycol, mimic the effect of Ca2+ and increase the asymmetry, whereas the second group, N.N'-dimethylformamide, formamide and p-dioxane, block the increase in asymmetry induced by Ca2+. However, all solvents in both groups act similarly in decreasing the flagellar beat frequency.

The effects of methanol and dimethylformamide on the asymmetry of reactivated sperm in the presence or absence of added Ca²⁺ are shown in Fig. 1. Methanol increases the asymmetry in both cases. On the other hand, dimethylformamide decreases the asymmetry induced by Ca²⁺ and has almost no effect on the slight asymmetry found in the absence of added Ca²⁺. The decrease in flagellar beat frequency induced by these two solvents is shown in Fig. 2. The preparations of sperm remain almost 100% motile until sufficient solvent has been added to lower the frequency from ~28 Hz to 18 Hz, beyond which the numbers of non-motile sperm increase. Table 1 shows the relative sensitivity of the sperm to the various solvents tested. With most of the solvents, the relative concentrations needed to produce the chosen standard effects on asymmetry and beat frequency were about the same; however, the action of formamide seems to be relatively selective for asymmetry.

The changes in symmetry and beat frequency induced by the solvents seem to be complete within the time of mixing and to remain constant for ~5 min. The effects of methanol and 2propanol seem to be fully reversible if the sperm are diluted 10-fold out of the solvent solution into fresh reactivating solution within ~5 min. The blockage of Ca2+-induced asymmetry by dimethylformamide and dioxane, however, is only partially reversed on dilution of the sperm into fresh reactivating solution containing 1 mM free Ca2+ but no solvent, although the beat frequency immediately returns to its original value. In the average of four preparations, the initial turning rate of 0.32 radians per beat fell to 0.11 radians per beat on addition of 0.9 mol % dimethylformamide and then rose to only 0.17 radians per beat after 50-fold dilution of the solvent. However, the asymmetric beating of such preparations could be fully restored by the addition of 3.2 mol % methanol, or 1.7 mol % methanol and 0.1 mM free Ca²⁺.

Brief digestion with trypsin desensitizes reactivated sperm flagella to Ca²⁺, so that the flagellar bending waves become nearly symmetric even in the presence of millimolar concentrations of free Ca²⁺ (refs 10, 11). This suggests that brief trypsin

digestion destroys one or more structures that mediate the $\mathrm{Ca^{2^+}}$ regulation of asymmetry, while leaving intact the ability of the flagella to propagate symmetric bending waves. We have compared the effect of mild trypsin digestion on the movement of reactivated sperm induced to beat asymmetrically by addition of 3.9 mol % methanol (in the absence of added $\mathrm{Ca^{2^+}}$) with its effect on a control sample containing reactivated sperm induced to beat asymmetrically by the addition of 1 mM free $\mathrm{Ca^{2^+}}$ but no methanol. After addition of 0.2 μ g trypsin per ml of reactivating

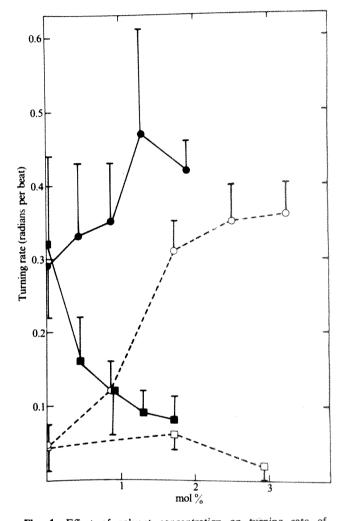


Fig. 1 Effect of solvent concentration on turning rate of demembranated sperm reactivated at 22-23 °C with 1 mM Mg-ATP²⁻ in buffered solution containing either 0.1 mM EGTA (open symbols) or 0.1 mM EGTA and added Ca2 symbols). In these two conditions, the actual concentrations of Ca²⁺ not complexed to EGTA or ATP are estimated to be <10⁻⁸ M and ~1 mM, respectively²³. Procedures for demembranating and reactivating sperm of the sea urchin, Tripneustes gratilla, were as described earlier for 'potentially symmetric sperm' (ref 8). \bullet , Methanol and free Ca²⁺ (~1 mM); \bigcirc , methanol and free $Ca^{2+}(<10^{-8} \text{ M}); \quad \blacksquare, \quad N,N'$ -dimethylformamide and free $Ca^{2+}(<10^{-8} \text{ M}); \quad \square,N,N'$ -dimethylformamide and free $Ca^{2+}(<10^{-8} \text{ M})$ $(<10^{-8} \text{ M}).$ Solvents were Glass Distilled grade from Burdick and Jackson, Inc., Muskegon, Michigan; equivalent results were obtained with highly purified grades from other sources. The solvents were diluted 1:1 with reactivating solution to minimize the heat of solution before addition to the sperm. Concentrations are given as mol % (mol fraction × 100). For comparison with other units, 1.0 mol % equals 2.2% (v/v) or 0.55 M methanol, and 3.3% (v/v) or 0.53 M dimethylformamide. Turning rates were determined from time exposures taken at a magnification of ×160 on Polaroid 3000 Type 107C film by measuring the angle of circular arc travelled by the sperm head during an exposure of known duration. This value combined with the flagellar beat frequency, determined using a stroboscopic flash unit, gave the turning rate in radians per beat. Each point represents the mean obtained from measurements of 10-30 sperm, and the error bars indicate the standard deviation.

^{*}To whom correspondence should be addressed at Kewalo Marine Laboratory, University of Hawaii, 41 Ahui Street, Honolulu, Hawaii 96813, USA.

Table 1 Relative effectiveness of solvents

Concentration required to induce asymmetric beating* (mol %)		Concentration required to block Ca ²⁺ -induced asymmetric beating [†] (mol %)			entration required to er beat frequency‡ (mol %)
Ethylene glycol Methanol 2-Propanol	4.5 2.5 0.5	p-Dioxane N,N'-Dimethylformamide Formamide	0.7 0.6 0.4	Ethylene glycol Methanol Formamide N,N'-Dimethylformam 2-Propanol p-Dioxane	4.6 3.2 2.5 side 1.5 0.6 0.6

^{*} The concentration (mol %) needed to raise turning rate to ~0.34 radians per beat in sperm reactivated with 1 mM ATP at 22-23 °C in a buffer containing 10 mM Tris-HCl, pH 8.1, 0.15 M KCl, 2 mM MgSO₄, 0.1 mM EGTA, 1 mM dithiothreitol and 2% (w/v) polyethylene glycol (molecular weight 20,000).

solution the waveforms became gradually more symmetric at about the same rate in both samples, until after 2-3 min the sperm swam in almost straight lines with apparently normal, symmetrical waveforms. The parallel elimination of both Ca2+induced and methanol-induced asymmetric bending waves by trypsin digestion suggests that these two agents share, at least in part, a common pathway in regulating asymmetry, and that the trypsin-sensitive structure is located in this shared portion.

While the effects of the solvents on asymmetry divide them into two opposing groups, their effect on beat frequency is in the same direction in all cases and may be due to a direct alteration of the kinetic properties of dynein ATPase¹². This second action of the solvents seems to be distinct from their action on asymmetry as it is unaffected by trypsin digestion, and it is also distinct from that of Ca2+, which, at up to 2 mM in the conditions used here, has only a small effect on beat frequency7.

The relative effectiveness of the solvents in altering the asymmetry of flagellar waveforms seems to be unrelated to their dielectric constants, which for solvents of the second group range from ~2 for dioxane to 109 for formamide¹³, while their relative effectiveness on asymmetry varies less than twofold.

Cilia and flagella contain calmodulin-like proteins that presumably act as Ca2+-receptors14-17. Binding of Ca2+ to

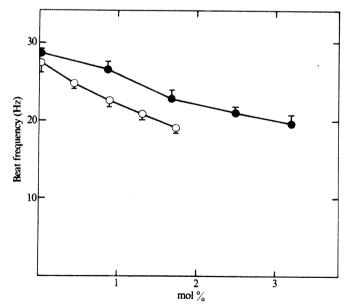


Fig. 2 Flagellar beat frequency of sperm reactivated with 1 mM Mg-ATP²⁻ at 22-23 °C as a function of added solvent: •. Mg-ATP²⁻ at 22-23 °C as a function of added solvent: ●, methanol and free Ca²⁺ (<10⁻⁸ M); ○, dimethylformamide and free Ca²⁺ (~1 mM). Conditions are as described in Fig. 1 legend. Each point represents the mean (±s.d.) obtained from measurements of 8-22 sperm.

bovine brain calmodulin induces the exposure of a hydrophobic surface on the protein which then becomes capable of interaction with various hydrophobic ligands in a manner that may mimic its interaction with the phosphodiesterase molecule in vivo 18. Organic solvents generally induce unfolding of protein structure by promoting the transfer of hydrophobic side chains from internal nonpolar domains of the native structure to the external organic solvent/water solution which provides a less polar environment than water alone 19. Our results could be explained by assuming that methanol and 2-propanol themselves induce the exposure of the hydrophobic surface in a way similar to the action of Ca2+, while dimethylformamide and dioxane (in the presence of elevated Ca²⁺) competitively inhibit the binding of this domain to its subsequent substrate. However, a common feature of the solvents of the second group is the presence of relatively polar, oxygen-containing moieties that could act as hydrogen acceptors and thus interact with proteins by competing for hydrogen bonds, and this provides a possible alternative mechanism for the action of these solvents²⁶. In any case, the fact that the effects described here were induced by concentrations of solvent that are low relative to those frequently used in studies of protein unfolding 21,22, suggests that these solvents have a specific effect on sensitive regulatory protein subunits. Thus, it may be profitable to investigate the effects of these two groups of solvents on other Ca2+-regulated systems as well as on the conformation of the solubilized Ca² receptor proteins.

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- Naitoh, Y. & Kaneko, H. Science 176, 523-524 (1972).
- Nanon, 1. & Kaneko, H. Science 176, 323-524 (1972).

 Murakami, A. & Takahashi, K. J. Fac. Sci. Tokyo Univ. 13, 251-256 (1975).

 Holwill, M. E. J. & McGregor, J. L. J. exp. Biol. 65, 229-242 (1976).

 Hyams, J. S. & Borisy, G. G. J. Cell Sci. 33, 235-253 (1978).

 Gibbons, B. H. J. Cell Biol. 84, 1-12 (1980).

- Brokaw, C. J., Josslin, R. & Bobrow, L. Biochem. biophys. Res. Commun. 58, 795-800
- Brokaw, C. J. J. Cell Biol. 82, 401-411 (1979)

- 8. Gibbons, B. H. & Gibbons, I. R. J. Cell Biol. 84, 13-27 (1980).
 9. Gibbons, B. H. & Gibbons, I. R. J. Cell Biol. 54, 75-97 (1972).
 10. Brokaw, C. J. & Gibbons, I. R. in Swimming and Flying in Nature Vol. 1 (eds Wu, T. Y. -T., Brokaw, C. J. & Brennan, C.) 89-126 (Plenum, New York, 1975). Brokaw, C. J. & Simonick, T. F. J. Cell Biol. 75, 650-665 (1977).
- Holwill, M. E. J. J. exp. Biol. 50, 203-222 (1969).
- Riddick, J. A. & Bunger, W. B. in Techniques of Chemistry: Organic Solvents. Physical Properties and Methods of Purification Vol. 2, 3rd edn (Wiley-Interscience, New York,
- 14. Jamieson, G. A. Jr, Vanaman, T. C. & Blum, J. J. Proc. natn. Acad. Sci. U.S.A. 76, 6471-6475 (1979)
- 15. Van Eldik, L. J., Piperno, G. & Watterson, D. M. Proc. natn. Acad. Sci. U.S.A. 77, 4779-4783 (1980)
- Garbers, D. L., Hansbrough, J. R., Radany, E. W., Hyne, R. V. & Kopf, G. S. J. Reprod. Fert. 59, 377-381 (1980).
- Gitelman, S. E. & Witman, G. B. J. Cell Biol. 87, 764-770 (1980).
- LaPorte, D. C., Wierman, B. M. & Storm, D. R. Biochemistry 19, 3814-3819 (1980).
 Tanford, C. in The Hydrophobic Effect 2nd edn (Wiley, New York, 1980).
- 20. Jencks, W. P. in Catalysis in Chemistry and Enzymology, 332-336 (McGraw-Hill, New
- Herskovits, T. T., Gadegbeku, B. & Jaillet, H. J. biol. Chem. 245, 2588-2598 (1970).
 Parodi, R. M., Bianchi, E., & Ciferri, A. J. biol. Chem. 248, 4047-4051 (1973).
 Caldwell, P. C. in Calcium and Cellular Function (ed. Cuthbert, A. W.) 10-16 'St Martin's, New York, 1970).

[†] The concentration (mol %) needed to reduce turning rate to half its initial value in sperm reactivated with 1 mM ATP at 22-23 °C in the same buffer as above, but with CaCl2 added (1.1 mM).

[‡] The concentration (mol %) needed to reduce beat frequency by 9 Hz, with conditions as in first column for ethylene glycol, methanol and 2-propanol, and conditions as in second column for p-dioxane, dimethylformamide and formamide.

Co-translational membrane integration of calcium pump protein without signal sequence cleavage

Keith E. Mostov*, Paul DeFoor†, Sidney Fleischer† & Günter Blobel*

* Laboratory of Cell Biology, The Rockefeller University, New York, New York 10021, USA

† Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235, USA

The calcium pump protein (CPP) or Ca2+-ATPase is the predominant integral membrane protein of the sarcoplasmic reticulum (SR) of skeletal muscle^{1,2}. CPP is a single polypeptide chain of molecular weight (MW) 119,000 (ref. 3) which loops in and out of the membrane at least three times⁴⁻⁸. Recently, a number of integral transmembrane proteins possessing a single hydrophilic domain on each side of the membrane have been observed to use a signal sequence, cleaved^{9,10} or uncleaved¹¹ initiate translocation of their ectoplasmic domain 12. Microsomal membranes when present during translation are capable of correctly integrating these de novo synthesized pro eins into the membrane^{9,11}. We have used this in vitro translocation system to investigate the integration of CPP into the microsomal membrane. We found that CPP is synthesized without a cleaved signal sequence and that it can be integrated into heterologous microsomal membranes only when these are present during translation, but not when they are present after completion of translation.

Translation of mRNA from muscles of 1-day-old rabbits in the wheat-germ cell-free system (in the absence of pancreas microsomal vesicles), followed by immunoprecipitation of the total translation products with sheep anti-rabbit CPP serum yielded a single product (Fig. 1, lane 2) of an apparent molecular weight identical to that of authentic CPP (lane 1).

It has recently been established that CPP translated in the reticulocyte lysate system in the absence of microsomal membranes retains the initiator methionine¹³. However, this experiment did not indicate whether CPP was synthesized with a signal peptide which would be proteolytically removed by the microsomal membranes. To investigate this directly, we performed a partial amino-terminal sequence analysis of CPP synthesized in vitro in the absence of microsomal membranes (Fig. 2) and found substantial agreement with the known sequence of CPP from the SR¹⁴ (Met in position 1; Ala in 3, 4 and 14; Cys in 12). It can therefore be concluded that CPP is synthesized without a cleaved amino-terminal signal sequence. This conclusion is firmly based on previous data which demonstrate that the wheat germ cell-free system per se, that is, not supplemented by microsomal membranes, is free of that signal peptidase activity which cleaves signal sequences addressed to the endoplasmic reticulum. This signal peptidase activity is introduced into the wheat-germ cell-free system only on supplementation of microsomal membranes^{9,15}.

From cell fractionation of muscle and subsequent incubation of cell fractions containing free or membrane-bound polysomes in a protein synthesizing system, it has been established that CPP is synthesized by the membrane-bound polysome fraction ^{16,17}. Based on the criterion of resistance to extraction by low concentrations of detergent ¹⁷, the newly synthesized CPP was integrated into the homologous microsomal membranes.

To investigate whether *de novo* synthesized CPP can be integrated into heterologous (dog pancreas) microsomal membrane vesicles, and if so, whether integration is coupled to translation, we carried out experiments in which microsomal vesicles were present either at the start or after completion of translation.

To assay for integration we had hoped to use protection from limited trypsin digestion as a criterion because tryptic digestion of CPP in SR vesicles is known to yield several discrete fragments that are resistant to further degradation, presumably because of their being embedded in the lipid bilayer4-8 However, we found that the generation of these characteristic tryptic fragments was not the result of integration of CPP into the lipid bilayer. Almost identical fragments were produced when trypsin digestion of authentic CPP was carried out in the absence (Fig. 3, lanes 5-7) or presence (Fig. 3, lanes 2-4) of the nonionic detergent Triton X-100. When the in vitro product was digested with trypsin in a wide variety of conditions, these fragments were never found; regardless of whether the CPP was translated in the presence or absence of microsomal vesicles, only much smaller tryptic fragments were observed (data not shown). This may indicate that the in vitro product never became integrated into the microsomal membranes or that it was

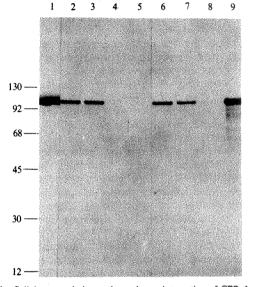


Fig. 1 Cell-free translation and membrane integration of CPP. Lane 1 is from a Coomassie blue-stained gel of authentic CPP2. Lanes 2-9 are from a fluorographed gel of in vitro translated products. Total mRNA was isolated from the limb and back muscles of 1-day-old rabbits¹¹ and translated in the wheat-germ cell-free translation system with ³⁵S-methionine¹¹. Sheep antirabbit CPP serum²⁸ and protein A-Sepharose were used to immunoprecipitate the translated CPP, and the immunoprecipitates were analysed on 7.5-15% polyacrylamide SDS gels and detected by fluorography. Lane 2 is an immunoprecipitate of a 20-µl translation. The specificity of the immunoprecipitation is indicated by the ability of excess (25 µg) authentic CPP to block the immunoprecipitation (lane 4) and the inability of normal sheep serum alone to immunoprecipitate the product (lane 5). Lane 3 is from a 50-µl reaction performed in the presence of microsomal vesicles from dog pancreas²⁹ . Note that the mobility of the in vitro product is not changed. In lane 6, a 100-ul translation performed in the presence of dog pancreas microsomes was incubated for 180 min at 25 °C, instead of the normal 90 min. After translation the pH was adjusted to 11.5 by adding 5 μ l of 1 M NaOH. After 15 min at 0 °C, the 105 μ l was layered over a sucrose gradient in a Beckman airfuge tube which consisted of 80 µl of 0.2 M sucrose and 20 µl of 2 M sucrose. The sucrose solutions contained the same salts as the translation mixture and were also adjusted to pH 11.5 with NaOH. The gradients were centifuged at 160,000 g for 15 min, then 120 µl were removed from the top (supernatant) and the microsomes collected from the 0.2 m/2 M interface and diluted to 120 µl with a solution which contained the same salts as the translation mixture, also adjusted to pH 11.5. Both samples were adjusted to 2% SDS, heated to 100 °C for 5 min, then neutralized and immunoprecipitated. Lane 6 is the immunoprecipitate of the microsomes while lane 7 is that of supernatant. To demonstrate that integration does not occur post-translationally, the same experiment was performed, except that microsomal vesicles were added only after the translation had been incubated for 90 min at 25 °C (at which point no further incorporation of ³⁵S-methionine into protein was detectable) and the incubation continued for a further 90 min at 25 °C. Lane 8 is the immunoprecipitate of the microsomes; lane 9 is that of the supernatant. As a positive control, we demonstrated that an integral membrane protein (the G protein of vesicular stomatitis virus) which is co-translationally but not post-translationally integrated into the dog pancreas microsomes9, will resist extraction in these conditions only when the protein is properly integrated into the heterologous microsomes (unpublished data). The positions of the molecular weight markers (in thousands) are indicated. Froteins used, in order of decreasing size were: Escherichia coli \(\beta\)-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase and cytochrome c.

integrated but did not assume the native conformation found in the SR. Alternatively, the characteristic tryptic fragments of authentic CPP may be the result of intermolecular association, because CPP exists as an oligomer, both in the membrane 18,15 and in detergent solution20. The in vitro CPP may be present in too low a concentration to form oligomers which might confer partial resistance to tryptic digestion.

As an alternative assay for membrane integration, we determined whether CPP could be extracted by alkaline solution (pH 11.5), as integral membrane proteins are non-extractable²¹⁻²³ By this criterion, about half of the de novo synthesized CPP molecules were integrated into dog pancreas microsomal vesicles, but only when these were present during translation (Fig. 1, lanes 6, 7)—not when they were added after translation (Fig. 1, lanes 8, 9). It remains to be shown, however, whether the observed integration into the dog pancreas microsomal vesicles was normal, that is, whether the folding of the polypeptide backbone in these vesicles is identical to that of its in vivo counterpart in muscle rough endoplasmic reticulum.

The observed coupling between synthesis of CPP and its integration into heterologous microsomal membranes are analogous to those observed previously for translocation of secretory proteins and integration of bitopic integral membrane proteins, suggesting a similar membrane-catalysed mechanism^{12,24}. The recent purification of endoplasmic reticulum-associated proteins²⁵ that catalyse translocation of secretory proteins across the endoplasmic reticulum membrane and the demonstration that these same proteins also catalyse the integration into the endoplasmic reticulum of bitopic integral membrane proteins as well as CPP²⁶ provide strong support for this notion. Furthermore, the efficiency of integration of CPP into the heterologous microsomal vesicles, as assayed by the resistance to alkaline extraction, can be increased to almost 100% by the addition of these purified proteins to the in vitro translocation system²⁶

A portion of this work has been presented elsewhere²⁷. We thank Drs J. Hemperly and B. Cunningham for use of, and assistance with, their Beckman sequencer. This work was supported by grants from the NIH (GM 27155 and AM 14632) and the Muscular Dystrophy Association of America. P.D. is an investigator of the American Heart Association, Tennessee affiliate.

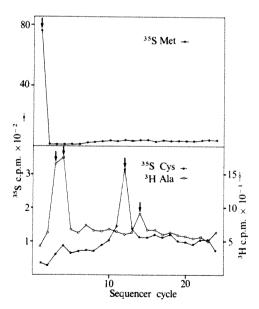


Fig. 2 Partial amino-terminal sequence determination of CPP translated in vitro in the absence of microsomal membranes. Translations (1 ml) were performed using either 35 S-methionine (1,000 Ci mol $^{-1}$) or 3 H-alanine (75 Ci mmol $^{-1}$) and 35 S-cysteine (700 Ci mmol $^{-1}$) (all NEN). Citrate synthase and oxaloacetate were present in the translation mixture to prevent the acetylation of the amino terminus of CPP30. CPP was immunoprecipitated, separated by gel electrophoresis, eluted from the gel and sequenced15

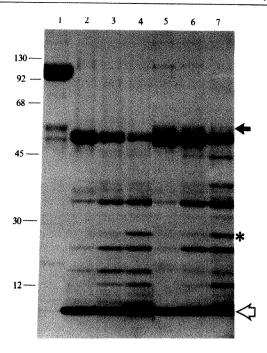


Fig. 3 Tryptic digestion of CPP in the absence or presence of Triton X-100. All samples were analysed by electrophoresis as described in Fig. 1 legend and the gel was stained with Coomassie blue. Each digestion contained, in a volume of 50 µl, 50 µg (protein) of purified SR vesicles, 1 M sucrose, 0.1 M NaCl, 20 mM Tris-HCl, pH 7.5, and the indicated amount of trypsin, pretreated with TPCK. Lanes 2-4 also contained 1% Triton X-100. Trypsin concentrations were: lane 1, 0 μ g ml⁻¹; lanes 2 and 5, 0.6 μ g ml⁻¹; lanes 3 and 6, 6 μ g ml⁻¹; and lanes 4 and 7, 60 μ g ml⁻¹. Digestions were carried out for 45 min at 23 °C and terminated by the addition of 10 µl Trasylol. Solubilization of the SR vesicles by Triton X-100 was ascertained by the inability of the detergent-solubilized vesicles to be pelleted by centrifugation conditions which were calculated to pellet particles with a sedimentation rate >40 S; intact SR vesicles were quantitatively sedimented by these conditions (data not shown). The band indicated by the solid arrow is calsequestrin, a peripheral membrane protein located in the lumen of the SR. It was protected from digestion by the intact vesicles (lanes 5-7) but not when the vesicles were solubilized by detergent (lanes 2-4). The asterisk indicates the position of trypsin. The heavy band indicated by the open arrow is Trasylol. The positions of the molecular weight markers are indicated as in Fig. 1.

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- MacLennan, D. H. & Holland, P. C. A. Rev. Biophys. Bioengng. 4, 377-404 (1975).
 Fleischer, S., Wang, C.-T., Saito, A., Pilarska, M. & McIntyre, J. O. in Cation Flux Across Membranes (eds Mukohata, Y. & Packer, L.) 193-205 (Academic, New York, 1979).
 Rizzolo, L. J., le Maire, M., Reynolds, J. & Tanford, C. Biochemistry 15, 3433-3436 (1976).
- Thorley-Lawson, D. A. & Green, N. M. Eur. J. Biochem. 40, 403-413 (1973) Rizzolo, L. J. & Tanford, C. Biochemistry 17, 4044-4048 (1978).

- Kitzolo, L. J. & Lantord, C. Biochemistry 17, 4044-4048 (1978).
 Saito, A., Wang, C.-T. & Fleischer, S. J. Cell Biol. 79, 601-616 (1978).
 Klip, A., Reithmeier, R. A. F. & Maclennan, D. H. J. biol. Chem. 255, 6565-6568 (1980).
 Allen, G., Trinnaman, B. J. & Green, N. M. Biochem. J. 187, 591-616 (1980).
 Lingappa, V. R., Katz, F. N., Lodish, H. F. & Blobel, G. J. biol. Chem. 253, 8667-8670 (1972).
- Ploegh, H. L., Cannon, L. E. & Strominger, J. L. Proc. natn. Acad. Sci. U.S.A. 76, 2273-2277 (1979).
- Bonatti, S. & Blobel, G. J. biol. Chem. 254, 12261-12264 (1979).
- Blobel, G. Proc. natn. Acad. Sci. U.S.A. 77, 1496-1500 (1980). Reithmeier, R. A. F., de Leon, S. & MacLennan, D. H. J. biol. Chem. 255, 11839-11846
- Allen, G. Biochem. J. 187, 545-563 (1980).
- Lingappa, V. R., Devillers-Thiery, A. & Blobel, G. Proc. natn. Acad. Sci. U.S.A. 74, 2432-2436 (1977)
- Greenway, D. C. & MacLennan, D. Can. J. Biochem. **56**, 452-456 (1978). Chyn, T. L., Martonosi, A. N., Morimoto, T. & Sabatini, D. D. Proc. natn. Acad. Sci. U.S.A. **76**, 1241-1245 (1979).
- Vanderkooi, J. M., Ierokomas, A., Nakamura, H. & Martonosi, A. Biochemistry 16, 1262-1266 (1977).
- Wang, C.-T., Saito, A. & Fleischer, A. J. biol. Chem. 254, 9209-9219 (1979).
 le Maire, M., Møller, J. V. & Tanford, C. Biochemistry 15, 2336-2342 (1976).
- Steck, T. L. & Yu, J. J. supramolec. Struct. 1, 220-232 (1973).
 Shanahan, M. F. & Czech, M. P. J. biol. Chem. 252, 6554-6561 (1977).
- Neubig, R. R., Krodel, E. K., Boyd, N. D. & Cohen, J. B. Proc. natn. Acad. Sci. U.S.A. 76, 690-694 (1979).
- Walter, P., Jackson, R. C., Marcus, M., Lingappa, V. R. & Blobel, G. Proc. natn. Acad. Sci. U.S.A. 76, 1795–1799 (1979).
- Walter, P. & Blobel, G. Proc. natn. Acad. Sci. U.S.A. 77, 7112-7116 (1980)
 Anderson, D. J., Mostov, K. E., Walter, P. & Blobel, G. (in preparation).
- 27. Mostov, K. E., DeFoor, P., Fleischer, S. & Blobel, G. Europ. J. Cell Biol. 22, 147 (1980).
 28. DeFoor, P. H., Levitsky, D., Biryukova, T. & Fleischer, S. Archs Biochem. Biophys. 200, 196-205 (1980).
- Shields, D. & Blobel, G. Proc. natn. Acad. Sci. U.S.A. 74, 2059-2063 (1977)
- Schmidt, G. W., Devillers-Thiery, A., Desruisseaux, H., Blobel, G. & Chua, N.-H. J. Cell Biol. 83, 615-622 (1979).

MATTERS ARISING

Flexural strength of cements

BIRCHALL ET AL.1 are to be congratulated on the improvements made in the flexural strength of cement, however, we question their scientific explanation which is given in terms of the removal of large size pores ≥100 µm; it is postulated that these pores are simple strength controlling Griffith flaws. We have shown2 that although notched specimens of ordinary cement paste tested in flexure seem to behave according to simple Griffith theory for specimens having a fixed beam depth, nevertheless, when the beam depth is altered different values are obtained for the fracture energy, R. In fact the apparent value of R increases with increasing specimen depth and R is not therefore behaving as a material constant, and a simple application of the Griffith equation is not acceptable. It is apparent from their conclusions that Birchall et al. have carried out measurements on beams of fixed overall depth. Note that in our experiments, specimens of differing size were cut from the same cured samples of cement and therefore the variation in R values could not result from changes in specimen microstructure from one sample to another. By extrapolation we deduced the behaviour of apparently sharp flaws in large cement samples and showed that the material can be regarded as intrinsically weak, for example, for a w/c ratio of 0.3 having an intrinsic strength of ~ 15 MPa, and that sharp flaws have an effective radius of curvature of ~0.5 cm; therefore flaws ≤0.5 cm in size will have a comparatively small effect on strength.

To improve strength it is necessary to increase the intrinsic strength by increasing the total number of effective chemical bonds per unit area of the material for example by reducing the total amount of unfilled space in the material, that is increase density. Birchall et al. quote a value for elastic modulus of 40 GPa for improved (MDF) cement—about twice that of OPC which suggests that its basic structural properties have been changed. We conclude therefore that it is a little misleading to attribute the strength increase solely to the elimination of large flaws and that the high strength has been achieved by a reduction in w/c ratio, good packing, compaction and drying out, all of which are expected to increase strength. The resulting MDF cement, although intrinsically stronger, is sensitive to flaws and will need to be free of macroscopic flaws to achieve the higher strengths; for example a 1-mm flaw will reduce the strength of MDF cement by a factor of 3, whereas a similar size flaw in ordinary Portland cement w/c = 0.3 will only reduce the strength by a factor of 1.6. Therefore, flaws must be eliminated during processing. However, as discussed above, simply eliminating flaws from an ordinary cement paste would not result in strengths as high as those quoted for the MDF cement.

J. E. BAILEY

Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH, UK

D. D. HIGGINS

Cement and Concrete Association, Wexham Springs, Slough SL3 6PL, UK

- Birchall, J. D., Howard, A. J. & Kendall, K. Nature 289, 388-89 (1981).
- Higgins, D. D. & Bailey, J. E. J. mater. Sci. 11, 1995–2003 (1976).

BIRCHALL ET AL. have shown that the flexural strength of a Portland cement paste can be increased to ~65 MPa. They termed the modified paste as "macrodefect-free" (MDF) paste and attributed the improvement to the removal of macro-defects. However, this interpretation may be queried.

Birchall et al. have observed that the flexural strength data of both the MDF paste and the ordinary Portland cement paste could be fitted to the Griffith equation.

$\sigma = (ER/\Pi C)^{1/2}$

where "tensile cracking stress (σ) is related to an effective critical crack length (C) which may be a pore; Young's modulus (E) and fracture surface energy (R) being taken as material constants". They found that E and R are 20 GPa and 19 J m⁻² for an ordinary Portland cement paste and 40 GPa and 30 J m⁻² for the MDF paste even though both the pastes have very similar porosities.

It appears that both the material constants E and R are so dependent on the pore size distributions that their product could be altered by a factor of 3. In that case one may wonder how the Griffith equation could be applied to these pastes. This uncertainty has been further emphasised in their Fig. 3 which shows that the MDF paste has higher strengths than the ordinary Portland cement paste even when both pastes have similar sized critical flaws. More probably Birchall et al. have altered the nature of the paste altogether by using rheological aids such as plasticizers and superplasticizers, which are generally organic products of some chain lengths; some even contain organic polymers. The addition of polymers to a cement paste increases its flexural strength as well as its flexural/compressive strength ratio from ~0.1 for a brittle failure to a higher value characteristic of a plastic failure. Note that the MDF paste

has also a flexural/compressive strength ratio of 0.3 (65/200).

It will not be very difficult to ascertain experimentally if the nature of the paste has been modified in which case the observed improvement may have little to do with the removal of macro-defects. Note added in proof: The patent application by Birchall et al.² shows that polymers have been used by them.

S. CHATTERJI

Byggeteknik, Teknologisk Institut. Gregersensvej, DK-2630, Tastrup, Denmark

- Birchall, J. D., Howard, A. J. & Kendall, K. Nature 289, 388-390 (1981).
- Birchall, J. D., Howard, A. J. & Kendall, K. European Patent Application No. 80301909.

BIRCHALL ET AL. REPLY-Bailey, Higgins and Chatterji have raised interesting questions, some perhaps prompted by the lack of detail in our short paper¹. We are aware of the detailed arguments relating to the fracture behaviour of cement paste and of the careful work by Bailey and Higgins on this. Our proposition is that, bearing in mind the heterogeneous and inconstant nature of cement paste, the Griffith criterion is an adequate explanation of the failure behaviour of the material. It has the advantage of predicting what steps should be taken to improve the material—the removal of macroscopic flaws—and our paper shows that this is demonstrably effective.

As Bailey and Higgins correctly point out, fracture tests on ordinary cement paste give a spread of values of R, the fracture energy, a parameter which appears to change significantly with test geometry and crack speed among other variables. We believe that these variations in R make it impossible by conventional experiments to distinguish the Griffith theory from more complex theories of fracture. The experimental scatter is too great. Essentially, what we have done is to extend the study into an unexplored range of flaw size, down to 10 µm, when the overall behaviour is seen to fit the Griffith theory satisfactorily.

It is suggested that the high value of elastic modulus (40 GPa) quoted in our paper for MDF cement indicates a higher number of bonds per unit area or a higher bond strength and a microstructure fundamentally different from that of normal paste. It is indeed implicit in our understanding of the mechanism of hydraand setting², that morphology will vary with the volume of space to be filled and hence with packing, and so on. However, a major point in our paper is that whereas elastic modulus is dominantly related to total porosity volume. flexural strength the

dominantly related to the size and proportion of macroscopic voids and, as illustrated in Fig. 4, is insensitive to total porosity volume. Thus high flexural strength is observed in specimens having a range of values of elastic modulus, 25–40 GPa.

Chatterji suggests that the properties of MDF cement are those of a polymermodified paste. However, the strength values of MDF cement far exceed those claimed for pastes made at modest temperature and pressure in the presence of a water-reducing additive, with polymer addition or by polymer impregnation. Furthermore, the failure mode of MDF cement is brittle with no evidence of plastic deformation. Thus the improved properties of MDF cement are attributable to the altered pore size distribution of the inorganic matrix and are, as we indicate. independent of the chemical nature of the inorganic cement matrix. In this context, other work (in preparation) has shown that MDF cements can indeed be further modified to increase significantly the fracture toughness, with the result that flexural strength is raised still further to levels exceeding 150 MPa. The absence of macroscopic voids is an essential prerequisite for the attainment of such very high strength.

> J. D. BIRCHALL A. J. HOWARD K. KENDALL

Technical Department, ICI Mond Division, Runcorn, Cheshire WA7 4QD, UK

- Birchall, J. D., Howard, A. J. & Kendall, K. Nature 289, 388-390 (1981).
- Birchall, J. D., Howard, A. J. & Bailey, J. E. Proc. R. Soc. A360, 445-453 (1978).

Can passive tactile perception be better than active?

PASSIVE tactile perception has invariably been found to be equal to or inferior to active tactile perception¹⁻³. An exception has recently been reported in an experiment by Magee and Kennedy4 who guided the finger of each blindfolded subject around raised-line drawings of common objects. These (passive) subjects were able to identify more of the drawings than the active subjects whose fingertip movements were unguided. Magee and Kennedy suggest that "the act of planning may draw on the limited resources of the haptic system so that less processing capacity is available to monitor and distinguish relevant from irrelevant kinaesthetic input".

We consider this to be an implausible explanation of active inferiority for the following reasons:

(1) The "act of planning" is more cognitive than sensory. No evidence is provided

to support the idea that, in this experiment, such planning can be expected to interfere with (or draw on limited resources of) the haptic (sensory) system. (2) The resources of the haptic system must be limited at some level of task complexity but the job it was required to do in this experiment should be well within its capacity. When all fingers are simultaneously engaged in palpating an object, the channel is apparently not overloaded. Why should movements on one fingertip tax the system to the degree that planning of such movements proves too much? Moreover, Shiffrin et al.5 present rather convincing evidence for an "unlimited-capacity nonattentional model of tactile perceptual processing". (3) Presumably, "relevant" kinaesthetic input comes from movements made when the fingertip faithfully follows the raised line and "irrelevant" inputs from errors (straying from the line). But surely deviations are important error signals which guide ensuing movements rather than act

either way.

(4) The comparison being made was not simply between active and passive haptic conditions. Rather, because haptic information was so (unusually) impoverished, conscious attention was necessary to build up a "picture"—for both passive and active subjects—but active subjects suffered the disadvantage of also having to plan movements.

as "irrelevancies". Information is gained

In a different experiment, it was shown that when passive and active subjects are yoked in a tactile maze exploration task, the performance of active subjects suffers because they have the responsibility of regulation (planning) of movements⁶. This experiment, and that of Magee and Kennedy, showed that it is possible to demonstrate interference at the cognitive level when information is transmitted by the haptic system—not that passive tactile performance is better than active.

BARRY L. RICHARDSON DIANNE B. WUILLEMIN Department of Psychology, University of Papua New Guinea, Port Moresby, Papua New Guinea

Matters Arising

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- 1. Gibson, J. J. Psychol. Rev. 69, 477-491 (1962).
- Landrigan, D. T. & Forsyth, G. A. J. exp. Psychol. 103, 1124-1130 (1974).
- Schwartz, A. S., Perey, A. J. & Azulay, A. Bull. Psychononomic Soc. 6, 7-9 (1975).
- Magee, L. E. & Kennedy, J. M. Nature 283, 287 (1980).
 Shiffrin, M. R., Craig, C. J. & Cohen, E. Percept. Psychophys. 13, 328-336 (1973).
- Richardson, B. L., Wuillemin, D. B. & MacKintosh, G. T. Br. J. Psychol. (in the press).

MAGEE AND KENNEDY REPLY—Richardson and Wuillemin argue that our experiments did not establish superior passive tactile perception in the identification of raised-line drawings. They base their claim on studies with tactile finger mazes in which they found passive exploration to be superior to active exploration, and were then able to show that passive superiority was due to a cognitive factor, that is, the act of planning exploratory activity. They argue that passive superiority with raised-line drawings is also due to this cognitive factor.

Modern theorists recognize that planning and anticipation are crucial to perception, and we too recognize this as a critical component of haptic perception. However, the crucial problem for present purposes is that Richardson and Wuillemin's conclusion is based on superficial similarity between two types of tasks rather than on fundamental principles of haptic perception.

Clearly, Richardson and Wuillemin's finger-maze learning could be solved as a verbal sequence of eight discrete left-right turns¹, a number within the bounds of short-term memory. In contrast, the identification of raised-line drawings involves complex shape information that is not discrete. Our subjects had to deal with a line continuously varying in location, direction and extent, that is, the task required the subject to integrate across time subtle spatial information that was difficult to encode verbally. Integrating all this information was not easy, as shown by the generally low levels of performance on this task. These low levels of performance indicate (contrary to Richardson and Wuillemin's assertion, made without supporting evidence) that our singlefinger task was not facile and was not well within haptic capacities. Richardson and Wuillemin's task has little to do with perception, while identifying shape and form is the sine qua non for haptic perception.

Our task was one of haptic perception in which we have shown passive superiority.

LOCHLAN E. MAGEE JOHN M. KENNEDY

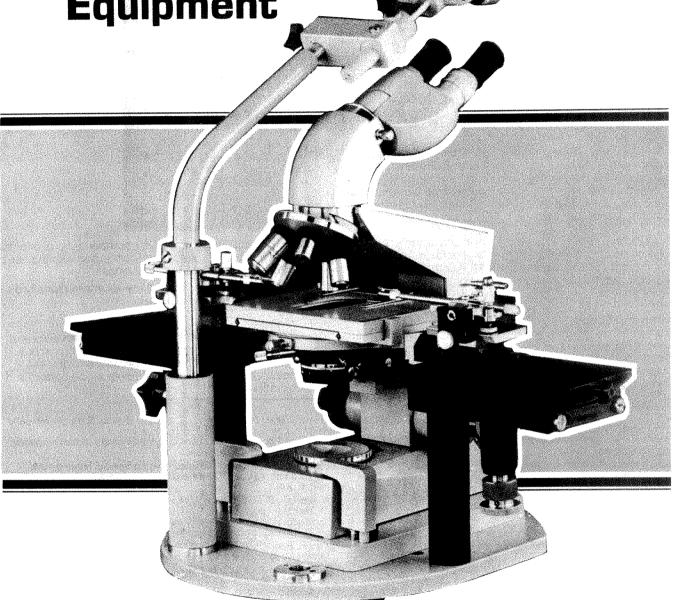
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1. Richardson, B. L., Wuillemin, D. B. & MacKintosh, G. T. Br. J. Psychol. (in the press).

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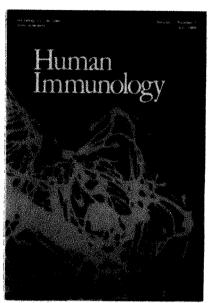
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BOOK REVIEWS

The genius phenomenon

Donald Michie

John von Neumann and Norbert Wiener: From Mathematics to the Technologies of Life and Death. By Steve J. Heims. Pp.547. ISBN 0-262-08105-9. (MIT Press: 1980.) \$19.95, £14

THE appearance of S. J. Heims's study of John von Neumann and Norbert Wiener is opportune. Technologies of computing, communication and control sprang from where the two men sowed, and are now taking us beyond range of our charts. Will the cybernetic age resemble other ages? Will scientists still be "on tap but not on top"? Or did the post-war influence exerted by physicists and mathematicians in American military circles presage new responsibilities for a stratum of society commonly regarded as temperamentally ill-equipped to bear them?

If so, it seems that we may have to accustom ourselves to receiving guidance from our emotional juniors on matters of life and death. The saving that "genius is to madness near allied" was always of course misleading. But if one were to decide to call infants mad for their helpless and grasping self-absorption, and adolescents mad because of their lonely assertiveness and intensity, then we could read meaning into the saving after all. The association between one or another form of emotional underdevelopment and extreme manifestations of creative excellence has often been remarked. It finds fascinating exemplification in Dr Heims's book about information science's two great founders.

Norbert Wiener no doubt started life as an infant; but if by some mischance he did not, then he later repaired the omission. Thus, Bertrand Russell, writing in 1913:

At the end of September an infant prodigy named Wiener, Ph.D. (Harvard), aged 18, turned up with his father who teaches Slavonic languages there, having first come to America to found a vegetarian communist colony, and having abandoned that intention for farming, and farming for the teaching of various subjects, [say] mathematics, Roman Law, and mineralogy, in various universities. The youth has been flattered, and thinks himself God Almighty — there is a perpetual conflict between him and me as to which is to do the

Socially unsure to the end of his life, Wiener's child-like attributes of generosity and utopian optimism struck all who knew him. There were also irritants — absentmindedness, simulation of sleep during the lectures or conversations of others, and the constant and overwhelming hunger for praise. The latter would impenitently break surface even when to all appearances it had

been totally transcended. A friend recalled a meal towards the end of Wiener's life:

... Wiener stopped and turned the conversation to Yilmaz (a physicist). Now people say that Wiener wasn't good at listening to people. Wiener was perfectly quiet as Yilmaz expounded his color theory ... then we went on to invariance, and science and epistemology; the conversation then took on a very general tone. I remember it was a beautiful, sunny day; it was a very heady experience; we were really getting along perfectly. Then at the end Wiener said to me: 'Was I a good listener?'.

I met Norbert Wiener at Oxford around 1950. N. A. Mitchison and I, with other graduate students, took him to Mitchison's rooms in Magdalen after he had talked to an undergraduate society. We settled him in an arm-chair, where he seemed to fall at once into a deep sleep. We began to discuss genetics among ourselves. This was a year or two before Crick and Watson had identified the genetic material in people's minds with DNA and had proposed the existence of a coding problem. Wiener's eves opened briefly: "How many bits of information, I wonder, are coded in the human chromosomes? Could one, do you think, get an upper bound by chemical arguments?". He closed his eyes. We exchanged glances, and having missed the whole of a very extraordinary point resumed our aimless talk.

Wiener's youthful attention was drawn by Russell to Einstein's 1905 paper on Brownian motion. Marc Kac wrote after Wiener's death:

In retrospect one can have nothing but admiration for the vision which Wiener had shown when, almost half a century ago, he had chosen Brownian motion as a subject of study from the point of view of integration. To have foreseen at that time that an impressive edifice could be erected in such an esoteric corner of mathematics was a feat of intuition not easily equalled now or ever. . . It gave us not only a new way of looking at problems but actually a new way of thinking about them.

The man who in the late 1940s founded cybernetics, supervised in the early 1960s a PhD student in a further development of this early work on stochastic processes. The student, Michael Arbib, has gone on to establish at the University of Massachusetts a Centre for Systems Neurosciences coherent embodiment of Wiener's dream of a mathematical school for the analysis of biological organization and its control. Every great scholar hopes to leave more than his writing. Wiener, in surveying today's work on formal models of brain function, would I think be satisfied both with the project itself and with its humanistic goals.





Norbert Wiener (top) and John von Neumann. Both "loved to dazzle and [were] preternaturally equipped to do so".

From first to last Wiener followed Descartes: "I would not engage in projects which can be useful to some only by being harmful to others". His penalty was to be the eternal mavenick — "calf or other animal not marked with an owner's brand".

Von Neumann's consuming drives presented a contrast to all this. Like Wiener, he loved to dazzle and was preternaturally equipped to do so: Leo Wigner referred to his brain as "a true miracle". But he made sure to dazzle as an insider. In the higher counsels of the Pentagon he showed a demonstrative hawkishness beyond that of the less rootless, less

intellectual, professional hawks of the military. In 1950 he was remarking: "If you say why not bomb them tomorrow, I will say why not today? If you say today at 5 o'clock, I say why not one o'clock?".

In connection with von Neumann's advocacy of preventive nuclear war, Steve Heims writes of his response when asked by Ulam to explain the "Hungarian phenomenon":

... he would say that it was a coincidence of some cultural factors which he could not make precise: an external pressure on the whole society of this part of Central Europe, a subconscious feeling of extreme insecurity in individuals, and the necessity of producing the unusual or facing extinction.

Edward Teller remarked of his youth that Hungary was foundering and if he wanted to survive he would "have to be better, much better than anyone else", echoed by another scientifically spectacular émigré, Davis Gabor: "Innovate or die!".

The Hungarian phenomenon may or may not be overemphasized in this book. But as concerns John von Neumann's desire to be not just on tap, but on top, Heims is replete with vivid documentation. A picture takes form of breath-taking intellectual exuberance and power lodged in a coldly ambitious yet sanguine personality, both complex and strangely undeveloped. When the author writes, in connection with the creation of nuclear weapons, of von Neumann's "compelling desire to serve and be part of the élite establishment, and to assume a modern American role reminiscent of the court astrologer or court engineer of a feudal military empire", we recognize it for the truth. We also recognize that von Neumann himself in all likelihood would jauntily have accepted the label for ornament. Among Johnny's attributed sayings was: "Only a man from Budapest can enter a revolving door behind you and emerge ahead of you".

Wiener's tangible legacy groups itself around the foci of neurobiology and cognitive science. Von Neumann's is to be found in the technology of brute-force computing, rather than in today's developments in artificial intelligence. Although aware of A. M. Turing's contributions to logic, to computer design and to the mechanization of human thought processes, von Neumann showed little sign of having received an influence. The notion of resource-limited computation, key to the brain's cognitive processes and their machine simulation, would surely have been alien to one whose reluctance to admit limits to his own calculational powers became a byword. A similar assumption of omniscient rationality lies at the root of the game-theoretic model of economic behaviour which was erected by him and Morgenstern, now displaced by H. A. Simon's notion of practically feasible, as opposed to ideal, calculations of selfinterest.

In one respect, Heims's book follows a popular error. Von Neumann is credited with sole intellectual rather than political authorship of the EDVAC design. This doubtless arises from the circumstance that the report of the EDVAC committee, which included Eckert and Mauchly, bore the sole signature of its chairman.

In general, however, Steven Heims's scholarship is both voluminous and detailed, and his personal biases, which are those of a liberal humanist, are not too obtrusive. The author succeeds least in his attempted tour of the pure and applied mathematics which these men built, in von Neumann's case outstanding in this

century. For this we must wait for another book. But the living substance of two great minds and of the convulsive epoch through which they lived is here recreated in 414 pages of narrative and 115 of notes. The book informs, stimulates and entertains. In its cumulative effect it also profoundly disturbs. It is right that we scientists should be induced to contemplate our professional responsibility for the repercussions of our trade. The labours of Dr Heims have fashioned a powerful instrument to this end.

Donald Michie is Professor of Machine Intelligence in the University of Edinburgh,

New dimension for archaeology in tool use

Ruth Tringham

Experimental Determination of Stone Tool Uses: A Microwear Analysis. By L. Keeley. Pp.320. ISBN hbk 0-226-42888-5; ISBN pbk 0-226-42889-3. (University of Chicago Press: 1980.) Hbk \$15, £10.50; pbk \$7, £4.90.

AMONG the spate of literature accompanying the rise of interest in prehistoric artifact use, two books stand out: Lithic Use-Wear Analysis, edited by Brian Hayden (Academic, 1979), and this volume by Lawrence Keeley.

Experimentation forms the most important aspect of Keeley's research, and it comprises the third and fourth — by far the most important — chapters of his book. The two preceding chapters discuss recent research on microwear and the method of the microscopic examination of the edges of stone tools.

For Keeley, experimentation is the "fabrication of replicas of archaeological implements and their use on various materials under controlled conditions" (p.3), the ultimate purpose of which is "the interpretation of particular archaeological pieces usually from archaeological occurrences limited in time and space" (p.5). The value of such replications is that they help to bring alive long-vanished patterns of behaviour, and suggest directions for more systematic research. The series of replications reported by Keeley in detail describe the formation of polishes on the surfaces and edges of flint flakes as a result of contact with different materials in different tasks. These polishes are then identified on archaeological specimens and inferences made about the material and the task of the prehistoric tool.

Keeley's replications, however, have limited implications for general issues of the evolution of human behaviour. In fact they are not really "experiments" in the definition of the term used in experimental sciences, in that they are not designed to test empirical hypotheses by the systematic and artificial manipulation of variables.

They do not explain the nature of the properties of the raw materials being tested. As Keeley is the first to point out (p.167), his replications do little to explain the variation in the character of the polishes formed on the flint. On the other hand, he criticizes as artificial such experiments as my own which do not replicate purposeful tasks but, under laboratory conditions, mechanically bring tool edge and worked material into contact in a repeated and standardized action. And yet to criticize such experiments as "artificial" misses the whole point of experimentation.

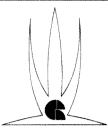
In spite of this inherent theoretical weakness in the book, there is no doubt that Keeley has made a methodological breakthrough in microwear studies by focusing on an essential aspect of the reconstruction of the utilization of stone tools — the correlation between the variability of polishing of flakes and that of the worked material.

The second major section (Chapters 5-7) reports the observations which Keelev made on prehistoric tools and the application of this microwear information to the interpretation of variability in three Lower Palaeolithic assemblages from southern England (Clacton, Swanscombe and Hoxne.) Here, as stated earlier in the book, Keeley's main aim is to reconstruct task-specific behaviour in his sites and assemblages. Yet his conclusions from microwear observations of the three assemblages do not directly address this problem. The observations provide information on the utilization of rock as a raw material resource rather than on the activities which the tools performed. On the basis of microwear information, Keeley concludes that the utilization of flint as a source of tool raw material was opportunistic and relatively unplanned in the Clactonian and Acheulian industries represented by his three sites. Selection of flakes for specific tasks, if such selection existed, was on the basis of the shape and

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angle of the edge, not on the shape and size of the flake itself. There is a most interesting discussion on the relationship between the role of hand-axes and flake tools in the stone tool assemblages, which of course is pertinent to our understanding of the changing use of stone as a raw material between the Lower and Middle Palaeolithic industries, hand-axes being much rarer in the latter. It is unfortunate that so far very little microwear information has been collected on hand-axes. As Keeley points out, "there is much potential for future research".

The implication of Keeley's conclusions in this section is that microwear data can provide unique information on the use of stone by allowing us to relate empirically the form of artifacts to their utilized parts. Furthermore, such data can provide the sole source of information on an aspect of the evolution of human behaviour which has received little discussion by archaeologists — the consumption of resources: their uses, maintenance and discard. But it seems that Keeley does not recognize this broader significance of his work.

Similarly, the two concluding chapters are somewhat disappointing. They discuss, respectively, areas for future research and the conclusions of the study so far, and both are characterized by a surprising modesty and lack of ambition. The conclusions of the microwear analysis of the prehistoric assemblages which Keeley (p.176) feels are the most important are that it has "... uncovered many fascinating details about the technology of the Lower Palaeolithic, not the least of which are the existence of plant 'gathering' knives and information about the methods of hide preparation employed"; furthermore, it has "uncovered some interesting relationships between the morphology and function of implements in the assemblages . . . ", and it has provided a "little tantalizing information pertinent to questions concerning the purposes of hand-axes in the Middle Acheulian . . . ". But is microwear analysis — all those hours and energy — destined to provide only the "fascinating", the "tantalizing" and the "interesting"? Likewise, the suggestions for future research tend to concentrate on particular details and problems.

In this book there is an enormous amount of valuable experimental and analytical information, which should have an impact on a wide public. Keeley warns about many of the traps of microwear analysis, including over-confidence and over-optimism. He fails, though, to mention the most dangerous one of all: that one can fall in love with one's flake edges, especially when they are viewed under the microscope, and forget why it is that we originally decided to spend so much time with them.

Ruth Tringham is Associate Professor of Anthropology at the University of California at Berkeley.

Natural history from Henry VII to Victoria

G. D. R. Bridson

British Natural History Books, 1495-1900: A Handlist. By R.B. Freeman. Pp.437. ISBN 0-7129-0971-0/0-208-01790-0. (Dawson, Folkestone, Kent/Archon, Hamden, Connecticut: 1980.) £20, \$39.50.

Modest proportions and unpretentious presentation combine to disguise this book's place as a milestone in the progress of British natural history bibliography. We have never had an equivalent of, for instance, Meisel's magnificent bibliography of American natural history down to 1865, though such works as Simpson and Henrey for botany, and Irwin and Lisney for ornithology and entomology, have made significant contributions. Otherwise one could turn to the German compilations of Engelmann, Carus and Taschenberg for a bibliography of general natural history and zoology down to 1880, amongst which is buried British material.

The compilation of a checklist of all British natural history books is a task for the experienced bibliographer and we are fortunate that one so well qualified as Dr Freeman should attempt the task. The result is a work that does great credit to him and outstanding service to the user. It is only a handlist but within that format lies much concealed scholarship and great attention to accuracy. This is enhanced by providing the full forenames of every author possible, by searching out and recording multiple editions, by providing a chronology of pre-1801 titles and by indicating in the index "those works which, in the opinion of the author, are the most important contributions to the subject of the entry". The 35-page subject index extends the value of the book beyond that of a handlist, though that still remains its basic function and as such it is remarkably comprehensive. One can find omissions among its 4,206 entries by crosschecking with works such as Jackson's guide to botanical literature or Taschenberg's general natural history listings. But, I must stress, so much is included that only specialists in book rarities will find fault.

What is more controversial is the exclusion of papers in periodicals and, unfortunately, any bibliographical

In celebration of the centenary of the British Museum (Natural History), the Museum and Cambridge University Press have published *Chance, Change and Challenge*, a two-volume illustrated compilation which will be of particular interest to sixth-formers and undergraduates. Volume 1, *The Evolving Earth*, deals with the origin and development of the Earth, and Vol. 2, *The Evolving Biosphere*, examines the evolution of life. Prices are: Vol.1 hbk £30, pbk £10.50; Vol.2 hbk £32.50, pbk £11.50.

reference to the periodicals themselves. This a handlist of books, but just when a preprint, offprint, reprint or plain tear-out becomes a separate book will give rise to much argument in many individual cases. So many papers from periodicals were reprinted, often with new pagination, in the nineteenth century that the full record of this transition literature will take many years to document with accuracy. The exclusion of bibliographical mention of periodicals is, in my view, the book's most serious omission and one that would not have required so much effort to rectify.

Librarians, bibliographers, historians, book-collectors, booksellers and naturalists will all want this work on their shelves for frequent reference, and several of those will wish to interleave and annotate their copies. They will find few typographical errors to correct, an odd binary entry to delete perhaps (for example Nos. 439 and 1,181), and a few cross-references to add here and there. At £20 the book is rather costly for such commonplace presentation but in terms of intrinsic value and likely duration of utility that cost will be amply rewarded.

G. D. R. Bridson is Librarian at the Linnean Society of London.



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Charles Neville

Biology of Insect Eggs. By H. E. Hinton. Pp.1,125. Three volumes. ISBN 0-08-021539-4. (Pergamon: 1981.) \$400, £167.

THIS posthumous work is monumental in both senses of the word. Professor Hinton tried courageously to finish it during his terminal illness, but the final stages of publication, including compilation and checking of the extensive references and index, had to be completed by his research assistant, Mrs Joyce Ablett, and by Dr Colin Mapes. Information on insect eggs was previously scattered throughout the literature. It now becomes available in one source, which is certain to become the standard work on the subject. But these three volumes constitute no mere review: they also contain an enormous amount of original information, based upon a lifetime of work divided between field and laboratory.

The egg stage of insects is no less important than those which follow. Yet most entomologists would be content if they could recognize even the adult stage of those species of flies found on cow pats. Howard Hinton could, however, with the aid of a hand lens, identify their eggs down to species in situ. As might be expected from one of the world's most knowledgeable entomologists, the text is packed with interesting examples of evolutionary adaptations.

Some of the most fascinating arise from the fact that, being small, insect eggs have a relatively large surface area. Whereas this is an advantage for oxygen uptake, it means that terrestrial eggs are prone to desiccation; conversely, eggs in aquatic surroundings are threatened by drowning. Professor Hinton shows how these factors explain the greater complexity of insect eggshells compared with those of larger animals, and relations between respiratory structure and function receive extensive coverage in the first volume. The inner layer of insect eggshells is filled with air, and it communicates with the outer surface through channels (aeropyles), some of which open on elevated turrets, or even horns. In some eggs, which might otherwise drown, trapped air bubbles function as gills which both provide oxygen themselves and give access to a further supply from the surrounding water. Such bubbles eventually shrink, however, and need replenishment. This is fine for motile stages but not for eggs, which cannot move. To solve this problem, some eggs which are laid in dung begin respiration by a shrinking bubble, and then change over to a constant-volume bubble trapped between hydrofuge hairs (a respiratory plastron). Hinton has shown how there are even more examples of plastrons in terrestrial eggs than amongst aquatic larvae. Plastrons are particularly

appropriate for eggs which are subjected to alternate drying and flooding, and have independently evolved in unrelated species many times. A further chapter describes how some eggs can absorb water via special organs (hydropyles).

Subsequent chapters in the first volume cover the interrelations between adults and their eggs (fecundity, oviposition and parental care). There are also chapters on the adult glands which secrete protective egg cases; on the proteins which form them; on eggshell proteins and their chemical cross-linking; and on how eggs interact with their enemies, often fooling them with deceptive colours or shapes.

The second volume describes the egg structure and adaptations of insects, order by order. Numerous keys to identification will prove invaluable to future workers, though, sadly, there was not time for a chapter on fleas. These two volumes are profusely illustrated: I counted 911 photographs, mostly original scanning electron micrographs, a technique which Howard Hinton used right from its introduction. In addition there are 32 tables and nearly 300 clear text diagrams, most of which are original.

The final volume provides the overwhelming bibliography (over 5,000 references) and separate indexes to species, authors and subjects. The work ends with a list of Professor Hinton's publications: these three volumes now complete it. His contributions to entomology thus end on a resounding note.

Charles Neville is Reader in Zoology at the University of Bristol.

Children who are left to themselves

P. E. Bryant

Developmental Psychology and Society. Edited by John Sants. Pp.389. ISBN 0-333-21340-8/0-312-19751-9. (Macmillan Press, London/St Martin's Press, New York: 1980.) £20, \$35.

D. W. WINNICOTT, a well-known child psychiatrist, is quoted as having remarked in a heated discussion that there is no such thing as a baby. He meant that it is absurd to treat babies in isolation. They are in constant contact with parents and others, and most of the significant things that they do are social in some way or other. This is a point which most child psychologists accept nowadays, but they still find it hard to incorporate into their work.

The clearest example is the question of the importance of language in intellectual development. It seems obvious that people talk to children and tell them things, and that this contributes significantly to the child's growing understanding of his world. Yet the effects of this sort of communication have been virtually ignored by child psychologists. Some, like Piaget, go to great lengths to show that language is of small importance to a child's development; others think that it might have some effect but in the form of "inner speech" - the child talking to himself. Nobody seems to realize that grown-ups actually talk to children. As usual, the social element is left out.

The great merit of the book which John Sants has edited is that it attempts to fill this gap. Nevertheless, the clearest point to emerge is how indirect our knowledge is of the effects of different kinds of social contact. Even the best chapters show this. Those, for example, by Roger Goodwin on

language and by Neil Warren on crosscultural psychology are particularly impressive. Both are comprehensive and entertaining. But the chapter on language has virtually nothing on people talking to each other — rather odd in a book with society in its title — and the cross-cultural chapter is more concerned with connections between "primitive" and child-like mentalities than with the transmission of culture from one person and generation to another. The topics seem to be social, but the meat of social behaviour — social interactions — is left out.

A more glaring example of discrepancy between appearance (the chapter heading) and reality (what lies beneath) occurs in Wallace's chapter on educational competence. Nothing is said in it about teaching the child, who is left to get on with his own development without any help from anyone else. This abandonment reflects a Piagetian bias, which also dominates a chapter by Furth on Piagetian perspectives. Nothing social there, and very little in a chapter by Archer and Lloyd on sex differences. The closest one gets to social interactions is in an interesting though speculative chapter by Trevarthen on very early physiological and social development, and by Isbell and McKee on social cognition.

None of the authors should be blamed too harshly for neglecting social interactions in a book on children in society. It is the fault of developmental psychology, which still has to get to grips with Winnicott's insight.

P. E. Bryant is Watts Professor of Psychology at the University of Oxford.

Electron Microscopy of Proteins

edited by J.R. Harris

Volume 1: September/October 1981, c.350pp., 0.12.327601.2.

Volume 2: December 1981, c.350 pp., 0.12.327602.0.

In the preface, Professor R.W. Horne, Department of Ultrastructural Studies, John Innes Institute, Norwich, England writes: "These volumes reveal the remarkable progress made in the field of morphology closely linked to biochemistry and biophysics. The contributors are distinguished experts within their own areas of research, which will provide the reader with an up-to-date account of the exciting and new developments in determining the structure of a wide range of proteins with the aid of the electron microscope.

Heterogeneity of Mononuclear Phagocytes

Proceedings of an International Workshop held in Vienna, July 15-19, 1980.

edited by Othmar Förster and Maurice Landy

July/August 1981, xxxviii + 538 pp., £16.40 (UK only)/\$39.50, 0.12.262360.6

The concept of the mononuclear phagocyte system was developed in the early 1970s, based on the observation that macrophages and other phagocytic mononuclear cells are derived from a common ancestor in the bone marrow. Various functions have been attributed to these cells. These include effector functions such a phagocytosis and the killing of micro-organisms; cytostatic and cytotoxic effects against tumour cells and parasites; the functions of an accessory cell against tumour cells and parasites; the functions of an accessory cell in the specific immune response; the secretion of biologically active molecules like enzymes, complement components, interferon and prostaglandins. This volume contains much recent research concerned with the correlation of these functions with the various subtypes of these cells: whether variation represents different stages of development or, alternatively, separate lines of differentiation or other functional differences associated with cell

Mechanisms of Sex Differentiation in Animals and Man

edited by C.R. Austin and R.G. Edwards

July/August 1981, xvi + 604pp., £37.00 (UK only)/\$89.00, 0.12.068540.X.

With the collaboration of Dr Ursula Mittwoch, the editors have written an introduction to the subject including one or two major topics that are not covered in the main body of the book. The subsequent papers, each written by recognized authorities in their particular fields, provide a readable, informative and contemporary description of current ideas and research in the study of sex determination. These include genetic and environmental mechanisms of sex determination, sexual endocrinology of fetal and perinatal life, and the nature and origin of sexual anomalies such as hermaphrodites, freemartins, mosaics and chimaeras, in both animals and man.

Eighth Sigrid Jusélius Foundation Symposium, Helsinki, Finland. June 1980.

Expression of Eukaryotic Viral and Cellular Genes

edited by Ralf F. Pettersson, Leevi Kääriäinen, Hans Söderlund and Nils Oker-Blom

July/August, xii + 324 pp., £20.40 (UK only)/\$49.00, 0.12.553120.6

Expression of Eukaryotic Viral and Cellular Genes contains recent and important papers by some of the most prominent molecular biologists in the world. There are articles about the structure of viral genes. (For example adenovirus, influenza virus and alphaviruses); The expression of viral genes (For example SV40 and adenoviruses, vesicular stomatitis virus and encephalo-myocarditis virus (EMC)); mechanism of translation of capped viral mRNAs: the structure and expression of transforming genes of RNA tumour viruses; the control of eukaryotic gene expression, including in vitro transcription of cloned ovalbumin genes, as well as cloning and expression of eukaryotic genes in heterotypic environments.

Handbook of Marine Mammals

Volume 1: The Walrus, Sea Lions, Fur Seals and Sea Otter

Volume 2: Seals

edited by Sam H. Ridgeway and Richard J. Harrison

Volume 1: July/August 1981, xvi + 236pp., £14.60 (UK only)/\$36.50, 0.12.588501.6.

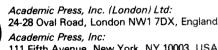
Volume 2: September/October 1981, xvi + 350pp., 0.12.588502.4

Interest in marine mammals has much increased in recent years as our knowledge of them has grown. In particular, the large cetacean brain and their supposed superior intelligence over other animals, in conjunction with their obvious diving and swimming abilities, have made marine mammals fascinating creatures to study. With the aid of distribution maps, identification pointers and spectacular photographs and drawings, as much as is known of each animal about its biology and life history is reviewed, and its identifying characteristics and distribution described. Similarities and differences are stressed and some mention is made of economical and commercial aspects. A full bibliography is included for each chapter.

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PRODUCT REVIEW

Fluorescence microscopes

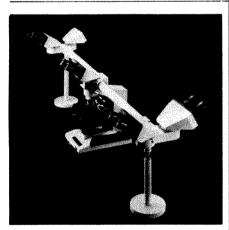
Among the new microscopes on display at Light Microscopy '81 will be the Fluoval 2, a research fluorescence microscope for incident and transmitted light fluorescence microscopy. This microscope from Carl Zeiss Jena Ltd. is suitable for most modern fluorescence microscopic techniques, including multi-staining. Another new microscope on display will be the incident and transmitted light microscope, Epignost 2E, which has long-working distance objectives and an image erecting tube for micro-assembly and micro-quality control work.

Circle No. 100 on Reader Enquiry Card.

Flatfield optics

A SERIES of new microscopes featuring DIN Micro Plan optics has been produced by Swift Instruments, Inc. These microscopes have standard features such as a clutch mechanism that operates automatically to prevent damage to the finely-balanced gear train, and a stage that is raised uniformly on precision ball bearings. The model M973-DM also features research-calibre DIN flatfield Micro Plan optics and a choice of centerable DIN condensers. The Swift DIN Micro Plan objectives are designed to give a flatter field and better resolution.

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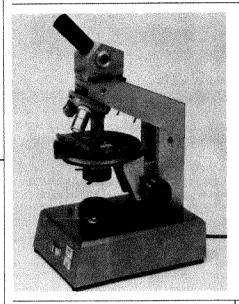
Multi-viewing attachment

AUNIT for multi-viewing, the MDO, is now available from Olympus for use with the new BH2 series microscope and others in the range. The MDO allows up to five observers to simultaneously view the same specimen. The image for each user is of equal brightness, with the same magnification and orientation as the one seen by the instructor. The modular design of the MDO allows for a comfortable working distance between each observer. Both brightfield and phase contrast observation are possible with this unit.

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PRODUCT REVIEW - MICROSCOPY

The Light Microscopy '81 exhibition, organized by the Royal Microscopical Society, is being held at Imperial College, London, UK from 7 to 9 July. The notes on these pages include information provided by the exhibitors. For further details circle the appropriate number on the reader enquiry card bound inside the journal.



Polarizing microscope

A NEW polarizing microscope for both incident and transmitted light application is being introduced by James Swift. In the design of the MP81, particular attention has been paid to eliminating unneccessary controls while retaining full facilities and allowing easy servicing. The MP81 is available as a transmitted, incident or combined transmitted/incident light microscope. Both incident and transmitted light illumination systems use highintensity tungsten-halogen light sources. The MP81 features research standard objective centering, ball-bearing stage, a Bertrand-lens monocular, wide-field eveniece with cross-hair, solid-state brightness control and a range of accessory items.

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Stains

A RANGE of over 200 dry stains and dyes, 53 staining solutions and a comprehensive list of buffers in tablet or concentrate form is available from BDH Chemicals. Other materials for microscopy include embedding and mounting media and immersion oils. A new catalogue, 'Stains and Microscopy Materials', gives comprehensive details of the whole range and lists stains and dyes by colour index number for easy identification. The range of stains and microscopy materials will be on show at the Light Microscopy exhibition.

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SEM for research

A SERIES of scanning electron microscopes has been announced by Bausch & Lomb. The Nanolab 2000 and 2100 instruments offer ease of operation, with the following features: access to the stage from above; beam profiling function to give maximum performance and long filament life, and true TV imaging up to ×100,000 with precision stage. Both Nanolab instruments provide resolution to 60 Å with a standard tungsten emitter and an optional LaB, electron source increases resolution attainable to 40 Å. The Nanolab 2100, designed specifically for research, allows continously variable accelerating voltage, dual magnification, 360° scan rotation, and a 2,500-line recording CRT. The Nanolab 2000 series has applications in metallurgy, quality control, electronics, geology, forensics, biology and medicine.

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Optics accessories

VARIOUS products from Anaspec will be shown at Light Microscopy '81, including optical microspectsometers, fibre optic data multiplexers, optical monochromators, diffraction gratings, and a complete range of TV facsimile transmission systems, image digitizers and slow scan

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Illumination systems

A BULLETIN from Dolan-Jenner Industries provides detailed specifications for their fibre optic illumination systems. As fibre optics transmit mostly visible light from an incandescent source, they act as heatabsorbing filters and provide cool light at the point of application. Several systems are illustrated, including single and dual branch, and annular configurations. Mounting stands, lenses and adapters for various microscopes are also described. A series of rigid miniature probes to supply illumination to difficult areas is also available. Charts are included to show working distance with and without lenses. Circle No. 107 on Reader Enquiry Card.

Image analysis

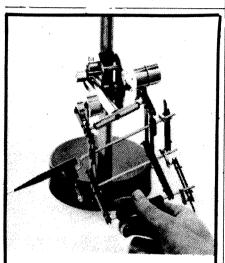
ONDISPLAY at Light Microscopy '81 will be an image analysis system from Graphic Information Systems Ltd. This system can quantify the constituent parts of microscopic images either directly from a light microscope or from a photograph.

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Histology

PRODUCTS relevant to semi-thin resin work are included in a new catalogue from Emscope Laboratories Ltd. This catalogue covers fixative reagents, processing solvents, embedding kits, resins and components, laboratory chemicals, light microscopy stains (dry and in solution), enzymes, mounting media and a new range of embedding, processing, sectioning and staining accessories.

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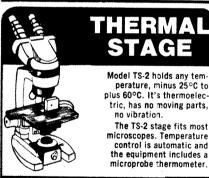


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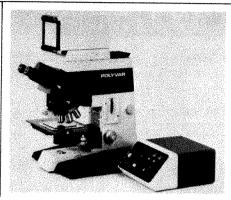


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INTENDED specifically for semiconductor industry, the Polyvar 66 microscope from Reichert-Jung is not only suitable for research applications, but can also be used for monitoring production processes. It is designed to accept a large interchangeable mechanical or motor stage with a specimen traverse range of 6×6 inches, and has an integrated, fullyautomatic camera system which makes hard copies of the image. The modular system of the Polyvar-Met 66 allows various techniques, such as viewing of overall structural/mechanical faults and examination of photochemical coating faults, to be selected and operational within seconds. The module required is plugged into the microscope stand.

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Light microscopes

MICROSCOPES intended for life and materials sciences will be shown by Carl Zeiss at Light Microscopy '81. These will include stereoscopic, inverted, industrial and axiomat modular microscopes, plus accessories for fluorescence, polarizing microscopy, photomicrography, photometry, image analysis and interference microscopy.

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50 Å scanning

A NEW scanning electron microscope, the model JSM-35CF from JEOL, will resolve 5 nm (50 Å) by using a new 'corrected field' objective. The instrument has an improved electron gun design to give a high quality image. Most laboratory sites will achieve 4 nm (40 Å) resolution with the standard tungsten hairpin emitter. An alignment wobbler allows rapid adjustment of the objective aperture, particularly important for high resolution results and for probe work with optional wavelength and energy dispersive detectors. The precision specimen stage is designed to take heavy specimens and will reset to position coordinates x, y, z, rotation and eucentric tilt to very high accuracy. Optional extras include split-screen imaging, scan rotation, derivative processing and alternative recording cameras.

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Slides and stains

A WIDE range of prepared microscope slides is available from Griffin and George Ltd. The sets contain selected slides for study of parasitology, protozoa and algae, for example. Griffin and George also supply a range of zoological, botanical and histological microscope stains.

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Motorized micrometers

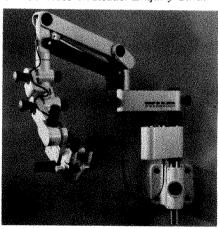
HIGH precision motorized and manual translation stages, rotators and optical mounts are available from Oriel Scientific. These include both stepping motor driven units with step size of 1 μ m, and d.c. motor units — the Motor Mike actuators. These are motorized micrometers which can replace the manual micrometers found in most stages and rotators. Control units are available for all drives.

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Surgical microscopes

Even the finest and lowest-contrast details can be clearly seen using the M600 series of surgical operating microscopes from Wild Heerbrugg. They provide a large, evenly illuminated field of view of striking depth. These instruments can be placed in any position with mm accuracy and have a considerable range of height adjustment. A continuously variable counterbalance allows the instrument to be guided with ease and precision, even with a ciné camera attached. Thus, the instrument can be adapted for uses such as photomicrography, filming or recording on videotape.

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Specimen preparation

A NEW product in the field of specimen preparation, the Polycut, will be shown at the Light Microscopy '81 exhibition. This heavy-duty microtome from Reichert-Jung is suitable for sectioning hard and/or large biological specimens and a wide range of industrial materials.

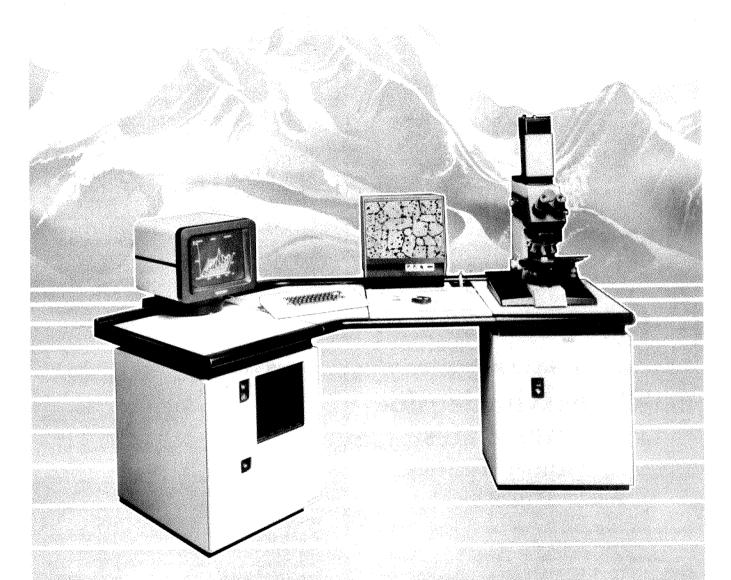
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READERSHIP: Electron Microscopy, Biology, Biochemistry.

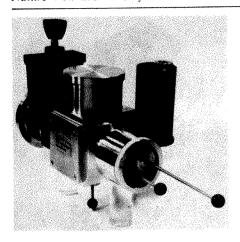
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System microscopes

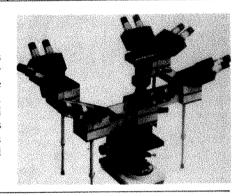
Easy viewing by means of an observation tube inclined 30° to the horizontal is possible using a new design of microscope from Olympus. The focusing knobs allow smooth, light movement and are positioned low to enable the hands to rest naturally during operation. To allow easy processing of a large number of specimens, the stage is in a low position. Ultra-low magnifications ($\times 1$ to $\times 4$) are possible using a new condenser and the illumination system allows observation under Köhlertype illumination from $\times 1$ to $\times 100$. The most advanced model in the series has the unusual feature of compensation for changes in supply voltage, maintaining a constant level of brightness.

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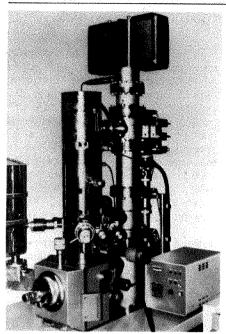
Teaching microscope

THE conference microscope, Balplan, from Bausch & Lomb is available in models with up to seven heads for teaching or conference situations. Designed to save space while allowing comfortable viewing, Balplan delivers crisp, bright images to all seven heads. Balplan Conference models are also available in 2, 3, 4 and 5-head configurations for both brightfield and phase contrast applications.

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LIGHT MICROSCOPY '81 — THE STATE OF THE ART International Conference and Exhibition on Light Microscopy Imperial College, London SW7 7-9 July, 1981 The Exhibition, is organised by the Royal Microscopical Society in association with 'Nature'. The Conference not only marks the inauguration of the Light Microscopy Section of the Royal Microscopy as it is now and of significant developments. The first day's programme is devoted to the instrument itself. The second day is concerned with imaging techniques, and "light" microscopy is interpreted liberally. The third day will deal with two aspects of measurements on images — photometric (in transmission, emission and reflection) and geometric. EXHIBITION In conjunction with the conference, there will be an exhibition of the latest products of the Light Microscope Manufacturers. This exhibition will be open, free of charge, to registrants and non-registrants. Times for non-registrants to visit the exhibition will be open, free of charge, to registrants and non-registrants. Times for non-registrants to visit the exhibition betails available from the Administrator: THE ROYAL MICROSCOPICAL SOCIETY 37/38 St. Clements, Oxford OX4 1AJ Telephone: Oxford (0865) 48768/721081



SEM system

A NEW lanthanum hexaboride (LaB_c) system for the DS-130 scanning electron microscope (SEM) is now available from International Scientific Instruments. It has a filament life of 500 h, although filaments have been known to last for > 1.000 operating hours. The DS-130 guarantees 30 Å resolution and is microprocessorcontrolled. The option of the LaB, system further enhances its capability for low voltage work in spatial modes such as backscattered electron imaging, cathodoluminescence and microanalysis. It is intended particularly for research on the surface of materials or for fragile or nonconducting specimens. Changeover from tungsten to LaB, emitter is at the touch of a button. This new system overcomes previous problems of short filament life and poor stability of LaB₆ emitters. A suitable vacuum level is obtained by either an ion or turbo pump.

Circle No. 120 on Reader Enquiry Card.

Photomicrography system

DEVELOPED for situations where automatic exposure metering is not required, the PFX System from Nikon is a manually-operated photomicrographic instrument with a direct projection optical system. The vibration-dampened, leaf-type shutter operates at speeds of 1/250th to 1 s with positions for bulb and time. A new ×2 ocular viewfinder or photomask eyepiece allows accuracy of focusing. Almost 100% of the light is available to image the film during exposure by the direct projection optical system. This is achieved by eliminating all relay lenses, prisms and reflective surfaces in the optical path. Thus, film images have greater contrast, sharpness and improved colour fidelity. The FX 35 manual camera back is standard for 35 mm applications.

Circle No. 121 on Reader Enquiry Card.

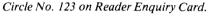
Heating/freezing stage

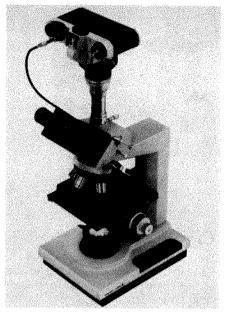
CHANGES in the properties of a wide range of materials can be observed in controlled conditions of heating and cooling using a compact apparatus developed by Stanton Redcroft. Two versions of the transmitted light heating/freezing stage (TLHFS) are available, one operating from the low temperature of -180°C to 550°C; the other from ambient to 600°C. Intended for use with any high quality microscope using long-working distance objective lens, the apparatus permits light to pass through the material under test by resting the sample on a sapphire window; this allows the characteristics of a material to be examined more fully than is possible with hot/cold stages using reflected light. Use of a polarized light source allows additional data to be obtained from materials such as organic polymers, pharmaceuticals and inorganic materials. An applications note on transmitted light hot stage microscopy can be obtained by circling the appropriate number on the reader enquiry card.

Circle No. 122 on Reader Enquiry Card.

Precision measurement

PRECISION measuring equipment from Vickers Instruments will be shown at Light Microscopy '81, including the M41 image shearing microscope with coincidence setting shear. Also on display will be a precise focus indicator which allows focusing of the M41 by bringing two images of a graticule line into coincidence. A range of conventional microscopes is also available, including the M16.





Triocular microscope

FOR USE in routine clinical laboratory tests. Bausch & Lomb have recently introduced the Galen II microscope, which also has applications in veterinary and medical schools. The Galen II features monocular, binocular and triocular heads with optional 35mm camera kit, exclusive dual diopter adjustments, and front-mounted illumination controls. Also available are a wide range of flat-field achromatic, planachromatic or phase contrast objectives, plus phase contrast and polarizing accessories. Optional accessories include a darkfield condenser, a choice of tungsten or tungsten-halogen illuminators, and various eyepieces.

Circle No. 124 on Reader Enquiry Card.

Stereoscan

THE Stereoscan 100 scanning electron microscope from Cambridge Instruments Ltd. has a wide range of uses but is particularly suited to routine applications. It is simple to install and use, compact and designed for ease of operation. The specimen chamber has internal measurements of $170 \times 270 \times 280$ mm, allowing 90° tilt on 127 mm-diameter specimens. This means that a wide range of specimens can

be studied intact. The Stereoscan 100 is capable of 70 Å resolution combined with a magnification of × 25 to × 200,000. It has a wide range of accelerating voltages (2, 3, 5, 10, 15 and 25kV) and low voltage option (0.5, 1, 2.5, 10 and 25kV). The ultra-clean vacuum is turbomolecular-pumped and operator aids include a choice of two automatic recording systems, TV and a wide range of slow-scan speeds. A large 9-inch viewing screen can be tilted to suit different operators.

Circle No. 125 on Reader Enquiry Card.



AT I OUT CEMEL TS

Awards

The Artois-Baillet Latour Health Prize—has been awarded to **Professor Sir Cyril Astley Clarke**. Sir Cyril has also recently been awarded the Linnean Society Medal 1981 for Zoology.

The government of France has named Lawrence Berkeley Laboratory scientist **George Brecher**, to the French National Order of the Legion of Honor for his work in haematological research.

I. I. Rabi of Columbia University is to be awarded the Michael I. Pupin Medal.

The prizewinners of the Feldberg Foundation awards for 1981 are **Prof. Lindemann** University of the Saarland; **Prof. J. Hughes** (Imperial College of Science and Technology London); **Prof. H. Kosterlitz** (Unit for Research on Addictive Drugs, University of Aberdeen).

Thomas Fryer (NASA's Ames Research Center) has been named NASA Inventor of the Year for 1980 for his work in medical electronics.

Winners of Brandeis University's 1981 Rosenstiel Award for "Excellence in Basic Medical Science Research" are Bengt I. Samuelsson (Karolinska Institute in Stockholm) and Elias J. Corey (Harvard University) for related research of substances that influence the body's vital organs; and Frank H. Westheimer (Harvard University) for contribution to research in biochemistry.

In recognition of fundamental work in the field of crop bioenergetics the Rank Prize funds have been awarded to: Dr Hugo Peter Kortschak; Dr Marshall Davidson Hatch, and Dr Charles Roger Slack.

Applications are invited for the Institution of Chemical Engineers Industrial Research Fellowship which carries an Honorarium of £1,000 and necessary travelling expenses. The Fellow will be expected to produce a report identifying recent advances in the subject and highlighting areas where future research would be beneficial. Applications to: Mr S.A.E. Buxton, Senior Assistant Secretary (Technical), The Institution of Chemical Engineers, 165-171 Railway Terrace, Rugby, UK.

The Fondation de Physiopathologie Professeur Lucien Dautrebande will award his next prize of about 1,500,000 belgian fr during 1982 for work on human or animal clinical physiopathology having therapeutic implications. Further information from the office of the Foundation, 35, chaussée de Liège, 5200—Huy, Belgium.

For the John Eggert Prize Foundation. Young scientists are encouraged to submit papers from the field of Imaging Science to Dr W. F. Berg, Hellstr. 7, DH-8127 Forch, Switzerland by 15 October 1981.

The British Vacuum Council seeks to encourage young scientists (under 27 years of age) by inviting entries for its annual C.R. Burch Prize of £100 for the best submitted paper on vacuum studies, surface science, thin films, or any related topic in which vacuum science or engineering plays an important role. Entries by 1 October 1981 to: Dr J.S. Colligon, Department of Electrical Engineering, University of Salford, Salford, UK

The Peter Debye-prize will be awarded by the University of Limburg, to a person or group of persons who are considered to have made a fundamental contribution to research in the field of immunology and oncology. Further information from the University of Limburg att. Drs V. H. Rutgers, secretary of the jury, Tongersestraat 53, PO Box 616, 6200 MD Maastricht, The Netherlands.

Appointments

Professor Max Whitten, Professor of Genetics at the University of Melbourne has been appointed Chief of CSIRO's Division of Entomology.

Dr. Angus D. McEwan has been appointed Chief of the CSIRO's new Division of Oceanography, he is currently a research leader in the Division of Atmospheric Physics.

Lord Todd, (Christ College, Cambridge) and Professor Thomas (University of Cambridge) have been elected Honorary Fellows of the Indian Academy of Sciences.

Meetings

8-10 August, Cryogenic Process Engineering, San Diego (CRYOCOURSE, PO Box 3081, Boulder, Colorado 80307, USA).

10-14 August, **1981 Cryogenic Engineering** Conference, San Diego (Dee Belsher, CEC, NBS, 1-4001, Boulder, Colorado 80303, USA).

17-22 August, **High Pressure in Research** and **Industry**, Uppsala (High Pressure Conference, c/o RESO, Box 1313, S-75143 Uppsala, Sweden).

31 August — 2 September, **British** Association Annual Meeting, York (Press Office, BAAS, 23 Savile Row, London W1, UK).

2-4 September, Uranium Institute Annual Symposium, London (Conference Associates UIS, 34 Stanford Rd, London W8, UK).

7-10 September, **EMAG '81**, Cambridge (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

9 September, **Asthma**, London (Society for Drug Research, c/o Institute of Biology, 41 Queen's Gate, London SW7, UK).

10-11 September, Concepts of Purity, Canterbury (Mr P.R.W. Baker, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, UK).

10-21 September, Leukotrienes and Prostacyclin, Erice (Prof. F. Berti, Istituto di Farmacologia e Farmacognosia, Universita di Milano, Via Vanvitelli, 32, 20129 Milano, Italy).

13-16 September, Surviving the 80s, Southampton (Conference 1981, Southern Science and Technology Forum, Building 25, The University, Southampton, UK).

14-15 September, Integrated Optics, London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

17-18 September, AGU Pacific Northwest Regional Meeting, Ellensburg (B. Bentley, PNAGU, Central Washington University, PO Box 1000, Dept of Geology, Ellensburg, Washington 98926, USA).

17-18 September, AGU 1981 Midwest Meeting, Minneapolis (Agu Midwest Meeting, 2000 Florida Ave, NW, Washington DC 20009, USA).

19 September — 2 October, Postgraduate Course on Pain Therapy, Vicenza (Agenzia Spiller, C.so Felice 151, 36100 Vicenzia, Italy).

20-25 September, Computer Ergonomics, Loughborough (HUSAT, Dept of Human Sciences, University of Technology, Loughborough, Leicestershire, UK).

21-23 September, **Turbulence**, Missouri-Rolla (Prof. G.K. Patterson, Chemical Engineering Dept, University of Missouri-Rolla, Rolla, Missouri 65401, USA).

21-24 September, Gas Fluidisation, Bradford (Mr D.B. Firth, I Chem E, 12 Gayfere St, London SW1, UK).

21-25 September, Clean Air and Pure Water — Tomorrow's Luxuries?, Jönköping (Elmia AB, Box 6066, S-550 06 Jönköping, Sweden).

22-25 September, Information '81: Forecasts and Realities, Oxford (ASLIB, 3 Belgrave Square, London SW1, UK).

22-23 September, Applications of Petroleum Geochemistry to Basin Studies, London (Dr J. Brooks, Exploratino Dept, The British National Oil Corporation, 150 St Vincent St, Glasgow, UK).

29 September — 2 October, Indoor Pollution, Health and Energy Conservation, Amherst (Mrs J. Curhan, Energy and Environmental Policy Center, John F. Kennedy School of Government, Harvard University, Cambridge, Massachusettes 02138, USA).

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QUEEN MARY COLLEGE University of London LECTURER IN ZOOLOGY

Applications are invited for this post in the Department of Zoology and Comparative Physiology. Preference be given to applicants specialising in vertebrate zoology although specialists in other fields will be considered.

Salary on scale £6,070 — £12,860 pa plus £967 London Allowance.

Forms and further details available from The Secretary, (N) Queen Mary College, Mile End Road, London E1 4NS, to whom applications should be (8948)A made by 31 July 1981.

IMMUNOLOGIST MOLECULAR BIOLOGIST

Postdoctoral positions available for 1) Molecular genetic studies of expression of immunoglobulin genes, and 2) for characterization of lumphocyte subpopulations as identified with monoclonal antibodies and separated by FACS.

Send curriculum vitae and names of three references to: K L Knight, PhD, University of Illinois Medical Center, SBMS Microbiology/Immunology, 835 South Wolcott, Chicago, IL 60612.

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UNIVERSITY OF **CAMBRIDGE CELL BIOLOGISTS**

Interested in postdoctoral work on cell surface properties and biochemistry, in situ nucleic acid hybridisation or electron microscopy required for projects on mammalian embryos.

Applications, with names of three referees, by 31st July, to: Dr M H Johnson, Department of Anatomy, Downing Street, Cambridge

(8982)A

INSTITUTE of Cancer Research: A Research Assistant is required to join the Leukaemia Unit at Sutton, Surrey, to investigate the immune status of patients receiving bone-marrow transplants. Previous experience in cell culture and mixed lymphocyte cultures essential. Salary in scale £4,677 — £6,222 pa plus London Allowance of £527 pa. Applications with a full CV (in duplicate) and the names of two referees should be sent to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/6.

(8990)A

Biochemists/ **Biotechnologists**



the world's foremost organisations dedicated to all aspects of new product and process development, with laboratories in the UK, Australia, Belgium and the USA and covering almost every scientific and engineering field.

Continuing expansion has created new vacancies in the field of biochemistry. Candidates will have a good first degree in biochemistry, preferably with a Ph.D. Expertise in the synthesis of enzymes, nucleotides, peptides or other related biochemicals will be an advantage.

Applicants should also be creative, adaptable and commercially aware. Experience in a commercial biochemical environment would be a decided asset.

Please write with career details to: Dr. R. C. Whelan, Operations Director, Patscentre International, Melbourn, Royston, Herts, SG8 6DX. Tel: Royston (0763) 61222.

Patscentre International Cambridge Division



(8979)A

GRIFFITH UNIVERSITY Brisbane, Australia

LECTURER -SCHOOL OF SCIENCE (Life Sciences - Plant Molecular/Cellular Biology)

A Life Science option is offered by the School of Science and includes an integrated coverage of Genetics, Microbiology and Physiology.

Applicants should possess appropriate experience in plant biological sciences, relating to molecular and/or cellular aspects. Depending on the expertise of the appointee, the main teaching duties will be in undergraduate courses in plant physiology (second year course), genetics, and microbiology (second and third year courses). It is anticipated that there will be opportunities to teach honours level courses. Pursuit of research is a requirement of the appointment and collaboration in group and interdisciplinary research projects is encouraged.

It is anticipated that the appointee will commence duties as early as possible in 1982. Apointment will be made at the lower end of the Lecturer scale, ie \$A19,821 - \$26,037.

Further details may be obtained from the School Administrator, School of Science, Griffith University, Nathan, Queensland, Australia, 4111, to whom applications (including curriculum vitae, names and addresses of two referees) should be sent by 31 August 1981.

Conditions of appointment and application procedure also available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H (8964)A

HOWARD READ

CORRESPONDENCE

K. PAULUS

Continued from p. 8

This is not the ideal test of "survival of the fittest"; it does not compare fitness and survival within one population. Instead, it compares populations, of which the later is descended from survivors of the earlier (at both the individual and the species level). The populations are separated by some millions of generations; this interval is long enough for comparison of the populations with an objective standard to reveal an increase in fitness. Could this cumulative increase in fitness be produced by anything except "survival of the fittest"?

D.G. STEPHENSON

Department of Geology. University of Keele, UK

- Rudwick, M.J.S. Br. J. Phil. Sci. 15, 27-40 (1964). Paul, C.R.C. in Patterns of Evolution (ed. Hallam, A.),
- 125-158 (Elsevier, Amsterdam, 1977).

University staffing

Sir - On behalf of my members I should like to make comment on the article, "Change wanted" in Nature of 11 June (p.442).

I do not wish to proffer an opinion on the second interim report from the Swinnerton-Dyer committee but I do want to protest most strongly about your suggestion that a cutback in non-academic staff would be quicker and should be considered first.

First, the financial savings accrued from such cuts would be a drop in the ocean in comparison with the salaries saved from the academic staff. We as technicians are aware of departments with an academic staff establishment which in no way reflects the actual number of students taught. It is the topheavy nature of such departments that needs careful consideration.

Second, the last sentence in the article asks "But is not the university an institution whose chief purpose is academic?". In order to maintain that purpose the academics need the back-up services of trained technicians to provide an efficient lab class and assist with research projects. An academic with a heavy teaching load trying to do research at the same time would either have to cut back the amount of teaching or give up a substantial part of his research projects in order to replace the technical services now provided.

As regards cleaners and porters, they already work in rather grubby conditions and I am sure that academic standards would not be improved if the academics had to clean their own rooms or provide an adequate supply of toilet paper in the lavatories.

In conclusion, we are aware that because of government cuts, savings must be found somewhere. But please do not point the finger at one group of staff. Far rather let each college put its own house in order and safeguard the jobs and careers of all its employees by looking at other areas of saving

The universities could also make a positive and voluble stand against the government cuts in an effort to maintain the opportunity of higher education for as many people as possible.

A.L. PRICE THOMAS

Branch Chairman, Association of Scientific, Technical and Managerial Staffs. Westfield College Branch, London NW3, UK Psychiatry on trial

Sir - Your ill-disguised dismissal of R.D. Laing (Nature 4 June, p.367) does little to clarify the many interconnected issues raised by the Sutcliffe case. Permit me to draw the following lessons from his trial:

(1) Escape into the protection of some illness, however well-defined or spurious, is no longer possible. Each of us needs to accept responsibility for our actions.

(2) The utter "normality" displayed by Sutcliffe during his trial now puts the onus on psychiatry to defend its labelling of unacceptable behaviour as "illness": may I remind you that there are psychiatrists amongst those who collude in the incarceration of Soviet dissidents.

(3) That anyone, particularly psychiatrists, should be surprised when the "common and pervasive" sexual abuse of, and violence towards women takes such an extreme form, is but a sad reflection on our society. Where I beg to differ from you is in not ascribing this to "psychiatric illness" but rather to the inevitable consequence of a pervasive morality. The sooner we stop hiding behind the comfort of psychiatric illness the better we shall see our own responsibility as members of the society that has nurtured Sutcliffe.

Clifton, Bristol, UK

Search for truth

SIR - Although not directly involved in the investigation of the origin of species, astronomers are, nevertheless, involved at the "sharp end" of research into origins as they seek to explore the Universe and as such I would like to comment on the leading article "How true is the theory of evolution? (Nature 12 March, p.75).

Darwin's theory of evolution, like the theory of special creation, is just that, a theory, which is incapable of being proved as fact by scientists, and also incapable of being falsified. Both theories therefore, if given the label of scientific theories, fulfil Popper's second criterion. Second, in order to assemble and evaluate evidence for particular theories, scientists, hopefully, try to be as objective as they possibly can; if not, then their credibility may well be called into question. However, most people would find it impossible to be totally objective and impartial in weighing up evidence. Each of us has prejudices which we are incapable of putting out of our minds as we seek to assess observed facts, so their interpretation can never be fully objective.

This problem is particularly acute when the origin of the species is being investigated. The whole question of the existence of God and as a consequence our accountability to him as God, past conflicts between church leaders and scientists, dissatisfaction with the implications of evolution on the one hand and with the role of the Bible and the church on the other have all made the investigation of the origin of the species a good deal more subjective than other areas of scientific investigation.

Creationists will do themselves a great disservice by choosing to bury their heads in the sand as scientific investigation proceeds in the future but equally so will evolutionists if they draw up behind a barrier of indignation at the thought of Darwin's theory never

achieving the status of fact.

Objectivity of investigation and interpretation is not only desirable but very necessary, for in the final analysis the truth will stand all investigations and still be the truth long after we are all laid to rest.

Department of Astronomy,

University of Manchester, UK

American Creation

SIR - The correspondence arising from the British Museum's cladistic activities has had one common aim — to avert the imminent threat of an upturn in creationism (Jukes, Nature 21 May p.186; editorial 28 May p.271). We are tempted to ask why evolutionism feels threatened by creationism, when the real controversy has not yet been stated openly. This does not lie in E. O. Wiley's question (Nature 30 April, p.730) "Does the phenomenon of evolution occur?", since the majority of creationists would not deny that evolution occurs, but in the question "Did the evolutionary mechanism provide the actual pathway from sterile Earth to living world?"

Any view of origins that does not invoke a supernatural Creator must conclude either that it did or that life arrived from space in some form, as proposed by Crick and Orgel, Wickramasinghe and Hoyle, and others. Either view must be accepted by faith, either in the propositions themselves or in the ability of science to provide proof in the future. The atheist, whose metaphysical presuppositions do not allow him to countenance any form of divine activity, is more close-minded than many theists, who would happily accept either theory or the alternative, special creation.

To take a narrower view, any survey of Bible-believing Christians would reveal a wide spectrum of conclusions. These would range from those who believe that God has worked through essentially neo-Darwinian evolution, to those "creationists" who find current evidence for Darwinian evolution unconvincing and conclude that special creation is consisten; not only with the scientific evidence but also with the whole of Scripture, the reliability of which can be verified experientially by the Christian.

It is often stated that many creationists take no account of the scientific arguments for evolution. Since the converse is also true, we will point to some of the issues we consider relevant. It is reasonable to point out that no plausible theoretical model exists which provides a mechanism for the spontaneous generation of nuclesc acids as informational macromolecules specifying polypeptides which themselves mediate the replication and expression of that information. The experiments demonstrating the formation of a variety of organic molecules from presumptive prebiotic soups fall far short of providing a pathway for chemical evolution. Again, it is self-evident that the fossil record leaves much to be desired and few biologists recognise the dependence of the geological column on radiometric dating methods based on questionable assumptions about initial conditions. The whole history of evolutionary thought is littered with the debris of dubious assumptions and misinterpretations, especially in the area of fossil "hominids". To come up to date, protein and DNA sequence data, generally viewed as consistent with an

evolutionary explanation of diversity, are invariably interpreted using methods which presuppose, but do not demonstrate, evolutionary relationships, and which use criteria that are essentially functional and teleological. Finally, there is a collection of isolated fragmentary pieces of evidence which are usually dismissed as anecdotal because they are irreconcilable with the evolutionary model. From the above and other considerations it is possible to argue a strong case for special creation.

If, however, we are to be banished to the lunatic fringe, we find ourselves in the company of men who are regarded as the founders of modern science - Boyle, Newton et al. These men formulated the laws of physics and chemistry, not by invoking the supernatural but by assuming it, in the conviction that all things were made by an intelligent and all-powerful Creator who had imposed order and meaning on his creation. So it seemed to them, and is it not reasonable to ask whether they would have begun to ask the right questions if it had seemed otherwise? It has, for example, been suggested by Needham that the reason for the failure of the Chinese to develop the scientific method of the West was that they did not see order in nature as ordained by a personal, rational being.

Science can be conducted only within a context or "world view" which inescapably determines its aims and directions. We, as scientists, must be aware that as long as our society looks to the great god of science for direction and meaning, our own world view will be shaped by our own science. If we assert untruth, half-truth or hypothesis as fact, society and we ourselves will be misled and we will be responsible for the cultural, political and ethical consequences. Truth was never determined by majority opinion.

We are dismayed by the methods and attitudes of American Creationism. The legislature cannot be used to establish truth, either scientific or spiritual. Furthermore we believe that the Bible, though accurate, was not written as a scientific textbook but written primarily to lead men to a personal knowledge of God through His Son. In that knowledge we are able to say: "By faith we understand that the world was created by the word of God" (Hebrews 11.3).

Chris Darnbrough John Goddard William S. Stevely

University of Glasgow, UK

Room for faith

Sir - Contrary to what your editorial on creationism and evolution might suggest (Nature 28 May, p.271), few "card-carrying believers" within the scientific community would hold to the "God of gaps" theory you describe. Few see any major conflict between science and religion. Most see them as providing different languages to describe the same phenomenon — the view that God created the world and that evolution was the mechanism by which he did it is not contradictory. "God created the world" cannot be held up as a scientific theory. It is scientifically and theologically unprovable. It has to remain a matter of faith. However, some of the corollaries of creationism: the insufficiency of mutation and natural selection to account for intra-specific diversity, catastrophic geology, a shrinking rather than an expanding active gene pool; are worthy of

some consideration within a scientific context. Indeed, it is surprising how rapidly some tentative theories in support of evolution have become scientific facts. The absurdity of recapitulation and the half-truth of horse evolution are still incorporated into school textbooks as irrefutable fact. Evolutionists often present their "beliefs" as dogmatic "fact" in a similar vein to creationists.

What is required is not that creation should gain equal time within the school curriculum of Arkansas or the exhibitions of the British Museum (Natural History); but that school pupils and general public alike are presented with the solid facts as far as they are known with both their limitations and their certainties (but not with conjecture or extrapolation). And that they be encouraged to draw their own conclusions; to observe the data, to construct their hypothesis and to test it against the new data as they emerge. In other words, they should be encouraged to learn and apply the scientific method. This should be the basis of all biology courses and museum exhibitions.

However, the controversy will not be stayed by the application of scientific method; because what one believes about the origin of life and of man has a grave effect on how one treats fellow man and how society functions.

NEIL K. MCBRIDE

Microbiology Department, Queen Elizabeth College, London W8, UK

Missing links

Sir — In your leading article "How true is the theory of evolution?" (*Nature* 12 March, p.75), you discussed the apparent inability of the Darwinian theory of evolution controlled by natural selection to explain the episodic nature of the fossil record.

It must be accepted that the fossil record of most evolutionary lines does indeed show changes taking place in discontinuous jumps rather than in a continuous manner. It is important to realize that even a continuous development resulting from the accumulation of many small changes may only rarely show itself as this in the fossils unearthed.

We are unlikely to find the fossil remains of more than one in a hundred million or so individuals. Most of the fossils which we see, therefore, are necessarily those of organisms which were common over large areas and which remained common and unchanged for long periods. For this to be possible the species concerned must have been well adapted to a particular niche in a stable environment. If the environment changes the niche must change, and may then be invaded successfully by another species, or even vanish altogether.

Once a species has filled a stable niche it can rarely gain by changing in anything but minor ways. In an environment which has been stable for a long time, all major neighbouring niches are likely to be filled by other successful organisms, so that individuals that differ from the species optimum are likely to be less successful than the norm. Natural selection will then act to slow down significant evolutionary change.

Rapid evolution can occur only where there is extremely intense intraspecific competition, as in the case of our own ancestors, or where a species is not well adapted to its habitat — for example in regions colder or wetter than the stable environment to which it was adapted, and/or with different fauna or flora. Population pressure in a successful species will constantly be supplying a trickle of individuals

to such non-optimal peripheral regions around the main habitat. In such a region a small mutation, useless or even mildly harmful, could be advantageous to survival outside the normal range. Again, this would be unlikely to help if the new area itself had a stable climax ecology where all important niches were already filled. It is in a small area with many empty niches, and perhaps cut off by some natural barrier introduced by climatic, volcanic or other phenomena, that a now nonoptimally adapted species may survive and find a whole series of minor mutations advantageous, eventually leading to major changes in structure and behaviour. During the whole of this development numbers will be limited, allowing a significant degree of genetic drift, and the intermediate forms may not persist for long, so that the chances of our finding fossils of such intermediates will be slight.

Not until the species has changed so much that it can successfully invade a new stable area when the barriers break down, as the rabbit did in Australia, can it become common and persistent over an extended period — and give us a chance to find its remains.

I am not trying to disprove the occurrence at some times in the past of large discontinuous jumps in evolution, or to prove that all evolution has occurred as a result of natural selection acting on a large number of random mutations, mostly small. All I am saying is that whichever of these is true the fossil record must be expected to be discontinuous.

"Missing links" may be truly missing, or simply so small in number and period of existence that they have not been found.

J.H. FREMLIN

Department of Physics, University of Birmingham, UK

Scientists for sale?

SIR — Increasingly, the members of the biomedical research community are being forced to give their activities a greater immediacy of application. The commercial pattern into which the work on genetic engineering and monoclonal antibodies is falling reflects this pressure. There could arise conflicts in the academic mind some of which merit public discussion.

In the past there have been no constraints, other than moral, on the free movement of individuals between research groups. However, now, many of the commercially useful activities, whether in industrial or nonindustrial laboratories, involve team work. These groups, like football teams, require continuity for success and their members, it can be argued, should be subject to the same restraints on movement from laboratory to laboratory as are footballers between teams. Specifically, contracts of employment should be considered as not only binding on the employer but also on the employee. It could even be maintained that changes within the period of a contract could be arranged by mutual agreement but only on payment of an appropriate transfer fee. Breaking of the contract by either employer or employee should be subject to the process of the law in the usual way.

As things stand at the moment, poaching of staff and of ideas on which considerable development money has been spent can occur without let or hindrance. A.J.S. Davies Institute of Cancer Research, Royal Cancer Hospital, London, UK

The Queen's University of Belfast CHAIR OF CHEMICAL ENGINEERING

Applications are invited for the Chair of Chemical Engineering. The salary attached to the appointment is £17,676 per annum with contributory pension rights under FSSU or USS.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: 18 September 1981. (Please quote Ref. 81/N) (8958)A

GUY'S HOSPITAL MEDICAL SCHOOL Renal Unit, Clinical Science Laboratories GRADUATE RESEARCH ASSISTANT

with experience in immunology and/or platelet work, required for one year for work on MRC supported research project.

Salary on scale £5,285 — £7,700, plus £967 London Allowance.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge SEI 9RT, quoting Ref. RM. Enquiries to Professor J S Cameron (Tel: 01-407 7600 ext. 2445). (8943)A

Public Health Laboratory Service Board Central Public Health Laboratory Food Hygiene Laboratory BIOCHEMIST

to join a small research group engaged in studies on the purification and characterization of various toxins associated with foodborne disease and to initiate studies on rapid methods for the detection of bacterial pathogens in food.

Applicants, male or female, should have an appropriate degree and post-graduate experience of a range of biochemical and immunological techniques; a higher degree would be an advantage. The successful candidate will be expected to show versatility and initiative to meet the future requirements of the laboratory.

The appointment will be at the Senior Microbiologist Grade. Current salary range £8,201 — £10,448 inclusive of London Weighting. NHS Terms and Conditions of Service will apply.

Applications, including full curriculum vitae and the names and addresses of two referees, should be sent to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT (Tel. 01-205 7041). (8961)A

Development Scientists-Recombinant DNA

Up to £10,000°

BUCKS

Amersham International Limited, formerly The Radiochemical Centre Limited, holds a major position in the development and supply of radioisotopes for research and medical applications. Many of our products are already used in molecular biology research and we have now set up a new group with the objective of investigating potential applications of recombinant DNA and developing new products.

We now have several attractive career opportunities for self-motivating and innovative scientists who have a strong background in one of the biological sciences and a good understanding and several years' practical experience of recombinant DNA techniques to help us meet these objectives.

One of the posts will particularly suit a scientist who has a thorough understanding of the application of radioisotopes in molecular biology and a background in biological chemistry plus research experience which has included handling and purifying nuclear acids and proteins.

Candidates must have an appropriate PhD qualification and a successful record of R & D work in an academic or industrial environment. Career prospects for the right candidates are very good in a British Company which has a world wide reputation and an excellent growth rate in a highly complex and competitive market.

Starting salaries will be up to £10,000* with the prospect of increases to £12,500+ and they will be reviewed in November. First class benefits will include full relocation assistance, where appropriate.

Please write with concise career details to P. L. Jones, Personnel Manager at the address below.

Amersham International Limited

White Lion Road Amersham Buckinghamshire HP7 9LL

(9000)A

Amersham

MIDDLESEX POLYTECHNIC RESEARCH FELLOW ROAD ACCIDENT STUDIES £6,960 — £10,929 pa inc

£6,960 — £10,929 pa inc (Burnham Researcher 'B' Scale) A temporary two-year post to carry out a research project into the effects of road improvement schemes on accidents, financed by the Science Research Council.

Candidates should preferably have a higher degree in a relevant subject—eg Civil Engineering, Mathematics, Statistics—with some experience of research in industry or in an institution of higher education. This post is not open to candidates who have previously held SRC funded Research Assistantships, but is open to those who have held SRC Studentships.

Write quoting ref A176C for post details, conditions of service and an application form, posting first-class to: Personnel Office, Middlesex Polytechnic, 114 Chase Side, London N14 5PN. For further information about the project, write to or telephone Dr C C Wright, Middlesex Polytechnic, Queensway, Enfield, Middlesex EN3 4SF. Telephone O1-804 8131 ext. 326. Closing date 13 July. (8946)A

UNIVERSITY OF BRISTOL Long Ashton Research Station HEAD OF PLANT SCIENCE DIVISION

An experienced plant physiologist or other plant scientist is required to lead and to manage the Plant Science Division, comprising work on environmental physiology, plant nutrition, plant biochemistry and growth regulation. The Division will need to collaborate extensively with others devoted to food and beverages, pomology and crop protection.

Appointment in the Deputy Chief Scientific Officer grade (at present £16,500 to £19,500 pa). Non-contributory superannuation scheme.

Essential qualifications are a Higher Degree and a well established reputation in relevant scientific disciplines. Furthermore, the post requires an understanding of the administration of research and the experience and personality to lead a team of approximately 38 expert staff.

This is a second advertisement; previous candidates will be considered without further application.

Further particulars from

The Secretary, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF. Written applications, including a full cv, all relevant details and the names of three referees must be received by 24th July, 1981.

SYNCHROTRON RADIATION RESEARCH MRC/SERC JOINT APPOINTMENT

The Medical Research Council and the Science and Engineering Research Council have agreed jointly to appoint a Post Doctoral Research Associate to work at the SERC Synchrotron Radiation Source (SRS) at the Daresbury Laboratory. This appointment provides a unique opportunity for the right man or woman to make an original contribution to an expanding field of research. The successful applicant would be expected to take responsibility for supporting the exploitation of the SRS in the field of biological and medical applications of small angle x-ray scattering and to participate in the research programme. Projects already approved include work on proteins, muscle and viruses. and the appointee would be expected to act as a focal point for these activities and to advise and assist other SR users in this area. An experimental station is being commissioned and will be available for some of this work but the appointee would be expected to take part in the design, construction and operation of further equipment. For this reason, the appointee should have a good understanding not only of the application of SR to biological problems but also of electronic methods of x-ray detection and data collection. The ability to work as a member of a team and to have an enthusiasm for the development of new techniques are essential qualities.

An appointment will be made at a salary between £8006 and £9015 per annum fixed according to age, qualifications and experience. The post will be available for a fixed term of three years and will be superannuable.

Closing date: 31st August 1981.

For further information please write to or telephone Warrington (0925) 65000 Dr. P. J. Duke (Ext. 460). Applications should be sent together with curriculum vitae and the names and addresses of two referees, quoting reference number DL/765/N to:

The Personnel Officer

(8977)A

DARESBURY LABORATORY

Science & Engineering Research Council Daresbury, Warrington WA4 4AD.

University of London
British Postgraduate Medical Federation
Cardiothoracic Institute
Cardiac Muscle Research Unit

Research Assistant Electron Microscopy/Cell Biology

Applications are invited for the above post to work with Dr N. J. Severs on membrane structure in cardiovascular muscle and endothelial cells. Freeze-fracture techniques are to be the principal tools in this project, and preference will be given to candidates with previous experience in electron microscopy. A keen interest in membrane structure and function is essential. The post is funded by the British Heart Foundation, and will be available for three years from October 1st 1981. Candidates should have (or expect to obtain) a first or upper second class honours degree. The successful applicant will have the opportunity to register for a higher degree.

The salary will be on the base point of the pre-doctoral research assistant scale, currently £5,285 + £967 London Allowance. The post is superannuable in the Universities' Superannuation Scheme.

Application forms from the Secretary's Office, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX, to be returned not later than 24th July, 1981. (8952)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF HUMAN GENETICS

Applications are invited for the post of

POST DOCTORAL RESEARCH FELLOW

in the above Department to study immunological approaches to therapy in Cystic Fibrosis with particular regard to Phagocytic cell function. Previous experience of cell separation techniques and with human phagocytic cells would be an advantage. The post is available for three years from a date to be arranged.

The salary will be on the IA scale for Research Staff which starts at £6,070, according to age, qualifications and experience.

Further details from Dr J A Raeburn, University Department of Human Genetics, Western General Hospital EH4 2XU (Tel 031-332 2471 ext 177), to whom applications should be sent not later than 3rd August, 1981.

Please quote Reference no. 5030. (8973)A

UNIVERSITY OF CALIFORNIA AT LOS ANGELES FACULTY POSITION

The University of California at Los Angeles has an opening for a tenure (Associate or Full Professor) faculty position in the Department of Atmospheric Sciences. The applicant's research specialization should be in the field of atmospheric radiation, preferably with emphasis on applications to the dynamics of planetary atmospheres. The candidate should have an exceptional record of accomplished research and will be expected to develop a high-quality, independent research program at UCLA. Demonstrated experience in graduate and undergraduate teaching is desirable.

Applications will be accepted until March 1, 1982. The appointment can be effective as early as July 1, 1982. Interested inviduals should submit a letter of intent to: Professor Hans R. Pruppacher, Chairman, Dept of Atmospheric Sciences, University of California, Los Angeles, CA 90024. Tel: (213) 825-1954.

The University of California is an equal opportunity/affirmative action employer. (NW719)A

THE ROYAL SOCIETY OF CHEMISTRY

EDITORIAL STAFF

A Chemistry Graduate is required for editorial work on the Society's publications. A good degree or equivalent in chemistry is essential, and a proven ability to write in clear concise English will be an asset. Further particulars and application forms may be obtained from: Mrs. S. Sheldon, Assistant Personnel Officer, The Royal Society of Chemistry, 30 Russell Square, London WC1B 5DT. Telephone: 01-580 3482. Closing date — 17 July 1981.

(8984)A

WESTMEAD CENTRE SENIOR RESEARCH OFFICER —

PULMONARY PHYSIOLOGY (Advert No: H34)

Salary: A\$19,132 - A\$21,703

Qualification: PhD with experience in pulmonary physiology.

Duties: Full time research in lung and chest wall mechanics, gas mixing and ventilation distribution, working with Dr L A Engel (formerly at McGill University). The position is available immediately for a 3 year period. Westmead Centre is a new 925 bed teaching hospital of the University of Sydney with excellent facilities.

Hours of duty: 0830 - 1700.

Enquiries and official application forms: Dr L A Engel, Respiratory Medicine Unit, Westmead Centre. Telephone: (02) 6336797.

Applications with full curriculum vitae and names and addresses of 2 referees to be addressed to: The General Superintendent, The Westmead Centre, PO Box 264, Westmead, NSW 2145, Australia.

(W371)A

THE UNIVERSITY OF LEEDS

DEPARTMENT OF FORENSIC MEDICINE

Applications are invited for a post of postdoctoral

RESEARCH FELLOW

in the Department of Forensic Medicine for work on Histological localisation of drugs involving the use of fluorescence techniques, microscopical methods and possible immunological techniques. The appointment will be made for a fixed period of up to three years from 1981.

Applications should have a PhD in Pharmacology or Biochemistry and/ or relevant experience.

Salary on the IA Range for Re-search and Analogous Staff (\$6,070 £10,575) according to age, qualifications and experience.

Informal enquiries be made to Professor D J Gee, Department of Forensic Medicine (Telephone 0532-33144 Ext 5684).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 90/10/D. Closing date for applica-(8963)A tions 23 July 1981.

POSTDOCTORAL Research Associates to study the role of calmodulin in cell functions. Specific projects include (1) adenylate cyclase and phosphodiesterase, (2) prostaglandin metabolism and enzymology, (3) calmodulin-binding proteins, and (4) immunocytochemistry. Successful applicants may begin immediately. Send curriculum vitae and three letters of reference to Dr Wai Yiu Cheung, Department of Biochem-istry, St Jude Children's Research istry, St Jude Child.
Hospital, Memphis, Tennesse (NW724)A Tennessee

UNIVERSITY OF KEELE DEPARTMENT OF GEOLOGY DEPARTMENTAL **DEMONSTRATORSHIPS**

Applications invited for post of Demonstrator, available from 1 October 1981. Appointment for one year in first instance, conditionally renewable up to three years. Successful candidates expected to assist with teaching of both elementary and advanced laboratory courses in various aspects of Geology (including Geochemistry and Geo-physics). Some postgraduate expe-rience in research or in industry essential and knowledge of igneous and/or structural geology an advantage.

Initial salary £5,285 on the Other Relate 1B scale with membership of the University superannuation

Further details and application forms from Professor G Kelling, Department of Geology, The University, Keele, Staffs. ST5 5BG (Tel. No. 0782-621111). Application forms (in duplicate) should be returned to the Registrar, University of Keele, not later than 10 July 1981. (8942)A

Silicon Processing Development, Implementation and Control

IBM United Kingdom Laboratories Limited at Hursley, near Winchester, comprises the company's largest development facility outside the USA. To join the section providing pre-production fabrication support to the IC design groups, we seek an engineer with at least 3 years' experience in silicon IC processing and device physics.

This team, which reports directly to the Director of Advanced Technology, is responsible for developing and operating new process facilities in the Hursley Laboratory, and for obtaining all relevant information and assistance from our manufacturing plants and development laboratories in Europe and the USA.

The successful applicant will be required to visit these locations, and also direct the work of up to three engineers and technicians. A graduate of Electronics or Physics, the ideal man or woman will have practical experience of computer-controlled test systems and of test data reduction.

As a Company making a substantial contribution to the country's economy - over £600m in the last 10 years - IBM employs 15,000 men and women at over 40 locations throughout the UK Some 4,000 work at our two manufacturing plants and another 1,500 here at the Hursley

In addition to an attractive salary, a substantial employee benefits package is offered which includes a non-contributory pension scheme, free life assurance and BUPA membership.

Please telephone Mike Shute, Personnel Officer on Winchester (0962) 4433, Extension 6413 for an application form or write to him at: IBM United Kingdom Laboratories Limited, Hursley Park, Nr. Winchester, Hampshire SO21 2JN. Please quote reference: N/92282.



UNIVERSITY OF QUEENSLAND Australia

DEPARTMENT OF MATHEMATICS

LECTURER IN **NUMERICAL ANALYSIS**

Applicants should possess a PhD degree and a strong theoretical background in mathematics together with experience in research in a major area of numerical analysis. 14 August 1981.

LECTURER IN **BIOCHEMISTRY**

Applicants should have a PhD in Biochemistry and have a strong commitment to both teaching and research in Biochemistry. 31 July

TEMPORARY LECTURER IN ZOOLOGY

(1.1.82 - 31.12.82)

Primary responsibility Vertebrate Anatomy and Introductory Parasitology for first year Medical students; fisheries biology for level 3 Science students and some graduate supervision. Possible extension of appointment until 31.8.83. 31 August

Salary: \$A19,821 - \$A26,037 pa.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (8992)A

UNIVERSITY OF PENNSYLVANIA

PHARMACOLOGY DEPARTMENT

The Department of Pharmacology has openings for up to six

FACULTY MEMBERS

Emphasis will be on recruitment at the rank of Assistant Professor, but a limited number of tenured positions are also available. Applicants must have an advanced degree and at least two years of post doctoral research experience. Preference will be given to individuals with background in molecular pharmacology, neuro-pharmacology, or analytical phar-macology, but applications from outstanding candidates in any area relevant to pharmacology will be welcome. One position may relate to problems in ophthalmology. These positions will be available over a period of 18 months beginning in October of 1981. Each appoint will include the use of newly renovated, air conditioned laboratory space and reasonable start-up funds.

Applications from female and minority persons are especially encouraged.

Inquiries, which should include curriculum vitae, list of publications, reprints or preprints of selected papers and at least three letters of reference, should be addressed to: Faculty Search Committee, Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, Pa. 19104. An equal opportunity/affirmative action employer. (NW715)A

UNIVERSITY OF HONG KONG LECTURESHIP IN **PHYSIOLOGY**

Applications are invited for a Lectureship in Physiology. The appointee will be required to participate in the teaching of physiology to dental and medical students. Applicants with higher degrees in Physiology or medical degrees will be given preference.

Applicants should have some research and teaching experience and a special interest in Cardiovascular Physiology.

Annual salary (superannuable) is: HK\$95,700 x 6,480 - 108,660 BAR $115,140 \times 6,420 - 160,080$ (£1 = HK\$10.80 approx.). Starting salary will depend on qualifications and experience. A medically-qualified appointee who has just completed his pre-registration year will be appointed as a Lecturer at the minimum point of the scale.

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowance, leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon-Square, London WC1H 0PF, or from the Appointments Unit, Secretary's Office, University of Hong Kong, Hong Kong.

The closing date for applications is 31 July 1981. (8969)A

Toxicologists

to evaluate data on Medical Aspects of Chemical Hazards

There are currently opportunities at two levels, to join the London-based Division which provides advice to Government Departments on all medical aspects of chemical contamination of the environment. A data bank is maintained on all subjects relevant to this work.

Up to £13,555

Work includes the evaluation of biological data in order to advise on possible health hazards associated with the following areas:-

Post I — Tobacco, tobacco substitutes and tobacco additives.

Post II — Food additives and contaminants.

Post II — Food additives and contaminants Candidates should normally have a first or second class honours degree in an appropriate subject plus a range of varied experience on work concerning chemical pollution and environmental protection. Experience in toxicological research and/or critical assessment of toxicological data is essential.

Appointment as Principal Scientific Officer £10,705 — £13,555 with starting salary according to qualifications and experience. *Ref:* S(B)677/2.

Up to £9,015

This post is concerned with the evaluation of

toxicological data relating to the exposure of man to chemicals in food. The successful candidate will join a multi-disciplinary team engaged on the preparation of monographs of toxicological data, background papers and briefing material for consideration by expert advisory committees in the UK and abroad. There will be opportunities to gain further practical experience in the Department's new toxicological laboratory at St Bartholomew's Hospital.

Candidates, normally aged under 30, should have a degree/HND/HNC in an appropriate discipline and at least 2 years post-qualification experience with a relevant biological/chemical background.

Appointment as Higher Scientific Officer £7,090 — £9,015 with starting salary according to qualifications and experience. *Ref:* SB/8/KE/2.

All salaries under review.

All successful candidates will be required to take the Diploma in Toxicology and will be given every encouragement in their studies, including day release.

In certain circumstances, assistance with removal expenses may be available.

For further details and an application form (to be returned by 24 July 1981) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote appropriate reference.

Department of Health and Social Security

(8978)A

NEW ZEALAND

PETROLEUM GEOPHYSICIST New Zealand Geological Survey

New Zealand is undergoing major expansion of its energy resource investigations including prospecting for hydrocarbons. The Department of Scientific and Industrial Research, the principal Government R & D Agency, and advisor to Government and industry in science and technology, has a vacancy in its Geological Survey for a seismic interpreter. The position, in the Petroleum and Basin Studies Section requires a person with a sound geological background primarily for regional analysis for the Basin Studies Programme.

Qualifications: A good 4 year bachelor's degree or higher, and at least 3 years petroleum exploration experience, are preferred.

Salary: A salary of up to NZ\$23,520 per annum is offered for this position, depending on qualifications and experience.

Further information, application forms etc may be obtained from the Chief Migration Officer, New Zealand High Commission, New Zealand House, Haymarket, London SW1Y 4TQ, quoting Imm 2/335/39.

Applications quoting Vacancy No. 2557 accompanied by a résumé should reach the above address by 30 September 1981. (8975)A

MEMORIAL UNIVERSITY OF NEWFOUNDLAND DEPARTMENT OF BIOCHEMISTRY PROSPECTIVE POSTDOCTORAL TRAINEES

graduate students and research fellows are encouraged to apply for research positions in the area of environmental and marine biochemistry, antifreeze porteins, membrane glycoproteins, intermediary metabolism, physical chemistry of lipids and proteins, experimental and community nutrition, microbiology, food sciences, food processing and food engineering. Grant-funded research in these areas is conducted in wellequipped laboratories by highly motivated research faculty. Salaries of postdoctoral and research fellows, other benefits and starting dates are negotiable. Fellowships of graduate students may be supplemented from grants and teaching assistantships.

Apply with vitae and two letters of recommendation to Dr S Mookerjea, Head, Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X7, Canada. (8941)A

ASSISTANT RESEARCH SCIENTIST

AT UNIVERSITY OF CALIFORNIA SAN DIEGO

Research on role of central nervous system. Steroid hormone receptors in control of blood pressure. Two years of postdoctoral experience in steroid and/or neurotransmitter receptor work preferred. Salary \$22,500 plus, depending on experience. CV to Prof. D. D. Fanestil, University of California, San Diego, M-023, La Jolla, CA 92093.

An Equal Opportunity/Affirmative Action Employer. (NW722)A

TUFTS UNIVERSITY SCHOOLS OF MEDICINE

DEPARTMENT OF PHYSIOLOGY

Tufts University has instituted a search for

CHAIRPERSON

Department of Physiology. The department of physiology is expected to be a center of research and scholarly excellence, as well as providing leadership in physiology instruction for the School of Medicine, School of Dentistry, School of Veterinary Medicine, and the Sackler School of Graduate Medical Science.

Individuals interested in being considered for this position should direct thier letters to Dr Seymour Reichlin, Chairman, Physiology Search Committee, Box 275, New England Medical Center Hospital, 171 Harrison Avenue, Boston MA 021111. Tufts has an affirmative action program, and encourages applications from women and members of minority groups.

(NŴ718)A

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF
ANIMAL PHYSIOLOGY
Babraham, Cambridge CB2 4AT

POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post doctoral appointment financed for a period up to 3 years by the National Research Development Corporation in one of the following fields:i) Chemical carcimogemesis and cell transformation in vitro;

ii) Hybridoma production. Both areas will involve study of animal and/or human cells secreting hormones or antibodies.

Candidates should have a 1st or upper 2nd class honours degree and at least 2 years post-graduate experience in molecular biology and/or immunology and aquaintance with cell culture techniques.

Salary in Higher Scientific Officer scale (under review) £6,075 to £7,999 pa. Starting pay according to experience non contributory pension scheme.

This post can be discussed in more detail with Dr W Mason on Cambridge (0223) 832312. Application forms from the Secretary of the Institute quoting reference NRDC1. Closing date: 31.7.81. (8999)A

THE MEDICAL RESEARCH **COUNCIL OF NEW ZEALAND**

VIRUS RESEARCH UNIT Dundedin, New Zealand

DIRECTOR

Applications are invited from either medically or non-medically qualified virologists for the position of Director of the Council's Virus Research Unit, Department of Microbiology, University of Otago, Dundedin. The Unit has an establishment of five professional and six technical positions. As a result of an increase in establishment, two junior professional and two technical posts are currently vacant. Council would expect the Director to initiate a research programme of full international standard built around any medically significant viral system. While it is desirable for a portion of the research effort to be orientated towards clinically significant problems, the prime object of the unit is the pursuit of fundamental research in virology.

Salary would be not less than that of Associate Professor in the University. A particularly well qualified individual could be appointed at the level of full Professor.

Current salaries are: Associate Professor (Science)

NZ\$33,050 per annum. Associate Professor (Medical) NZ\$40,778 — \$45,987 per annum. Professor (Science) NZ\$35,564 — \$44,505 per annum.

Professor (Medical) NZ\$45,250 -\$50,464 per annum.
A 5% General Wage Order is

expected to apply.

Further particulars are available from the Registrar, University of Otago, Dunedin, New Zealand, or the Association of Commonwealth Universities (Appts.), 36 (Square, London WC1H 0PF. 36 Gordon

Applications close on 31 July, 1981, or as soon as possible (8970)A thereafter.

NATIONAL BLOOD TRANSFUSION SERVICE **Lancaster Centre**

Quernmore Road, Lancaster invites applications for the post of Senior Medical Laboratory Scientific Officer. Candidates should possess the Fellowship of the I.M.L.S. and preferably have the Advanced Certificate in Blood Group Serology or appropriate scientific degree with relevant experience.

The successful applicant will assume supervisory duties in connection with technical work carried out in various departments at the Centre.

Some evening work is envisaged and the appointee will be invited to participate in the emergency on call rota

Salary scale £6,129 to £8,097 per annum, increase pending. National Health Service conditions and superannuation scheme.

Applications, together with the names and addresses of two referees should be addressed to the Consultant in Charge to reach the Lancaster Centre by 14th August, 1981 (8988)A

UNIVERSITY OF PENNSYLVANIA

DEPARTMENT OF HUMAN GENETICS

THREE TENURE TRACK **POSITIONS**

to begin at the Assistant Professor level are available in the Department of Human Genetics. Candidates (MD or PhD) trained in modern molecular genetic techniques and with a record of research in genetics are urged to apply.

One position is to be filled by an MD researcher who would be qualified to hold a joint clinical appointment. Areas of interest in molecular approaches include: evolution, development, gene regulation, molecular cytogenetics, polymorphism and molecular mechanism of disease.

Send résumé to: Dr R Schmickel, Dept of Human Genetics, Room 195 Med Labs Bldg/G3, University of Pennsylvania, Philadelphia, PA 19104. An Equal Opportunity/ Affirmative Action Employer.

(NW726)A

UNIVERSITY OF ABERDEEN DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

RESEARCH ASSISTANT

Applications are invited from Physicists with experience in medical physics required for a project funded by the Cancer Research Campaign to study the improved detection of early metastatic disease by combined use of radionuclide tomographic imaging and serum biochemical markers, working with Dr P P Dendy.

The appointment will be tenable until 30 September 1982 in the first instance, to commence as soon as

Salary on Range IB Scale £5,285 -£7,700 per annum, with appropriate

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by Friday 17 July 1981. (8945)A

UNIVERSITY OF NOTTINGHAM

CANCER RESEARCH CAMPAIGN LABORATORIES POST-DOCTORAL

CELLULAR IMMUNOLOGIST

Applications are invited for a postdoctoral post supported by the Cancer Research Campaign to join a group studying cellular immune responses to tumours. This includes the analysis of natural cell mediated immunity and its modulation by interferon and related substances.

The appointment will be initially for a period of twelve months. Salary will be in the range £6,070 to £10,575 per annum.

Applications in writing, enclosing a curriculum vitae including 2 referees, to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD, not later than 31st July 1981. Ref No 789. (8998)A



RESEARCH SCIENTIST MARINE SEISMOLOGY

Salary: To \$39,889

Ref. No.: 81-NCRSO-EMR-10

Energy, Mines and Resources Canada Geological Survey of Canada (GSC)

Dartmouth, Nova Scotia

The Atlantic Geoscience Centre located at the Bedford Institute in Dartmouth has a vacancy for an experienced seismologist to lead the marine seismic programs of the Atlantic Geoscience Centre. The successful candidate will participate in and co-ordinate the GSC involvement in international, multi-institutional marine seismic reflection and refraction programs; will initiate, conduct and interpret the results from seismic programs of local, national or international scope. Programs are directed towards the investigation of the structure and origin of continental margins off Eastern Canada and the Arctic. This seismologist will be required to contribute original and creative research work.

Qualifications

Graduation with a Doctorate degree or a lesser degree with research experience and productivity equivalent to a Doctorate degree from a recognized university in one of the earth sciences (or a related field with considerable earth science experience) with previous seismological experience preferably in marine seismology and a demonstrated capacity for original geophysical research, its organization and coordination.

Knowledge of the English Language is essential.

Clearance No.: 111-077-001

Additional job information is available by writing to the address below:

Toute information relative à ce concours est disponible en français et peut être obtenue en écrivant à l'adresse suivante:

How to apply

Send your application form and/or résumé to: Joan Girling **National Capital Region Staffing Office Public Service Commission of Canada**

300 Laurier Avenue West Ottawa, Ontario K1A 0M7 (613) 593-5331

Closing date: July 31, 1981

(NW714)A

Please quote the applicable reference number at all times.



UNIVERSITY OF OXFORD UNIVERSITY LABORATORY OF PHYSIOLOGY

Departmental Demonstratorship

Salary: £5,285 - £8,105 (National Lectureship Scales)

The Department of Physiology invites applications for a Departmental Demonstratorship. The initial appointment will be for three years, with the possibility of renewal for a further three-year period. Departmental Demonstrators take part in all aspects of the teaching of physiology to medical and non-medical undergraduates, but are expected and encouraged to conduct their own research. Applications are invited from able candidates in any area of physiology or related disciplines.

Applications (3 copies) including the names of two academic referees should be sent by 30 September 1981 to: The Administrator, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT. (8947)A

Newcastle Area Health Authority (Teaching)
Royal Victoria Infirmary
Regional Genetics Advisory Service

SCIENTIFIC OFFICER BASIC GRADE

Graduate required immediately for work in active cytogenetics service laboratory involved in all aspects of diagnostic cytogenetics including leukocyte culture, amniotic fluid cell culture, fetal cultures and bone marrow analysis. Previous experience preferable but not essential. For further information please contact Professor D. F. Roberts, telephone Newcastle (0632) 25131, Extension 658.

Salary on Whitely Council PTA Scales: Probationary 1st or 2nd Class Honours Degrees: £5,346 \times 5 increments to £6,363 pa. Other Degrees: £4,839 \times 7 increments to £6,363 pa. Post-Probationary Scale: £5,553 \times 7 increments to £7,110 pa.

Applications giving full personal details, together with the names and addresses of two referees should be sent to the Senior Administrative Assistant (Personnel), Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, quoting Ref. No. 81/41. Closing date: 17 July 1981.

University of Kent at Canterbury Space Sciences Laboratory GIOTTO COMET HALLEY MISSION

A new team sponsored by the European Space Agency and financed by the SERC is being formed under the direction of Dr J. A. M. McDonnel to develop a Principal Investigator experiment for the interception of Comet Halley 1986. The team will design and co-ordinate the development of a sensor cometary dust impact system in co-operation with the Rutherford and Appleton Laboratories (RAL) and 11 co-investigators in Europe and the USA. Applications are invited for the following vacancies.

PROJECT MANAGER to be responsible to the Principal Investigator for the design, development, test and integration work to be carried out at the University in co-operation with RAL and ESA, Holland. Applicants should have experience in the aerospace and electronics fields. Initial appointment is for 3 years with probable extension until 1986. The salary will be between £8,515 and £12,305 within the IA/II range for research and analogous staff.

ELECTRONIC DESIGN ENGINEER (Graduate Research Associate) to design and develop high reliability, low-power circuitry. Experience in electronic design engineering would be an advantage. Salary range £5,285 — £6,880 on the Grade IB scale for other related staff.

ELECTRONICS TECHNICIAN GRADE 3 to develop and fabricate prototype flight circuits and to test manufactured flight systems. Salary range £4,672 — £5,066 on the Grade 3 scale.

Successful applicants would be expected to travel within Europe. The appointments will be made as early as possible and applications giving details of qualifications and experience and the names of two referees should be submitted to the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH, from whom further particulars may be obtained. Please indicate the post applied for and quote reference A18/81. Closing date 31 July 1981.

UNIVERSITY OF PAPUA NEW GUINEA (Port Moresby)

Applications are invited for the post of PROFESSOR OF CHEMISTRY

A major responsibility of the Department is in undergraduate teaching. This includes relatively elementary service teaching, but also more advanced courses for science and chemistry students. Applicants should have a strong interest in the problems of teaching chemistry in Papua New Guinea at all levels covered in the University. Department is committed to the training of National Academics and a significant contribution will be expected in this area of avanced training. Present research in the Department includes: chemical education; chemistry of natural products; general environmental and analytical chemistry; heavy metal contamination of the environment; kinetics and reaction mechanisms of co-ordination complexes; marine chemistry: nutrient composition of the winged bean; phytochemistry of medicinal plants; X-ray crystallography. The interests of the Professor need not be confined to these areas, but should be in the general area of application of chemistry to Papua New Guinea and its environment. Applicants should have wide research capabilities and extensive research experience in at least one appropriate area. Previous experience of teaching and research in a developing country in a tropical environment will be considered an advantage.

Salary: K 20,995 pa. Three-year contract; gratuity; support for approved research; rent-free accommodation; family passages; baggage allowance; leave fares after 18 months service; education subsidies; salary continuation scheme to cover extended illness or disability. Applicants who wish to arrange secondment from their home institutions will be welcomed.

Detailed applications (2 copies), including a curriculum vitae, a recent small photograph, a written statement outlining their views on the role of chemistry in a developing tropical country and naming 3 referees, should be sent to the Assistant Secretary (Staffing), University of Papua New Guinea, Box 4820, University PO, Papua New Guinea to arrive no later than 3 August 1981. Applicants resident in UK should also send I copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London WIP ODT. Further details are available from either address.

(8949)A

THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF CHEMISTRY

RESEARCH FELLOWS

Applications are invited for appointment as postdoctoral Research Fellow in the Research School of Chemistry. Appointments will be for two years in the first instance Current major research interests in the School are:

Organic Chemistry (Professor A I J Beckwith, Professor L N Mander Mr R W Rickards, Dr J K Macleod Dr L Radom): including free radica reactions; mechanistics and synthetic organic chemistry; organic chemistry biologically active compounds ICR and mass spectrometry; ESR spectroscopy; theoretical organic chemistry. Inorganic Chemistry (Professor B G Hyde, Professor A M Sargeson, Dr M A Bennett, Dr G E chemistry. Robertson, Dr S B Wild): including solid state inorganic chemistry synthesis, structure and reaction mechanisms of transition metal complexes: biomimetic chemistry organo-transition metal chemistry X-ray crystallography; resolution and reactions of chiral arsines and phosphines. Physical and Theoretica. Chemistry (Professor D P Craig, Dr J Ferguson, Dr R Bramley, Dr T R Welberry): including molecular and crystal theory; spectroscopy and photochemistry; photophysics and EPR; X-ray diffraction and theoretical studies of disordered materials Analytical Chemistry: Miss B J

Appointments will be made primarily within these research groups, but proposals for independent research will also be considered Further particulars on the appointments may be obtained on request.

Salary range \$A19,821 — \$A25,871 per annum. Current exchange rates \$A1 = 58p = \$US1.11. Reasonable travel expenses and assistance with housing for appointees from outside Canberra. Superannuation benefits are available. There is no application form.

Applicants should supply to the undersigned by 31 August 1981 a curriculum vitae, list of publications and statement of research interests together with two passport-sizec photographs, the names and addresses of three academic referees and the probable date on which the post doctoral Research Fellowship, if awarded, could be taken up. G E Dicker, Registrar, PO Box 4, ACT 2600, Australia.

Further particulars can also be obtained from the Association of Commonwealth Universitie: (Appts.), 36 Gordon Square, Londor WC1H0PF. (8938)A

THE UNIVERSITY OF ALBERTA

DEPARTMENT OF IMMUNOLOGY

has two vacancies for

POST-DOCTORAL FELLOWS

to work on the following research projects: a) cell-mediated and humoura immune responses to virus-transformed tumours, involving analysis of tumou rejection in vivo in a murine model; b) characteristics of cytotoxic T Cell responses to minor H antigens.

Send resume and names of referees to Dr L M Pilarski, Department of Immunology, University of Alberta, 845E Medical Sciences Building, Edmonton Alberta, Canada T6G 2H7. The University of Alberta is an equal opportunit employer. (NW720)A

UNIVERSITY OF SOUTHHAMPTON RESEARCH TECHNICIAN IN ORGANIC CHEMISTRY

A technician is required by the Department of Chemistry for support in the Research Laboratories. The work will involve synthetic chemistry and supervision of gc, hplc and spectroscopic equipment, leading to a wide range of experience.

Applicants should have an appropriate science qualification (at least ONC/TEC or equivalent) and relevant background experience.

The appointment will be made on grade 4 salary scale £5,284 — £6,078 or grade 5 £5,695 — £6,650 per annum, depending upon age, qualifications and experience.

Applications (two copies) giving date of birth, details of qualifications and experience together with the names and addresses of two referees should be sent to Mr C N Saull, Staffing Dept., The University, Southampton SO9 5NH as soon as possible quoting reference no. 462/T. (8989)A

UNIVERSITY of Arizona, faculty position at any academic rank. The Department seeks applications from candidates with expertise in basic science and ability to apply this expertise to problems in food science and food production. Basic science areas such as ultrastructural analysis, recombinant DNA, cell culture, or physical chemistry are of particular interest, but all areas will be considered. The successful candidate will be expected to develop a vigorous, independent research program in their speciality area. 12-month position, 90% research, 10% instruction. Applications including the names of three referees should be sent to: Darrel E Goll, Nutrition and Food Science, University of Arizona, Tucson, Arizona 85721 USA. Equal Opportunity/Affirmative Action Employer. (NW725)A

UNIVERSITY OF TORONTO DEPARTMENT OF MEDICAL GENETICS ASSISTANT PROFESSOR Tenure Stream

Applications are invited from MD's or PhD's with postdoctoral research training and a strong background in the molecular genetics of higher organisms or the application of molecular genetics to medical or related problems.

Appointee would be expected to lead an independent research program in area of expertise and participate in the teaching of molecular and cell biology. Salary is commensurate with experience.

Applications, giving names and addresses of three referees, should be sent to Dr Andrew Becker, Department of Medical Genetics, University of Toronto, Toronto, Canada M5S 1A8 before July 31, 1981. (NW716)A

QUEEN ELIZABETH COLLEGE Kensington (University of London) LIBRARIAN

Applications are invited from graduates with appropriate qualifications for the post of Librarian which becomes vacant on 1 October 1981. A scientific background is most desirable as the College in its teaching and research spans the physical and biological sciences. The Librarian will be responsible for completing the on-line computerised circulation system and for applying other up-to-date information technology.

The salary will be within the scale £12,305 — £15,410 pa, plus London allowance of £967 pa.

Application form and further particulars from the College Secretary, (N) Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH. Tel: 01-937 5411 ext. 209. Closing date: 24 July 1981. (8950)A

INSTITUTE OF CANCER Research: A post-doctoral post is available for a period of three years in the Division of Cell and Molecular Biology, Chester Beatty Research Institute, Fulham Road, SW3, (Head, Dr R A Weiss). The project involves the study of the genetics of neoplastic transformation using cellular and molecular genetic techniques. Experience in somatic cell genetics, cell biology or mole-cular genetics would be advantageous although not essential. Starting salary in range £6,070 to £6,880 pa plus London Allowance of £967 pa. Further information may be obtained from Dr C. J Marshall, Tel: 01-352 8133, ext 262. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref 300/G/5. (8997)A

THE UNIVERSITY OF ALBERTA

DEPARTMENT OF IMMUNOLOGY requires a

PROFESSIONAL RESEARCH ASSISTANT

to conduct a research project which involves analysis of the SV40 — TSTA and the immune response to SV40 — induced tumours. Applicants should possess a PhD with at least two years post-doctoral experience with expertise in the area of the biochemistry of cell surface proteins with emphasis on the cross-linking of proteins via bifunctional reagents. Salary \$20,000 — \$22,000 depending on experience.

Résumés should be sent to Dr L M Pilarski, Department of Immunology, University of Alberta, 845E Medical Sciences Building, Edmonton, Alberta, Canada T6G 2H7. The University of Alberta is an equal opportunity employer. (NW721)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

John Curtin School of Medical Research Department of Human Biology FFI LOW

The successful applicant will be required to take charge of the Department's laboratory on metabolic disorders and biochemical genetics. Demonstrated ability to carry out original investigations and supervise research in these fields will be essential and experience with the study of inherited haematological disorders will be an advantage.

Closing date: 31 October 1981.

Research School of Physical Sciences RESEARCH FELLOW IN SOLID STATE PHYSICS

The Department of Solid State Physics has two main fields of research — spectroscopy of non-metallic solids and the physics of crystalline and amorphous metals. It has two lasers (c.w. and pulsed) with associated dye lasers and is well equipped for studies in all aspects of solid state spectroscopy (EPR, Mossbauer, ultraviolet, visible and infrared) and in cryogenics and thermal properties of condensed matter. There are excellent facilities for materials preparation and supporting mechanical and electrical workshops.

Appointment will be from early 1982. Applications are welcome from those able to take leave from their own institutions. Closing date: 1 October 1981.

RESEARCH FELLOW IN THEORETICAL PHYSICS

The Department's main current interests are in theoretical aspects of nuclear physics, elementary particles and field theory, statistical mechanics, transport processes, solid state physics, and plasmas. It wishes now to make a non-tenured appointment in theoretical nuclear physics. Appointment will be from 1982. Applications are welcome from those able to take leave from their own institutions.

Closing date: 1 October 1981.

Term of appointment for Fellow will be for 5 years in the first instance with the possibility of reappointment after review to retiring age; for Research Fellow 3 years with the possibility of extension to 5 years. Salary on appointment will be in accordance with qualifications and experience within the ranges: Fellow \$A23,104 — \$A30,952 pa; Research Fellow \$A19,821 — \$A25,871 pa. Current exchange rate: \$A1=0.58np=\$US1.11.

Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation benefits are available for applicants eligible to contribute. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Registrar PO Box 4, Canberra ACT 2600, Australia, or from the Association of Commonwealth Universities (Appts.) 36 Gordon Square, London WC1H 0PF, before submitting applications. (8939)A



FRENCH PHARMACEUTICAL GROUP SANOFI

Cellular Pharmacology Research Opportunities

Applications are invited from CNRS or INSERM Scientists, or other French or Foreign Research Organizations, State Doctor or PhD, for creation of a stable team of Research in CELLULAR PHARMACOLOGY SANOFI RESEARCH.

This team will be settled in Toulouse and will work in close relations with CNRS Laboratory of Basic Pharmacology and Toxicology in Toulouse.

Salary at least at the level of Chargé 5 of CNRS dependent on qualifications, experience and scientific quality of publications.

Applications with curriculum vitae to: Staff Department, Sanofi Recherche, 195 route d'Espagne, F 31036, Toulouse.

(W373)A

Materials Science Technician

£5,751 — £7,134 p.a. inc.

An interesting opportunity at the Polytechnic's Enfield location, providing a full range of services for the Materials Science Laboratories. Responsibilities would include day-to-day management, preparation and presentation of materials for microscopic examination, and the identification, labelling and cataloging of new materials.

A good HNC or equivalent qualification in physics or materials science is expected, although at least five years industrial experience in one of those fields may be acceptable.

Write quoting ref: D315D for further details and an application form, posting first-class to: Personnel Office, Middlesex Polytechnic, 114 Chase Side, London N145PN. Closing date 13 July. (8987)A

Middlesex Polytechnic

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHYSICS RESEARCH SCIENTISTS

A three-year postdoctoral research position is available for work on a joint programme with British Petroleum under the direction of Dr A Howie, FRS for the application of high resolution scanning transmission electron microscopy to catalysis problems. Salary £6,475 — £7,290 pa.

Applications from physicists, chemists and materials scientists, preferably having some experience of electron microscopy, should be sent by 31 July 1981 to the Secretary, Cavendish Laboratory, Madingley Road, Cambridge CB3 OHE, from whom further information can be obtained. (8960)A

UNIVERSITY OF READING

European Science Foundation — Additional Activity in Taxonomy Applications are invited for the following staff to participate in the establishment of a EUROPEAN TAXONOMIC SYSTEMATIC AND BIOSYSTEMATIC DOCUMENTATION SYSTEM project under the auspices of the European Science Foundation:

1. INFORMATION SCIENTIST to play a major role in devising a

computerised information system for plant taxonomic and related data on a European basis. The successful candidate will have a PhD and substantial experience in information science/systems design and with

an interest in plant systematics. Starting salary up to £12,860 p.a.

2. PLANT TAXONOMIST to work closely with the Information Scientist and be responsible for collecting and assessing data, both published and unpublished, on the taxonomy, systematics and biosystematics of European plants. The successful candidate will have a PhD and experience in plant systematics, with an interest in modern methods of data handling. Starting salary up to £8,515 p.a.

Both appointments are for up to three years in the first instance and are superannuated under USS. They will be based in the Department of

Botany, Plant Science Laboratories. Apply, quoting Ref. R.11A, for application form and further details to Personnel Officer, University of Reading, Whiteknights, Reading RG6 2AH. Application forms to be returned by 15 August 1981.

ASSOCIATESHIPS

UNIVERSITY OF ADELAIDE

Invites applications for three

RESEARCH ASSOCIATESHIPS (X3503, X3504 & X3505)

which it proposes to award in 1981. Each award may be held in any department of the University.

Applicants should have gained their postgraduate qualifications elsewhere than at The University of Adelaide. In those disciplines where a PhD is a recognised indication of research ability such a degree will be required. In other areas, appropriate evidence of research ability will be required.

An Associateship will be tenable for two years and will not be renewable; no age limit governing eligibility has been specified but normally a 10 year limit since the award of the most recent higher degree qualification will apply.

Travel Provision: The University will provide, for a person taking up appointment from outside South Australia, an economy class air fare to Adelaide by the shortest practicable route. There is no provision for transfer of personal effects.

Salary Scale: A17,083 - 497(1) - 500(1) - 499(1) - 493(1) - 498(1)\$A19,570. There is no provision for superannuation.

Applications in duplicate must be made on the prescribed form. Forms and a leaflet outlining conditions of appointment may be obtained from the Secretary, Research Sub-Committee, Registrar's Office, The University of Adelaide. Applications must be lodged with the Registrar of the University, GPO Box 498, Adelaide, South Australia, 5001, not later than 1 August, 1981. (8940)O

CONFERENCES and COURSES

CHARLES DARWIN CENTENARY CONFERENCE 27 June — 2 July 1982

Darwin College Cambridge is promoting this Conference on the centenary of Darwin's death to survey the present state and prospects of evolutionary theory. The Conference will be primarily scientific rather than historical, but not intended only for specialists. The Conference will be strictly limited in size: early application is advised.

Speakers include -

Introduction: S. Brenner

Evolutionary History: E. Mayr, M.J.S. Hodge, D.L. Hull, G.E. Allen

Molecular & Cellular Evolution: L.E. Orgel, F. Jacob, D. Phillips M. Eigen, W.F. Bodmer, C.R. Woese, P.H. Clarke, A. Wilson

Evolution of Whole Organisms: R.C. Lewontin, E. Nevo. J.L. Harper, S.J. Gould, A. Hallam, G. Isaac, F.J. Ayala Evolution of Social Behaviour: R.W. Burkhardt, J. Maynard Smith, T.H. Clutton-Brock, P.P.G. Bateson, R. Dawkins, E.O. Wilson, B.A.O. Williams

Registration Fee £40: full board and lodging about £30 a day. Details and application forms from Hon. Secretary, Darwin Centenary Conference, Darwin College, Cambridge CB39EU, U.K. (8996) C

BIRKBECK COLLEGE (University of London)

DEPARTMENT OF ZOOLOGY

MSc IN ENTOMOLOGY

This two-year part-time evening course is designed primarily for entomologists but also allows teachers and others to extend their training in a scientific discipline and to become conversant with the tools of the current research. Topics covered include: sensory and neuromuscular physiology, respiratory physiology, osmotic and ionic regulation, insect genetics, taxonomy and economic entomology.

For further details contact the Departmental Secretary, Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX

(8966)C

BIRKBECK COLLEGE (University of London)

DEPARTMENT OF ZOOLOGY

MSc IN COMPARATIVE **PHYSIOLOGY**

A two-year part-time evening course suitable for zoology graduates wishing to specialise in the comparative aspects of the adaptations of animals to their environment. Topics covered include: osmotic and ionic regulations; respiratory and circulatory physiology; endocrinology; neurophysiology. Students are introduced to a wide range of modern physiological techniques.

For further details contact the Departmental Secretary, Department of Zoology, Birkbeck College, Malet Street, London WCIE 7HX.

(8967)C

Kingston Polytechnic

MSc in EARTH SCIENCE AND THE ENVIRONMENT

The third intake for the two year part-time course will be in January 1982.

Send for further details to the Secretary, School of Geology, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366.

Applications should be submitted by 20th July 1981.

(8981)C

STUDENTSHIPS

LIVERPOOL POLYTECHNIC DEPARTMENT OF BIOLOGY RESEARCH STUDENTSHIPS

Applications are invited for Post Graduate Research Studentship to work on "Thallium Uptake by Plants".

Applicants should have an appropriate first degree with CNAA.

Applications in the first instance to Dr N. W. Lepp, Department of Biology, Liverpool Polytechnic,

Byrom Street, Liverpool L3 3AF.

Closing date two weeks after the appearance of this advertisement.

(8957)F

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

RESEARCH STUDENTSHIP

Applications are invited from individuals holding, or expecting to obtain, a good honours degree in Physiology or Pharmacology for a Research studentship tenable in the Department of Physiology, St. George's Hospital Medical School.

The successful applicant will work on a research project concerning the effects of alcohol on the nervous system of animals. Further details are available from Dr T W Stone, Department of Physiology, St George's Hospital Medical School, London SW17 ORE (Tel: 01-672 1255 ext 4657), to whom applications should be sent along with CV and names of two referees.

(8972)F

CHELSEA COLLEGE UNIVERSITY OF LONDON SRC CASE STUDENTSHIP

Applications are invited from andidates with a first or upper second class honours degree or equivalent qualification for a three year CASE Studentship on the Pharmacology of Enkephalinase

The research will be supervised by Dr S L Hart in collaboration with Dr I W Smith at the Wellcome Labora-

Applications, including curiculum vitae and names of two eferees should be sent as soon as possible to the Secretary, Pharnacology Department, Chelsea College, Manresa Road, London College, N SW3 3TW.

ARC UNIT OF NITROGEN FIXATION University of Sussex and Open University SERC CASE **STUDENTSHIP**

students with an interest in Bio-Organic Coordination Chemistry iolding, or expecting, a first or upper econd class honours degree this ummer, are invited to apply for the ibove studentship.

It concerns the application of 15N imr spectroscopy and other echniques to chemical systems elevant to biological nitrogen ixation, most experimental work to be carried out at the UNF.

The joint supervisors are Dr J Mason (Open University) and Dr R L Richards (Unit of Nitrogen Fixation, Jniversity of Sussex, Brighton BN1 IRQ) to whom applications, together vith the names of two referees should e sent as soon as possible. (8980)F

UNIVERSITY OF SUSSEX

SCHOOL OF **BIOLOGICAL SCIENCES** Falmer, Brighton BN1 9QG

SRC CASE AWARD

'Protein Metabolism in Animal Tissues During Pregnancy and Lactation'

A studentship is offered in collaboration between Dr V M Pain at the above address and Dr R Vernon at the Hannah Research Institute, Ayr, Scotland.

Applicants, or those seeking further details should contact Dr Pain (0273 606755 Ext 53) or Dr Vernon (0292 76013 Ext 227) as soon as possible. (8953)F

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE (DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES) CASE STUDENTSHIP

Applications are invited from candidates holding a First or Upper Second Class Honours degree for a 3-year SRC CASE studentship to work on the development of forage

The research will be supervised by Professor W J Whittington in collaboration with Dr J T Walker of Germplasm Resource Management Limited, Loughborough.

Applications, including curriculum vitae and names of two referees should be sent as soon as possible to Professor W J Whittington, University Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE125RD. (8976)F

UNIVERSITY OF **BIRMINGHAM NEUROCOMMUNICATIONS** MEDICAL SCHOOL CASE STUDENTSHIP

Applicants with a good honours degree (I or II (i)) in Biochemistry/ Pharmacology are invited to apply for a CASE studentship for research on 'Taurine and central nervous system inhibitory processes'

Curriculum Vitae with the names of two referees to Dr W E Davies, Nuerocommunications, Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TJ.

UNIVERSITY OF DURHAM DEPARTMENT

OF BOTANY Applications are invited for SERC CASE STUDENTSHIPS

as follows:

1. In collaboration with the Scottish Horticultural Research Institute to investigate the physiology of high pod setting new genotypes of the Vicia faba. (Supervisor Professor Boulter).

2. In collaboration with ICI Pharmaceutical Division to characterise biochemically the mechanisms of peptide transport in Candida albicans. (Supervisor Dr J

Candidates should have or expect to obtain a First or Upper Second Class Honours degree or equivalent qualifications.

Applications, naming two referees, should be sent to Professor D Boulter, Department of Botany, Science Laboratories, South Road, (8995)F Durham DH13LE.

BEDFORD COLLEGE (University of London)

DEPARTMENT OF ZOOLOGY

Applications are invited from candidates holding or expecting to graduate with a First or Upper Second Class Honours degree in Zoology or an appropriate related discipline for an

SRC CASE STUDENTSHIP

The award is available for research on "Geographic variation in Callosobruchus maculatus", a pest of leguminous seeds both on the plant and primarily in store, in collaboration with the Tropical Products Institute, Slough. The project will involve a bionomic study of distinct strains of the beetle from various tropical origins, with a view to identifying differences of potential importance in control of the pest in field situations.

Applications including a full curriculum vitae and the names of two referees should be sent as soon as possible to Dr P F Credland, Department of Zoology, Bedford College, Regent's Park, London NW14NS (01-486 4400), from whom further information may be obtained. (8968)F

PLYMOUTH POLYTECHNIC

FISH BIOLOGY **RESEARCH IN DEVON**

- 1) A NERC funded PhD studentship is available to suitably qualified candidates to work with Dr M. J. Manning and Dr J. E. Harris on developmental aspects of immunity and tolerance in fish.
- 2) A NERC funded Research Assistantship with the opportunity to register for a higher degree is available to work with Dr M. J. Manning on the rationale of immunising young fish against disease.
- 3) Plymouth Polytechnic offers a one-year MSc Degree by course-work and research in Applied Fish Biology with NERC-funded studentships for some selected candidates.
- 4) Plymouth Polytechnic also offers PhD (3 year) or MPhil (2 year) postgraduate research programmes in areas which include Parasitology, Immunology, Microbiology, Cell Biology and Genetics.

For further details please write

Dr M. J. Manning, Dr J. E. Harris or Dr R.A. Matthews, Department of Biological Sciences, Plymouth Poly-technic, Drake Circus, Plymouth PL48AA.

Details of other research opportunities in the Department are obtainable from Dr L. A. F. Heath, Head of Department of Biolog-(8944)F ical Sciences.

BIRKBECK COLLEGE

(University of London) DEPARTMENT OF ZOOLOGY Applications are invited for a three year

SRC CASE

RESEARCH STUDENTSHIP

to study neurophysiological and behavioural aspects of food selection by larvae of Spodoptera species (Lepidoptera) in collaboration with the Centre for Overseas Pest Research (Dr R F Chapman).

Applicants should have, or expect to obtain this summer, a first or upper second class honours degree in a biological discipline.

Applications, with curriculum vitae and names of two academic referees should be sent, as soon as possible, to Dr W M Blaney, Department of Zoology, Birkbeck College, Malet Street, London WC1E 7HX. (8965)F

THE UNIVERSITY OF WOLLONGONG New South Wales

DELHI PETROLEUM POSTGRADUATE AWARD IN GEOLOGY

Applications are invited from graduates with good honours degrees or equivalent in geology for a postgraduate award in the Department of Geology.

The Award is for research in the area of source rock and maturation studies in the Cooper and Eromanga Basins.

The award is available for full-time candidates undertaking research leading to the degree of Doctor of Philosophy and may be held for a maximum of 3 years subject to satisfactory performance, work and conduct. An award holder receives an annual stipend of A\$7,500 pa.

Further information is available from Professor A. C. Cook, Department of Geology.

Applications, giving the names and addresses of two referees, should be forwarded to the University Secretary, The University of Wollongong, PO Box 1144, Wollongong, 2500, NSW, Australia, no later thay July 31st, 1981. (8955)N

FELLOWSHIPS

UNIVERSITY OF MANCHESTER

DEPARTMENT OF BOTANY
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications invited for postdoctoral fellowship, tenable for maximum of three years, to work on factors controlling sporocarp development in the aquatic fern Azolla and other aspects of its biology. The fellowship is supported by the Overseas Development Administration and the International Rice Research Institute (IRRI), Manila, Philippines, and the Fellow will spend a few months of each year at IRRI.

Experience of electron microscopy (SEM and TEM) an advantage. Initial salary £6,880 to £7,700 pa.

Applications to Professor E G Cutter, Department of Botany, The University, Manchester M13 9PL, by 20th July, 1981. (8974)E

EMBO

European Molecular Biology Organisation LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1981 AWARDS

Next deadline: August 15, 1981

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but subject to review of progress by the selection committee, they are usually renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is August 15, 1981. Successful candidates will be notifed of their awards immediately after the meeting of the selection committee which is on October 23, 1981.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.

(W370)E



Department of Haematology

POST DOCTORAL FELLOWSHIP

Applications are invited for the above post in the Department of Haematology, Trinity College, Dublin

The successful candidate will work in the field of bone marrow culture and marrow transplantation. Previous experience in tissue culture is desirable, not essential.

Applicants should submit a full curriculum vitae, together with the names of two referees, to:

Dr. S. R. McCann, Central Pathology Laboratory, St. James's Hospital, Dublin 8. Tel: Dublin 782088.

(8986)E

POSTDOCTORAL FELLOWSHIPS available starting 1 July 1981. Experience in protein purification and/or RNA-DNA hybridization analysis preferred. (i) To study estrogen receptor mediated events related to gene transcription and (ii) to study the mechanism of alterations in gene expression related to prostate neoplasia. Send curriculum vitae, graduate transcripts, and three letters of recommendation to: Roy G. Smith, PhD, The University of Texas Medical School at Houston, 6431 Fannin, Suite 6018, Houston, Texas 77030. An Equal Opportunity Employer. (NW717)E

RESEARCH IMMUNOLOGIST BRITISH HEART FOUNDATION SENIOR FELLOWSHIP

Applications are invited for the post of Immunologist attached to the Papworth Heart Transplant Research Unit which is funded by the British Heart Foundation. The principal research activities will be directed towards studying the effects of presently used anti-thymocyte globulins and other immunosuppressive agents, and on the potential use of monoclonal equivalents.

The Fellow will have access to clinical material at Papworth Hospital but will be based in the Immunology Division of the Department of Pathology at Adenbrooke's Hospital, Cambridge. The appointment will be for 3 years in the first place. Salary will be according to age and experience on the University Lecturer Scale, or for medically qualified candidates the salary will be equivalent to that of a Clinical Lecturer.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Mr P Wilburn, Superintendent, Department of Pathology, Tennis Court Rd, Cambridge CB2 IQP. (8959)E

UNIVERSITY OF WARWICK POSTDOCTORAL FELLOWSHIP IN MASS SPECTROMETRY

To work in the Department of Chemistry and Molecular Sciences with Professor K R Jennings on the mass spectrometry of explosives and related compounds. The project involves the study of the negative chemical ionisation spectra of nitrocompounds under a variety of experimental conditions. The appointment will be for one year in the first instance. Salary up to £7,700 in the Research Range IA scale: £6,070 — £10,575 pa, depending on age and experience. Informal enquiries to Professor Jennings (Tel: 0203-24011 ext. 2232).

Applications should include a full curriculum vitae and the names of two referees to be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL as soon as possible. Please quote Ref. No: 46/A/81/O. (8985)E

POSTDOCTORAL Fellowship: To study hormonal regulation of vitamin D metabolism in mammals. Theoretical and/or practical experience required. Contact: Dr E M Spencer, Children's Hospital of San Francisco, 3700 California Street, San Francisco, Calif. 94118. Equal Opportunity Employer, M/F/H. (NW723)E

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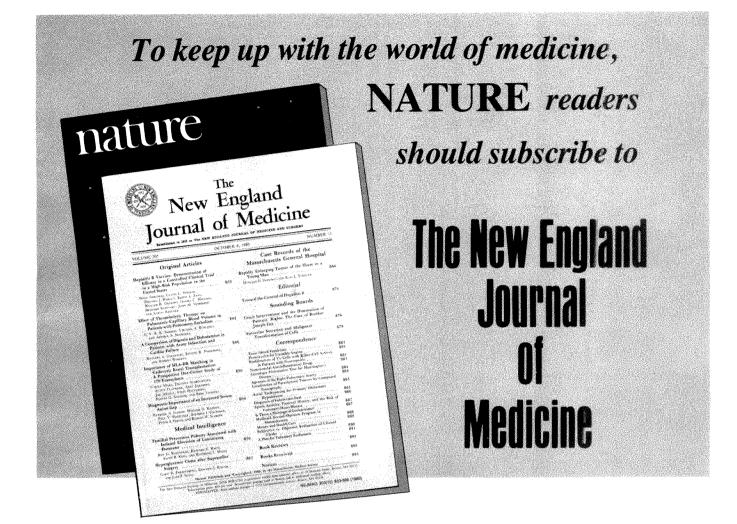
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UNIVERSITY OF EDINBURGH Department of Physics

RESEARCH ASSOCIATESHIP IN EXPERIMENTAL HIGH-ENERGY PHYSICS

Applications are invited for a Research Associateship (funded by SERC) within the high-energy physics group, which is engaged in hadron experiments at the CERN accelerators. Applicants should normally have three years' postgraduate research experience. The appointment will be made at an appropriate point on the 1A scale for research staff (currently starting point in the range £6,070 — £6,880 per annum).

Applications, with the names and addresses of two referees, should be sent, as soon as possible, to Dr D. J. Candlin, Department of Physics, James Clerk Maxwell Building, The King's Buildings, Mayfield Rd, Edinburgh EH9 3JZ, from whom further details may be obtained. Please quote reference No. 5028. (8954)O



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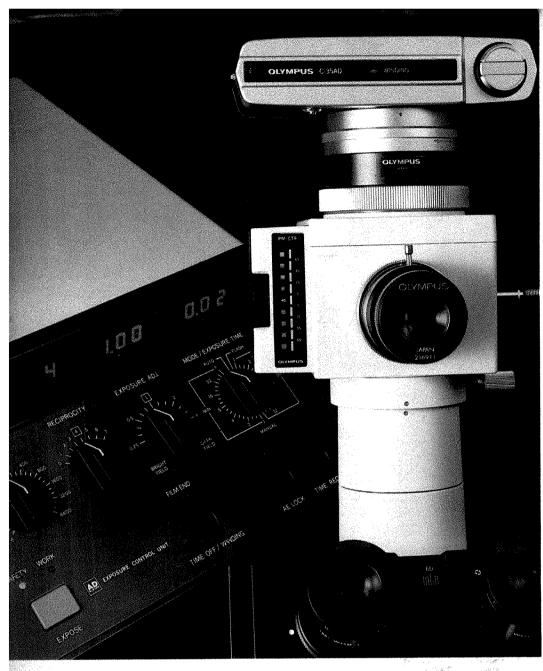
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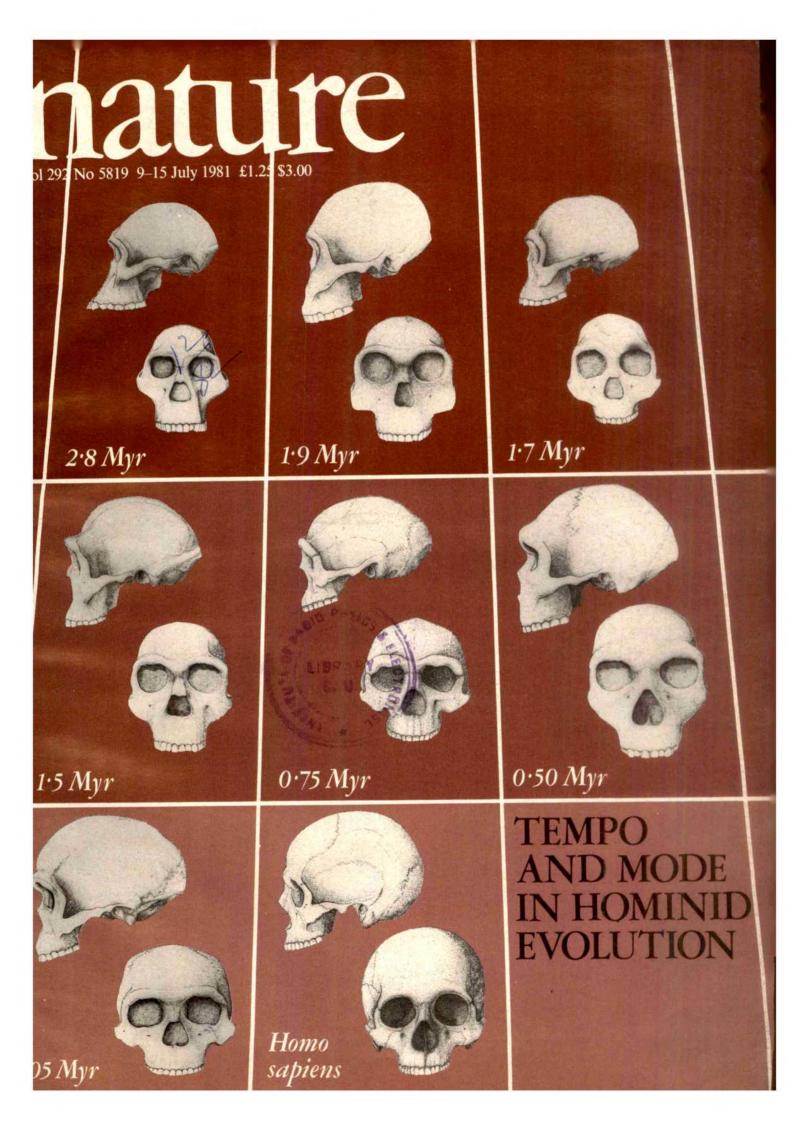


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¹Wells, R. D., Hardies, S. C., Horn, G. T., Klein, B., Larson, J. E., Neuendorf, S. K., Panayotatos, N., Patient, R. K. and Seising, E. (1980) Methods in Enzymol. 65, 372-347. The performance characteristics of RPC-5 ANALOG make it the resin of choice for molecular biologists interested in nucleic acid chromatography because it provides:

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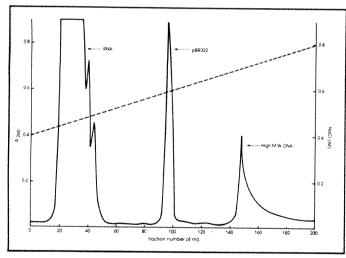


Figure 1. RPC-5 ANALOG chromatography of a total nucleic acid extract of a bacterial lysate from E. coll (HB101) harboring an amplified plasmid (pBR322). The nucleic acids were added to the column in 0.2 M NaCl, 10 mM Tris-HCl (pH 7.2) and 10 mM EDTA and eluted with an increasing linear salt gradient (0.4 - 0.8 M NaCl).

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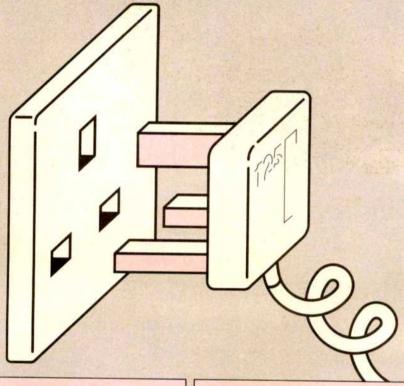
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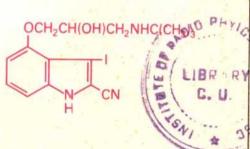
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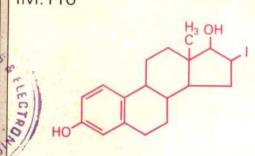
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(1) HOCHBERG, R.B. Science, **205**, pp. 1138-1140, 1979

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EDITORIAL OFFICES

London
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff

Alun Anderson Sara Nash
Philip Campbell Isobel Collins Judy Redfearn Isobel Collins Konrad Guettler Konrad Guettler
Tim Lincoln
Naomi Molson
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Washington News Bureau 801 National Press Building, DC 20045 Telephone: (202) 737-2355 Telex: 64280 David Dickson (Washington News Editor)

Publisher: Elizabeth Hughes Marketing Director: Ray Barker International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney Promotion Manager: Jonathan Earl

New York 15 East 26 Street, New York, NY 10010 Telephone: (212) 689-5900 American Publisher: Robert Ubell American Advertising Manager: Henry Dale Marketing Manager: Sheila Kane

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British universities on the brink

The threat of execution is said to help to clarify the mind. The British university system, which has woken up to the threat of trouble ahead only within the past year, will not even profit by knowing where it stands from the announcement last week of the allocations of funds to individual universities for the three years ahead (see page 99). The dilemma is constitutionally appropriate. The University Grants Committee has dealt with the universities as it should — self-governing institutions as they like to think they are, they have been told to manage their own affairs as best they can within the funds made available for the next few years. But some options the universities are individually and collectively denied. Because the grants committee has coupled its financial allocations with specific instructions about the numbers of domestic and European Community students individual universities may in future accommodate, it will not be possible for them to work their way out of trouble by being more productive. (If ever the universities have time, the legality of this requirement might usefully be tested in the courts.) And because the universities that have come well out of the past week's lottery are unlikely, self-governing as they are, to throw in their lot with the less fortunate, it is probable that half a dozen of the places most seriously affected will have to sink or swim on their own. Some of them, faced with budget cuts of 25 per cent or so (Aston in Birmingham and Salford for example) will be lucky if they do not sink, and will even now be asking whether that is the surreptitious intention of either the government or the grants committee.

For while the committee's announcement last week will certainly leave its mark on the British university system, there are still too many loose ends for comfort. What will in the end most affect the universities whose budgets are most drastically to be cut will be the lack of any formal mechanism for paying off redundant members of university staffs. Although it is agreed that the universities on which the cuts fall hardest will be able to adjust only be getting rid of tenured academics, no funds are available for providing them with compensation. And although Dr Edward Parkes, the chairman of the University Grants Committee, told a House of Commons select committee last year that some £200 million would be needed to bring about the impending adjustment, merely £20 million has been set aside for such purposes (out of the universities' collective subvention). But should not academics suffer the indignities and impoverishments that seem certain to afflict some three million other unemployed people in the United Kingdom before the year is out? This is what the enemies of the universities are asking. Some such calculation may account for the spleen with which the government has pursued the universities since its election more than two years ago. The trouble is that tenured academics most probably have rights at common law against universities that dismiss them, and that the amounts involved will far exceed the sum that Dr Parkes has put aside. In the end, either the universities concerned will have to exercise the right of self-governing institutions to go bankrupt or they will have to be rescued with more public money than the government will save with the cuts now decreed.

Another conspicuous loose end is that the grants committee has delivered its advice ("instructions" is more appropriate) without saying how its decisions have been reached. Are the lucky universities those with the lowest unit costs, the best track records in research grant applications, the lowest staff-student ratios, the best records in the recruitment of students, or what? There are reasons why too detailed a disclosure of the criteria that have been used would be damaging to individual universities. As things are,

however, the committee should forgive those now unwillingly dependent on its allocations of funds for suspecting that often mere gossip or even prejudice has determined its decisions. This charge, no doubt a slur, is nevertheless inescapable. If it had been prudent, or even merely politically expedient, the committee would several months ago have invited universities to agree on the criteria by which its decisions should be made. Even if the universities had failed to agree among themselves, the result would have been a licence to do what has now been done. As things are, without having consulted in advance, Dr Parkes has put his committee in the unenviable position of being regarded by the universities — his strongest supporters — as a dispensable bulwark between themselves and the public purse.

Four other questions remain unanswered. First, is it right that decisions of such gravity for the universities should be made in the absence of a mechanism for settling policy on higher education as a whole (polytechnics included)? Second, should British taxpayers be expected to put up with apparently arbitrary limits on the provision of university education, regardless of the ability and the willingness of qualified institutions to provide it? Third, what will be the consequences of last week's decisions for the pattern and even the quality of research? Finally, is it now to be supposed that higher education has become, as educationists have become too fond of saying, "irrelevant"? These questions will be much discussed in the months ahead.

Promising Americans

The United States Administration will not lightly be forgiven for the hash it has made of plans for international collaboration in this year's budget. President Ronald Reagan's budget director, Mr David Stockman, seems just the kind of man to implement a promise "to get the government off the people's backs". It is also a technical triumph that he and his colleagues were able to produce a coherent replacement for President Carter's budget during their few weeks in limbo, between the election last November and Inauguration Day. But in the process the budget team was plainly unreasonably dismissive of the consequences overseas of its proposals. And while Congress is doing what it can to repair some of the damage, at this stage it cannot put everything to rights. Certainly Congress will not be able to exorcise the impression that overseas obligations take second place, in the evolution of United States strategy, to domestic exigencies.

The trouble is well illustrated by two very different examples from the budget for the next financial year (which begins on 1 October). First, and simplest, is the case of the overseas budget of the National Science Foundation, used largely for supporting a surprisingly modest programme of direct collaboration overseas. (That large funds are still to be spent by other agencies, the State Department for example, is beside the point.) Like the foundation's educational budget, the overseas programme was drastically curtailed in Mr Stockman's budget. Nobody seems to have appreciated that the National Academy of Sciences was counting on this part of the foundation's budget for its contribution to the International Institute of Applied Systems Analysis, of which it is (with the Soviet Union) the principal member, and from which it is required by its obligations to give a year's notice of resignation. It now seems likely that the House of Representatives has found a way of letting the foundation dispose more flexibly of its budget, so that the academy will not have to

take a begging-bowl around to pay its dues on 1 January, but the future of its contributions remains in doubt. Mr Stockman may have concluded that the United States derives little benefit from the work of the international institute (which is probably true) and that even the institute's disappearance would be welcome (which may also be true). But international obligations cannot be overturned as easily as that. If they were formal treaties, duly ratified by the Senate, no budget director would think of striking out the associated costs. Do agreements on scientific collaboration deserve less careful consideration?

The trouble about the United States commitment to the International Solar Polar Mission is a different case. This potentially valuable scientific exploration of the Sun away from its equatorial plane has been planned for the past several years between the European Space Agency and the National Aeronautics and Space Administration, each of which was to have launched a satellite. There were howls of protest from Paris when Mr Stockman's budget omitted this item (see Nature 5 March, p.1) and science administrators in Western Europe have since fallen into the habit of complaining to their opposite numbers from the United States whenever they encounter them. Indeed, the Europeans are in danger of making too great a fuss; what is at stake is a single if expensive project, not an understanding affecting the survival of an institution, and in any case a single satellite in a polar orbit about the Sun at the next solar maximum will be a lot better than none. Even here, Congress is doing what it can to help (see page 102). It remains to be seen how the issue will be decided — but surely there are no grounds for flirting with the resuscitation of the Halley mission (in which every agency in the world capable of launching spacecraft will be participating) while a smaller sum of money would help to fulfil an international promise.

From the frying pan ...

The battle for the control of the British telecommunications network is almost over; there is a decent chance that the bill with which Parliament has been struggling for the best part of a year will become law in the next few weeks. But the war is only now beginning. In the past few weeks, several groups of private interests have been cheekily staking claims to set up in competition with the telecommunications monopoly. Cruelly as it must seem to the public monopoly (trendily known as British Telecom), other British public monopolies and nationalized industries are prominent among the would-be vultures. Thus British Rail (a wholly owned nationalized industry) and British Aerospace (wholly owned until nearly half its shares were sold off earlier this year) are talking of using the railway network as the başis for a competing trunk telecommunications network. Even more cheekily, Cable and Wireless, the state-owned company originally set up to operate telephone networks overseas, is talking of entering the domestic market even though it has not yet been sold off to the public (as the government intends). Yet British Telecom is plainly not intending to put up with whatever indignities the months ahead may bring. Some weeks ago, it put out a sombre warning of the dangers of connecting to the existing telephone network terminal equipment supplied by others than itself. Last week, it published a much more intelligent document a reply to the study commissioned by the Department of Industry from Professor Michael Beesley, which concluded that no great harm would be done, but rather good, if private interests were allowed to rent circuits from British Telecom and to use these for providing services that could then be resold to others (see Nature 23 April, p.619). The argument will now begin.

The resolution of these and related issues is crucially important for the future use in Britain of a technology as full of promise as politicians have been saying this past long year. And what happens in Britain may even be instructive elsewhere; experience in the United States of the erosion of the Bell System's monopoly is not everywhere valid and is in any case confusing, while governments elsewhere in Western Europe are in much the same state of indecision as afflicts the British government. But the

British experience is likely to be less helpful than it might have been. The most reprehensible feature of the Telecommunications Bill is that nobody will be allowed to chip away at the British Telecom monopoly without the consent of the Department of Industry. In practice, it will be for civil servants and British Telecom between them to decide what shall be allowed and what prohibitied. Thus although the present government has promised that within three years others than British Telecom will be entitled to sell terminal equipment to telephone users (and the illicit trade is already gathering strength), a change of government could change all that. And the latest document from British Telecom, intelligent though it is, demonstrates again what has been clear all along — that there is an urgent need for an explicit set of rules and some independent tribunal for administering them.

What Beesley said was simple. British Telecom would not come to a sticky end if it were required to follow the practice forced on the Bell System in the United States, and to lease trunk circuits from its own network to private operators who might then resell telephone services. Everybody's model is the American company MCI Communications Inc., whose customers are able to make trunk telephone calls between many (but not all) cities in the United States for roughly half the cost charged by Bell. Beesley acknowledged that such arrangements would rob British Telecom of revenue but not necessarily of profit, and argued that the business is not, in any case, a zero-sum game - symbiosis between the rump of the monopoly and private operators is just as likely as out and out parasitism of British Telecom. British Telecom protested at the time that Beesley's recommendations would "skim the cream" from its own business, and repeats that argument in last week's document — but in an especially revealing way. It repeats its fear about the cream, asserts that leasing its network to others would not improve the technical quality of services provided to customers (which is untrue) and goes on to say that such arrangements would compel it to "rebalance its tariffs in order to make individual sectors pay for themselves". The simple answer to the last complaint is "Whyever not?" But British Telecom is counting on the unwillingness of British politicians to fall in with "rebalancing" if the result means higher charges for any important sector of the British electorate.

The government has only itself to blame for the embarrassment it will now be caused. British Telecom, in its reply to Beesley, is disarmingly open about its present practice. It charges extra for use of the trunk network so as to subsidize local telephone calls and rural telephones. It urges the "material benefits" of providing a uniform service at uniform prices, and says that one of the consequences of liberalization would be increased rentals for telephone lines, "hitting hardest at the less well-off customers who make the fewest calls". Just in case the government fails to get the point, British Telecom guesses that the cost of a telephone call from a public coinbox would increase fourfold. Politically, the argument is telling. It is, however, false. The annual accounts of British Telecom are at present most obviously distorted by the government's requirement that the monopoly should finance the development of its network mostly out of current revenues. If the government decided otherwise (as it should) all tariffs could be reduced. But there is in any case no justification for the present distortion of telecommunications tariffs by British Telecom's self-assumed social obligations. Why should the interests of the telephone users "who make the fewest calls" increase the telecommunications costs of more serious users, mostly from business and industry? British Telecom should instead be required to follow economic pricing policies - charges for different services related to their marginal costs Parliament then invited to pay for socially valuable but otherwise uneconomic services (as is already done with railway services). The difficulty is that none of this can be accomplished without an independent and public examination of what the costs should be. The government itself is not sufficiently expert and is politically too suggestible. Thus the ironical outcome of the first serious attempt to liberalize the British telecommunications monopoly is likely to be to put the clock back ten years, and to throw the management of these vital services back into the political arena.

Congress stirs non-proliferation row

Testimony on Iraq raid backfires

A serious row affecting the future of the Non-Proliferation Treaty has broken out between the United States government and the International Atomic Energy Agency in Vienna. At issue is the appearance before two congressional committees in the past two weeks of a defector from the agency's safeguards inspectorate, Dr Roger Richter (33). At a meeting of the board of the agency in Vienna this week, Dr Sigvard Eklund, director-general of the agency, said that Dr Richter's evidence to the Senate and House committees on Foreign Relations (on 18 June and 1 July respectively) had involved the disclosure of confidential information in breach of his contract of employment, and that the agency was taking legal advice.

Dr Richter's appearances have been dramatic, to say the least. According to Dr Eklund's statement on Monday, Dr Richter last showed up for work in Vienna on 15 June. The following day, Senator Alan Cranston, chairman of the Senate committee on Foreign Relations, announced in Washington that Dr Richter, having resigned for the occasion, would be giving evidence three days later. Dr Richter's resignation was received by telex in Vienna on 18 June, according to Dr Eklund; it was not, however, accepted, but Dr Richter was instead fired.

Dr Eklund in his statement said that Dr Richter had worked for the agency since February 1978, and that he had been assigned to the section of the agency concerned with supervising safeguards in the "south and south-east" sections of the agency's territory, including both Iraq and Israel, in March 1979. Dr Richter's evidence to the congressional committees consisted most conspicuously of the assertion that the agency's safeguards were not adequate to detect violations of the Non-Proliferation Treaty by Iraq.

Dr Richter was dissuaded by Senator Cranston from quoting from a letter he had written a year ago to the US State Department, in which he had alleged that "the IAEA safeguards are totally incapable of detecting the production of plutonium in large scale materials-test reactors..." such as that destroyed by the Iraeli raid on Tamuz on 7 June.

One of the reasons why the agency has taken umbrage is that neither house of Congress has taken its denials seriously. On Monday, Dr Eklund told his board that the authorities in Iraq had been approached immediately after the Israeli raid on Tamuz

and visited the site on 18 June. They were unable to visit the main reactor because of the extent to which it had been damaged (and the insistence of the Iraqi authorities on a personal accident indemnity). The associated research reactor and the stockpile of enriched uranium was however inspected and found in order.

For the agency, the incident obviously raises serious questions about the fiduciary responsibilities of its safeguards inspectors. One requirement of the Non-Proliferation Treaty is that information gathered by inspection teams should be kept confidential. The fact that a defecting inspector should have told all to Congress on the day of his formal resignation is a serious blow to the system.

In the United States, Dr Richter's evidence to the Senate and the House has had an equally profound effect, and may impede the Administration's declared intention of liberalizing restrictions (made necessary by the Carter Anti-Proliferation Act) on the export of nuclear technology. The nuclear industry has been especially critical of the act's requirements that

recipient nations, even those that had signed the treaty, should go further than merely accept the Vienna safeguards before becoming eligible.

Part of the reason why the Carter Act has been controversial among potential recipients of United States' nuclear technology is that one condition for their signature of the treaty, in the early 1970s, was the promise that nuclear powers would assist with the development of peaceful nuclear technology.

Opinion is divided in Washington about the strength of Dr Richter's testimony.

In Congress, however, Senator Charles Percy, chairman of the Senate Foreign Relations Committee, says he has been shaken by Richter's evidence.

The chief casualty is likely to be the Administration's determination to reform the anti-proliferation policy it inherited in February. Even President Reagan startled the nuclear industry when he acknowledged at last week's press conference that signature of the Non-Proliferation Treaty did not necessarily imply compliance.

British universities transformed by budget

A major reshaping of the British university system was decreed last week, when the University Grants Committee sent letters to each of the 51 universities in Britain giving details of their recurrent grants for the next three academic years. But the full implications of what the committee has decided will not be clear until the details have been analysed by the Committee of Vice-Chancellors and Principals, to which the universities have separately (but in confidence) provided copies of the letters they have received from the committee.

Two features of the new pattern are however apparent. By the beginning of the academic year 1984-85, the total number of students from the United Kingdom and elsewhere in the European Community is to be no greater than 249,000, five per cent less than last year (the base year for all the committee's calculations) and 7.5 per cent less than in the current academic year. And the total recurrent grant for the universities, paid on the recommendation of the University Grants Committee, will fall from £972 million in the current year to £808 million in 1983-84.

As well as publishing a general statement of what it was about, the committee last week sent individual letters to universities including what is called "advice" about the teaching activities that should be continued (and sometimes strengthened) but also, in many cases, abandoned. Most recommendations of this kind, which only the bravest universities will ignore, concern the arts or social sciences. But some universities have also been "invited" to

abandon teaching their brand of biology.

The University Grants Committee (which has no formal mechanism for dealing with enquiries from the press) is not prepared to say how its decisions about individual universities have been arrived at. It seems, however, to have sought to preserve excellence and minority areas of study and to encourage what is known as "thrift" while maintaining regional balances. Unit costs appear to have been influential in the case of the University of Bath which, while boasting of a diversified programme of studies linked broadly (and sometimes loosely) with industry, also boasts of the lowest costs per student in Great Britain, and has been the most generously treated university of all - its income is cut by merely 7 per cent

It is also known that the committee, in making specific recommendations to the Department of Education and Science for grants to individual universities, took advice about the performance of universities in competition for research grants from the research councils. One vice-chancellor, at least, is glad to think that his university's relative immunity from impending frugality stems from his academics' success in raising more than £3 million a year by way of grants.

Vice-chancellors at the newer technological universities most severely affected by the cuts complain, however, that in its calculations of external research support the grants committee has paid too little attention to research support provided by industrial companies as distinct from research councils. They also

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point, with justifiable surprise, to the committee's formal endorsement (in its letter for general circulation) for courses of study intended to foster closer relationships between students and industry and the presence of at least three former colleges of advanced technology ("universities in waiting" in the early 1960s) among those now worst hit (Salford, Aston in Birmingham and Bradford).

The delivery of the committee's letters to universities comes at an awkward time, with the summer vacation almost everywhere begun. The committee has agreed that aggrieved universities should have a right of "consultation", which will, nevertheless, have to be exercised quickly. The committee of Vice-Chancellors and Principals is hoping within the week to put together a document showing how the pattern of the university system will be changed, but even that calculation will be jeopardized by uncertainty about the recruitment of overseas students (who pay higher fees) in the next few years. Even moderately gloomy forecasts suggest that the total reduction of university income may be as much as 17 per cent when allowance is made for that deprivation.

French universities

New appointments

While Mrs Margaret Thatcher squeezes the British universities, the departure of another lady over the channel has French universities sighing with relief. Madam Saunier-Seîté, Minister of the Universities under President Giscard d'Estaing, set out to centralize power over appointments and the allocation of degrees, and to weaken the role of some of the smaller regional universities. Now, under a gentlemanly new minister of the new government, M. Alain Savary, that is being reversed.

M. Savary says he wants dialogue with the universities, and dialogue he seems bound to get. Within a few days of the election of President Mitterrand, 100 lecturers at the University of Paris signed a declaration condemning the previous minister's "scandalous" methods of making university appointments and demanding a more democratic approach. The two principal education unions, the Syndicat National de l'Enseignement Supérieur and the Syndicat Général de l'Education Nationale, also weighed in with a joint statement warning that conservative and technocratic forces were still in control of the universities, and that they would have to be overthrown.

The chief target seems to be the Conseil Supérieur des Corps Universitaires, which according to its timetable should meet this month to consider this year's new appointments to the few university posts available. The council, strengthened by Saunier-Seîté, interviews candidates and makes its decisions in private, without right of appeal, complain the Paris 100; moreover

its council is said to be predominantly conservative and to give a poor hearing to candidates offering a novel approach to teaching or unfamiliar combinations of subjects. Certainly, the council — as now constituted — is an obstacle to university autonomy, and M. Savary, while not referring to it directly, has said he wishes to restore university autonomy and to set up new decision-making methods which will be "very decentralized".

On another tack, Savary also seems set to restore some of the second and third-level degree courses whose status as such was removed by the previous minister. A partial list of approved courses for 1981-82 was released last week. It was determined almost entirely by assessment procedures set in train the previous year and, conscious of its shortcomings, M. Savary has announced that the universities are free to appeal against the decisions (where a course has been cancelled) or to make new proposals. But he has called for "a sense of self-discipline" among the professors: there is not to be a free-for-all in which every wild proposal will meet approval.

Savary also says that appeals may not last into next year. The device is a stop-gap measure. For the long term, M. Savary plans to enter "without delay" into discussions, with all who are interested, over new mechanisms for the accreditation of courses.

Robert Walgate

US biomedical research

Against the tide

Washington

Democrats in the House of Representatives have been having little success in trying to reverse budget cuts proposed by President Ronald Reagan, but they may gain a rare victory on the issue of support for biomedical research training.

Focus of the dispute is the National Research Service Awards (NRSA) scheme, which provides about 10,000 grants annually to support postgraduate and postdoctoral research workers. The Reagan Administration is proposing that such grants should no longer contain institutional support to cover general overheads at research institutions, which would mean a cut of more than 25 per cent in grant allocation. Medical schools and universities complain that without this support — about \$50 million a year — they will not be able to sustain an adequate base across all areas of research training.

The medical schools won a preliminary round earlier this year, when both houses of Congress rejected the Administration's proposal to drop institutional support provided through the awards scheme as a budget saving for the fiscal year 1981, which began last October.

Less expected was their success in the debate on the 1982 budget in the House. The defection of a number of conservative Democrats to the Senate side resulted in

defeat for proposals submitted by the House leadership, and victory for amendments presented by Republicans.

For example, the House Science and Technology Committee had proposed deleting funds for the construction of the liquid metal fast breeder reactor at Clinch River in Tennessee, transferring much of this money to research in solar energy and conservation. The full House, however, rejected this proposal, restoring the Clinch River funds and severely reducing the solar energy budget.

In biomedical research training, however, the cuts proposed for 1982 brought a stream of protests from the research community. In a letter to Representative John D. Dingell, chairman of the House Energy and Commerce Committee which has responsibility for the budget of the National Institutes of Health, 58 separate medical and research associations warned that the cuts would be "severely harmful".

The lobbying seemed to pay off. Mr Dingell's committee recommended to the full House that the NRSA budget be raised to \$194 million from the proposed \$147.3 million.

The Republican-run Senate, however, has already passed a budget bill containing the lower figure proposed by Mr Reagan. In addition, the Senate suggests an upper limit on biomedical research supported by the National Institutes of Health of \$3.7 million, a move which the medical associations describe in their letter as "arbitrary, unprecedented and unnecessary."

Negotiations now have to take place between the House and the Senate before both sides can agree on a common bill. At the same time, there is a parallel debate going on over the budget for the Department of Health and Human Services which is responsible for the funding of the National Institutes of Health.

In particular a key Senate Committee — Labor and Human Resources — is in deadlock. The committee's previous chairman, Democrat Senator Edward Kennedy, backed by other Democrats and two Republicans, is proposing an additional \$50 million for research training awards. The current chairman, Republican Senator Orrin Hatch, is opposed to the committee taking a public stance in defiance of the President's recommendations; but he has promised that if the committee approves the lower figure, he will intervene to see if it can be raised.

Medical school lobbyists, such as the American Association of Medical Colleges, intend to keep up the pressure to have the funds restored. Dr Lamont-Havers of the Massachusetts General Hospital told a meeting of the American Association for the Advancement of Science that the proposed cuts reflected "a deep bias within the Office of Management and Budget" against the biomedical research training programme.

David Dickson

UK atomic energy

Design delays

British hopes of building a pressurized water reactor (PWR) seem now to rest on Dr Walter Marshall, chairman of the UK Atomic Energy Authority, who was last week appointed by the Department of Energy as chairman of a "task force" intended to stifle squabbles about the project — and to help reduce the cost. This development is a consequence of mounting impatience among Marshall and his colleagues on the four-man "nuclear industry group" with the delays that have accumulated in the PWR programme; they put their complaints in a letter to the Department of Energy three weeks ago.

As yet, the National Nuclear Corporation—the putative constructor—has not completed the reactor design, which should have been sent to the Nuclear Installations Inspectorate in the spring. Like a schoolboy late with his homework,



Marshall the catalyst

the corporation was able to manage by the deadline only a "reference design" that omitted details of the containment vessel and the emergency core cooling system. The missing information has not yet materialized because the corporation (and the Central Electricity Generating Board, which will have to pay for the reactor) have had cold feet about the cost.

Several modifications of the basic Westinghouse design have added to the cost. The generating board has asked for relatively easy access to the steam supply parts of the system to reduce maintenance time and the radiation exposure of workers. The containment building for the reactor was intended to have a double-walled construction, some concrete shielding was intended to be extra thick and there were to have been four (rather than two) emergency core cooling systems.

However, critics of these changes say they substitute concrete and equipment for analysis. Good chemical control of the steam supply system — for example, by purging the steam supply system before

dismantling for refuelling — can reduce radiation doses to levels acceptable to the generating board, say the critics, without using more concrete. (The board has indicated that it will not accept levels higher than those at the Heysham advanced gascooled reactor, a relatively clean power plant.) Some American utilities already use the hydrogen peroxide method in their PWRs. So this week a team of National Nuclear Corporation and generating board scientists has flown out to inspect such "best practice" in American reactors, and make its own measurements of radiation.

Similarly, the net benefit of quadrupling the emergency core cooling system could be obtained more cheaply by looking closely at the failure rate and significance of individual components of the system. The containment building will also probably be simpler, and similar to the optional construction designed by the Bechtel Corporation which is acting as consulting engineers to the National Nuclear Corporation.

What has worried Marshall, and now apparently the Department of Energy, is that the pursuit of perfection at the National Nuclear Corporation has been time-consuming as well as potentially expensive. His task force will function not as a decision-making body but as a forum in which the designers can be shamed into making up their minds.

All of the questions to be tackled relate to safety, and most of them involve trade-offs against cost. Marshall hopes that the outcome will be a reactor with a construction cost (per kW of generating capacity) only 60 per cent of the cost of the advanced gas-cooled reactor. He may have to be satisfied with less. Although some of the refinements of the Westinghouse design may have to be abandoned, Marshall is confident that the reactor can be built within British safety criteria.

The plan now is that the final design should be with the nuclear inspectors in the autumn. With a lot of luck, it may still be possible for the government to hold its promised public inquiry on the project before the end of 1982, almost exactly a decade after the electricity board first designated the site at Sizewell for the project. Marshall's chumminess with the minister at the Department of Energy with special responsibility for nuclear energy, Mr Norman Lamont, will help to simplify the timetable. Robert Walgate

Large Space Telescope

View from Munich

The contract for the European Coordinating Facility for the Large Space Telescope has been awarded by the European Space Agency (ESA) to the European Southern Observatory (ESO) in Garching, near Munich. The other contenders for the prize were the Royal Observatory at Edinburgh, the Institute of

Soviet second sentences

Dr Andrei Sakharov has this week issued two new appeals to scientific colleagues abroad on behalf of fellow human-rights activists.

The first is on behalf of Aleksandr Bolonkin, a mathematician arrested in 1972 on a charge of disseminating false propaganda - in fact, for having circulated the samizdat "Chronicle of Current Events". For this, Mr Bolonkin received a sentence of four years' prison camp and three years' internal exile, while the state Attestation Committee refused to confirm his doctoral degree. After serving his time in camp, and just before the sentence of exile expired, Bolonkin was rearrested and sentenced to a further three years in the camp. Then, a few weeks ago, just ten days before this second sentence was to expire, he was charged again.

Such reconvictions of political prisoners due for release have been frequent in the past two to three years, and many now fear that such a fate may await Dr Sergei Kovalev, whose seven year prison term (on the same charge as Bolonkin) expires next December (to be followed by three years' Siberian exile). Kovalev, whose life is reported to be in danger, is the subject of Sakharov's second appeal, which takes the form of an open letter to Dr Linus Pauling. Included in the appeal are Tanya Osipova, Dr Kovalev's daughter-in-law and a member of the Moscow Helsinki Watch Committee, who was recently sentenced to 5 years labour camp and 5 years exile, and her husband, Ivan Kovalev, against whom it is understood similar charges of subversion are being prepared. Tanya Osipova's plight, says Sakharov, is particularly serious, since she has to serve her sentence in an ordinary criminal camp, whose regular inmates traditionally bully and exploit the political offenders to gain favour with the camp authorities.

Space Astrophysics at Frascati and the Institut d'Astrophysique and the Observatoire de Paris in a joint proposal.

ESO (whose member states are Sweden, Denmark, the Netherlands, Germany, France and Belgium — to be joined by Italy and Switzerland next year) moved last year to the Munich site from Geneva. It manages the observatory at La Silla, Chile, where the main instrument is a 3.6-metre reflector.

There is more than enough room at Garching for the space telescope institute. ESO now operates an imaging processing unit, incorporating a VAX 11/780 computer. Another VAX is to be installed, along with 15 staff members, who will be expected to spend half their time on their own research and half on coordinating the European space telescope observations.

The detailed arrangements for the use of the telescope have yet to be worked out between the US National Aeronautics and Space Administration (NASA) and ESA.

ESA's decision has ruffled several British feathers. The Edinburgh proposal was based on the Starlink image processing system, which also uses the VAX computer and links six British universities. A confidential report by an ESA subcommittee, set up to evaluate the proposals, is said to have highlighted the scientific merits of the British scheme but put it in second place because the location was "far off from most member countries". Problems with pay differentials and apparent weaknesses in management and archiving were also mentioned, but these objections are dismissed by some British astronomers, who consider that a system based on Starlink would provide the best facility for European astronomers. Such a system could, however, be developed with advantage at ESO. Philip Campbell

Curien on top

The council of the European Space Agency (ESA) has unanimously elected Professor Hubert Curien, president of the Centre National d'Etudes Spatiale, the French space agency, to be its next chairman. In a break with tradition, the council has also elected two vice-chairmen Dr Harry Atkinson, a British delegate to ESA, and Dr H. Grage, a Danish delegate. The hope is that the three new appointees, who represent separate national interests, will between them steer the agency onto a truly European course as it negotiates its future for the next ten years.

Despite the unanimous vote, however, Professor Curien's election was not without dissent, some delegates fearing that it might give too much weight to French arguments for the further development of the Ariane launcher. Britain had argued that it was time for a British chairman. the last one having been Sir Harrie Massey who chaired the European Space Research Organisation, ESA's predecessor, in the early 1970s. But other countries feared that the possible British candidates would be too partisan. An attempt by Italy and Switzerland to bring John Adams, ex-joint-director of CERN, the European centre for high-energy physics, into the competition failed on the grounds that he is not a delegate to the ESA council. John Adams had declined to apply for the post of ESA's directorgeneral when it became vacant last year.

In the event, the compromise has been to elect the British and Danish vice-chairmen to work with Professor Curien. With the current uncertainty over ESA's future programme, they are bound to play a more vital role than ESA chairmen in the recent past.

Judy Redfearn

US space research

Halley again?

Washington

A faint glimmer of hope that there may, after all, be a mission to Halley's comet filtered from the House of Representatives last week. The House has voted to include \$5 million in the budget to keep the project alive during 1982. But this is less than the \$25 million which scientists at Jet Propulsion Laboratory (JPL) of the National Aeronautics and Space Administration (NASA) say is necessary in the 1982 budget for the first stage of a \$350 million project.

The Republican-dominated Senate in passing a parallel bill last month did not include money for a Halley mission because it had not been requested by the Administration. Even if the proposed mission survives the compromise bill which the two legislative bodies must now negotiate, it still has to go through the appropriations process in which budgets rather than programmes are agreed.

Scientists at JPL are hoping to convince President Ronald Reagan that not mounting the mission would be a serious blow to national prestige, given that the European Space Agency (ESA), Japan and the Soviet Union (in partnership with Comecon countries and France) are preparing their own plans.

Dr Ray Heacock, JPL's choice as project manager for the Halley mission, said last week that the \$5 million would be sufficient to fund the project for the first three months of the next fiscal year. After that the President, if the mission is approved during negotiations on the 1983 budget for the agency, could direct NASA to reprogramme some of its 1982 funds.

The proposal has some strong supporters, particularly among those who feel that space science activities in NASA have been unfairly squeezed by the agency's preoccupation with the space shuttle.

The original plans have also been scaled down considerably. NASA had initially talked of a spacecraft which would travel alongside the comet on its way to a rendezvous with the smaller Tempel 2 comet. The latest plans are for a more modest mission using reserve equipment from previous planned missions to launch a spacecraft through the comet's tail within 600 to 1,000 kilometres of the nucleus.

There remains hope, however, that NASA may at least be able to resurrect its full participation in the International Solar Polar Mission, originally planned to fly two spacecraft in complementary orbits over the poles of the Sun. The agency's decision, at the prompting of the Office of Management and Budget, to eliminate funding for one of the spacecraft generated a storm of protest from European allies which are building the other.

The House authorization bill passed last week added \$15 million over the Admini-

stration's request to allow NASA to continue construction of its spacecraft. This decision is likely to be supported in negotiations with Senate counterparts, where exastronaut Jack Schmitt is responsible for overseeing NASA programmes. The recommendations from the Appropriations Committee would also permit the project to continue, and given the importance which top State Department officials have attached to maintaining international commitments, it seems unlikely that the Senate Appropriations Committee, which meets to discuss NASA's budget next week, will object.

At the same time, NASA is unlikely to accept ESA's offer to build the second spacecraft, made during the negotiations to salvage the mission. Representative Don Fuqua, chairman of the House Science and Technology Committee, said that he was opposed to this proposal, because it was unfair to expose European contractors to the vagaries of US policy, and because even though the price-tag would be lower. spending the money in Europe would still result in a loss of US jobs and profits. TRW, the company selected by NASA as contractor for its own spacecraft, is now said to have found ways of reducing its costs considerably, a move likely to increase the mission's chances of survival

David Dickson

Trypanosomiasis

Question of breeding

Schemes for breeding cattle resistant to trypanosomiasis are to be hatched at a research institute being planned in the Gambia (West Africa) with support from international aid agencies and foundations. The objective is to throw light on why N'Dama cattle in Africa appear to be genetically more resistant to infection by trypanosomes (also the infectious agent of African sleeping sickness) than are the more common Zebu cattle, and to find ways of propagating this resistance.

Much of the enthusiasm for the new institute comes from the President of the Gambia, Sir Dawda Kairaba Jawara, who was trained as a veterinary surgeon in Glasgow in the early 1950s, and who became leader of the People's Progressive Party in 1960. A preliminary meeting was held at Bellagio last year, and a meeting in the Gambia in May this year worked out a timetable on which further decisions must be made in time for a final decision about the project by January 1982.

Among international research projects, the Gambian research centre is unusual in that the African Development Bank seems to be prepared to take the lead in providing funds. Other interested parties include the European Community and the British government, the Food and Agriculture Organization and the World Health Organization of the United Nations, and research institutes in the World Bank

network, including the International Laboratory for Research in Animal Diseases in Nairobi. A technical appraisal of the project is being carried out this month in the Gambia, and should be completed in October.

The mechanism of trypanotolerance in N'Dama cattle is far from clear. The capacity for tolerance obviously has a genetic basis, but the older cattle are the more free from trypanosomes in the blood, suggesting that tolerance is acquired in response to trypanosomal antigens — and that even N'Dama cattle might benefit from multiple vaccines. Part of the intended research programme will be to look for genetic markers for the easy identification of resistant cattle. The long-term objective is to prepare the ground for setting up breeding centres for the propagation of potentially resistant cattle.

The initial costs of the centre are estimated at \$3.5 million, with annual running costs of \$1.5 million.

Bulgarian wildlife

Shooting for keeps

Plovdiv

Bulgaria, which this year is celebrating its thirteen hundredth anniversary of statehood, has for a number of years been committed to an ecological policy aimed at restoring its range of wildlife to the level indigenous in the country a millenium ago. During the past few years, extensive herds of roe and red deer have been built up, moufflons have been reintroduced and a small herd of bison has been established based on stock remaining from a former royal park. And now, during the past month, a second herd has been formed in the national game and forestry park at Preslav; fourteen animals, including four pregnant cows, were transferred from the existing reservation and will eventually be joined by animals from Poland and the Byelorussian SSR.

This faunal re-establishment policy is part of an overall reafforestation drive. During the long Turkish occupation, which ended just over a century ago, a large proportion of Bulgaria's primaeval forests were destroyed. But shortly after the establishment of the Bulgarian People's Republic in 1944, a major tree-planting programme was started and considerable attention given to conservation. During the Second World War, Bulgarian game herds multiplied virtually unchecked, so that stocks became sufficient for the new regime to incorporate into its forestry policy a role for the human gun-bearing predator.

According to a report presented last month at the Plovdiv symposium on "Wildlife and the Environment" (part of the "Expo-81" World Hunting Exhibition), this policy is working well. Although every adult Bulgarian citizen is entitled to join the Hunting and Fishing

Union, thereby acquiring the right to hunt game, stocks are flourishing. The latest census gives 14,000 red deer (which have spread to almost all suitable habitats throughout the country), some 117,000 roe deer, which have moved permanently into arable areas and established a field ecotype, some 30,000 wild boar, 2,700 fallow deer, 2,000 moufflons, 1,500 chamois and 700 bears.

However, some small game species have suffered, apparently because of changing ecological conditions. The hare population, in particular, suffered a considerable decline in the late 1970s. The Hunting and Fishing Union, however, implemented a strict conservation plan—which included a total ban on hunting for two years—and the latest figures show a considerable increase.

Managing forests as a combined hunting and conservation resource does not seem to produce major conflicts of interest in Bulgaria. The various hunting clubs affiliated to the Hunting and Fishing Union are assigned specific tracts of the state forests, and are expected to assume many of the traditional duties of the gamekeeper - including the distribution of fodder in hard winters. No paid keepers are employed - the club members perform such tasks on a voluntary rota basis, which gives them a good insight into the problems of forest management. (Foreign tourist hunters, of course, are provided with the normal complement of beaters and ghillies at the cost of some £500 per week.)

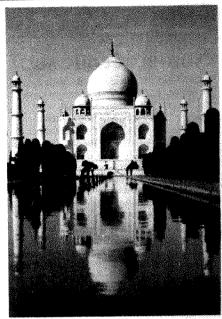
Nevertheless, Bulgaria has not been free from ecological mistakes in recent years, and this makes experts wary of introducing totally new species. Some years ago, for example, it was proposed to introduce American trout alongside the native Bulgarian variety, with which, it was confidently predicted, they would not compete. In the event, however, the newcomers attacked and virtually wiped out the local trout. Nevertheless, Bulgarian fisheries experts are prepared to consider innovations, and have reported some success with the bester - a non-migratory hybrid of beluga (white sturgeon) and sterlet, first produced in the Soviet Union about ten years ago.

India's environment

First steps

New Delhi

To protect the world-famous Taj Mahal, the Indian government is to close two coal-fired power stations in Agra, about 85 kilometres from the capital, New Delhi. This is one of the first concrete actions of India's new Department of Environment, aimed at saving the seventeenth century white-marbled monument from being blackened by smoke billowing out from the power stations. The new department has also persuaded the railway authorities to switch from coal to diesel power in their Agra workshops.



Agra's penance

These actions seem to be only a beginning for India's environment programme. According to the Ministry of Agriculture, some 175 million of the country's total 304 million hectares of land are subject to environmental problems. These include serious water and wind erosion over 150 million hectares, and shifting cultivation, waterlogging, saline and alkaline soils in the remaining area.

India is annually losing more than 6,000 million tons of topsoil through water erosion, and the total area subject to periodic floods now stands at 40 million hectares, an increase of 100 per cent in the past 10 years. Soil erosion is causing premature silting-up of tanks and reservoirs in which India has invested a massive \$12,500 million.

Another major problem is the large-scale deforestation in the Himalayas and other hilly areas of the country. And the 19 national parks and 202 wildlife sanctuaries covering more than 2.3 per cent of the geographical area of India are inadequate to protect the many endangered habitats and threatened species, especially as most of the sanctuaries suffer from a lack of any scientific, or any other kind of effective management.

One of Indira Gandhi's first concerns when returned as Prime Minister after the election of January 1980 was to stem the degradation of the environment, and this led to the setting up of a fully-fledged government department to tackle the problems. The government is expected to announce far-reaching recommendations shortly, but for the time being there is a campaign to create awareness among the general public on environmental matters. This summer, for the first time, "environmental camps" are being held all over the country to involve young people in tree planting schemes, and to put a similar message over to the inhabitants of the rural areas where the camps will be based.

Sunil Saraf

CORRESPONDENCE

Non-academic staff

SIR — I was disturbed by your swipe at the "ancillary staffs" in your comment on cuts in the budget for London University (Nature 11 June, p.442). The planned reductions in staff, in such a short space of time, show little concern for the careers and livelihoods of individuals, but are also uneconomic. The average cost to the nation of each unemployed person is estimated at £5,000 per year in lost taxes and insurance contributions and unemployment benefit paid out. This excludes the loss of purchasing power, lost VAT income and lost productivity.

You wonder whether the universities' chief purpose is academic — but the universities create, directly and indirectly, wealth. Foreign students bring money into the country — this may now be lost to Canada, the United States and New Zealand. With that go many exports, both "visible" and "invisible" (such as the "influence" which the Foreign Office has always laid great store by).

The efficiency of universities is obviously affected by lack of equipment — and also by lack of staff. Academics in general earn more than the other staffs. If non-academic staffs are reduced in preference to academics, the tasks now performed by academics will become more expensive. Their teaching and/or research time will be eaten into.

Britain spends less on pre-school education per head than any of her competitors. Since the 1972 White Paper on education, the staff-student ratio in universities has increased by 10 per cent. During a recession one should be prepared for the recovery, otherwise recession will soon return. The cutbacks in education (and other areas) may permanently damage competitiveness.

M. HOOPER (University technician)

London N15, UK

The Tamuz raid

SIR — I do not object to the condemnation of Israel in your issue of 18 June ("Making Israel atone for Tamuz" p.523). On the contrary, I believe that articles such as these serve a useful purpose in that they strengthen and unify the world Jewish community. Moreover, condemnations are always preferable to condolences. It is remarkable, however, that a scientific journal such as *Nature* should tarnish its image by publishing an unscientific report that is replete with conjecture, ill-conceived logic, half truths and distortions.

To the question "Why should oil-rich Iraq be interested in nuclear power?", the scientific answer given by Nature is "Why not?". A long apologetic discourse follows rationalizing the presumed logic of creating civil nuclear technology in Iraq. How foolish! The Iraqi spokesmen themselves never bothered to contrive such a rationale for their reactor. President Hussein makes no bones about it — he wants bombs. He calls upon the world to "help Arabs acquire nuclear weapons — which are essential for world peace"; he also states that it is a "rational move for Arabs to try to acquire a bomb" — New York Times, 24 June, p.1.

The tears shed about the damage done to the "international reputation of the nuclear non-proliferation treaty" also amuse me. How

effective are the inspections? In the case of Iraq these were conducted on a limited basis, only by representatives of Communist bloc nations. Congressional testimony by Roger Richter, former American inspector of the IAEA (19 June 1981), shows how Iraq would have easily fooled reactor inspectors. Indeed, Iraq and some other nations are signatories of the treaty, but are we talking about responsible democratic societies? How much would it take for ruthless dictators, that suppress and murder political dissidents in their own countries, to abrogate an agreement? Perhaps it is time for the current ineffectual treaty to be replaced by a more meaningful one. We should all devote our efforts to promoting effective policies that would dictate limitation to the world arms race, and stop nuclear proliferation, rather than pontificate and lecture Israel about morality.

For the sake of future generations, the scientific community has the responsibility to do everything in its power to curb the real culprits in this case; the corrupt Western powers who are prepared to place nuclear energy at the disposal of madmen. In their hearts decent people thank the heroic Israelis for undertaking this necessary mission. Nuclear power must not be handed to demented killers.

IRVING LISTOWSKY Albert Einstein College of Medicine, New York, USA

Sir — Your editorial of 18 June (p.530) demonstrates clearly that *Nature* doesn't have offices in Israel. I take issue with your naive call for atonement on Israel's part for destroying Tamuz. Nations do not atone for legitimate acts of self-defence especially those committed during a declared war.

Egypt and Israel are at peace. The Egyptian nuclear programme is obviously needed for power production. For Iraq, peace with Israel is available for the asking. *Nature* should note that Iraq has been hostile to Israel since before Israel's independence. Iraq has not atoned for the mistreatment and expulsion of Iraqi citizens who happened to be Jewish.

It seems that you think that Israel should place its trust in the United Nations or the IAEA or the Big Powers or some other institution. Why? Did the UN stop the blockade of the straits of Tiran, did the United Kingdom and France obtain passage for Israeli shipping through the Suez Canal and when will Europe stop acquiescing in the Arab economic boycott of Israel? The credentials of world institutions do not call for trust.

Your editorial fails to mention the prospects for peace in the Middle East which were increased tremendously by the Israeli strike. It demonstrates that the cost of enmity to Israel is much higher than her enemies previously thought.

I realize that the cost to *Nature* of publishing a pro-Israeli editorial may be prohibitive in terms of lost advertising revenue and retaliatory terrorism but your respected journal should not dirty its image by ignoring history and simple logic to defend an attempt to destroy a nation.

PAUL ROTHBERG

State University of New York, Stony Brook, New York, USA

No badger vaccine

SIR — M.J. Chapman (Nature 28 May, p.278) commented on various aspects of the Zuckerman report on badgers, cattle and tuberculosis. It would be inappropriate for me to deal with the medical aspects to which Mr Chapman refers other than to note that my reading of these parts of the report differs from his.

I should, however, like to comment on the question of badger vaccination. The only vaccine for tuberculosis currently in use is made from a modified (attenuated) strain of Mycobacterium bovis and is used only in humans. In trials, the vaccine has given unpredictable results and is of no value at all as a treatment for infected cases. It has been tried in cattle and found to be neither effective nor practical. Its effect on badgers, whether healthy or tuberculous, is not known.

The mass vaccination of badgers seems to me, however, to be unpromising. While BCG vaccine can be given orally, very large doses are required compared with the amount given by intradermal injection. Oral application on the scale required seems quite impracticable. Furthermore, to be effective any vaccination of badgers would in all probability have to be carried out before they had been exposed to infection. Recent work has found that 13 out of 49 young cubs removed from setts in one of the problem areas were already infected. This suggests that vaccination would need to be done soon after birth which is, unfortunately, impracticable in the wild.

Mr Chapman asks for consideration to be given to the views of "many zoologists" who doubt the basis of ministry policy. I can assure him that we are in contact with many zoologists, both directly and through the minister's consultative panel, and that the ministry's own zoologists are closely involved in the work.

It is not my impression that there is a body of informed zoologists who differ fundamentally with the policy that we are following. In this connection I refer Mr Chapman to the statement issued earlier this year by the Nature Conservancy Council in which they endorse Lord Zuckerman's advice.

W.H.G. REES

Ministry of Agriculture, Fisheries and Food, Tolworth, UK

Elementary error

SIR — In Nature 16 April, p.538, in an article by Jasper Becker about accidents and problems at the French reprocessing plant at Cap de la Hague, mention is made of the hypothetical release of caesium-137 and rubidium-106 (if the cooling equipment for the radioactive waste storage reservoirs at La Hague had remained inoperative for ten hours).

Is the isotope number of rubidium perhaps in error, or the element? I do not find rubidium-106 listed in the CRC Handbook of Chemistry and Physics, 1980-81 edition.

KAY DREY

University City,
Missouri, USA
The (hypothetical) offending element is ruthenium-106 — Editor

NEWS AND VIEWS

A cycling index for ecosystems

fom Robert M. May

I have heard it said that the River Thames passes through two people on its way to the sea. While I am sure this is an extravagant exaggeration, such recycling is typical of the flow of nutrients in many ecosystems.

Finn¹⁻⁴ and others have presented a formal scheme for characterizing the patterns of flow of energy or nutrients through a complex ecosystem. In particular, Finn1 has shown how to compute a 'cycling index', which gives a quantitative measure of the extent to which flows recirculate within the system. This cycling index is the fraction of the total flow through the system that derives from cycling, expressed as a ratio to the fraction of the total that derives from flow straight through the system. Thus defined, the cycling index can clearly range from zero (no recycling anywhere in the system) to an arbitrarily large number (recycling overwhelming direct flow).

As derived by Finn and co-workers, these concepts apply to conservative systems at equilibrium, such that the books balance for overall inflows versus overall outflows. Finn observes, however, that the methodology may be extended to nonsteady systems. The actual meaning of the 'compartments' that make up the system can vary greatly (trophic levels, species, individuals); although this certainly bears on the interpretation of results, it makes essentially no difference to the formalism.

Finn has analysed the cycling of energy in several ecosystems^{1,2}. One example is part of a marine ecosystem (exhibiting marine coprophagy) and has four compartments5,6: the shrimp Callianassa major, the faeces of C. major and associated bacteria, coprophagous benthic fauna, and the faeces of the benthic fauna and associated bacteria. Here the cycling index, deriving from recycling between the third and fourth compartments, is 10.24. The second example describes energy flow in the Cone Spring ecosystem⁷, and has components for primary producers, detritus, bacteria associated with the detritus, detritus feeders and carnivores. Here the cycling index is 10.16. The third example draws on the extensive studies of the Hubbard Brook ecosystem8,9. Here there are essentially no energy loops, whence2 the cycling index is

Insofar as one can generalize from these three examples, it seems that energy can be recycled to a small but significant extent. Such recycling of energy is not in conflict with thermodynamic principles (energy, of course, cannot be degraded more than once), but arises because living systems can 'use' energy without degrading it. This is the case, for example, with the energy associated with structural or storage elements (such as cellulose, proteins or fats), which can either be catabolized or be used again as structural elements on transfer to the next compartment.

Finn² has also analysed the model of Likens and co-workers8 for the Hubbard Brook ecosystem, to estimate the patterns whereby various nutrients flow from the above-ground biomass through the belowground biomass and the forest floor to the mineral soil and the available nutrient pools. The cycling indices suggest significant differences in the degree of recycling, with the elements K, Na, N, Ca, P. Mg and S having indices around 0.83, 0.76, 0.76, 0.62, 0.60, 0.59 and 0.51, respectively. Finn² comments that "this order does not appear to relate to whether or not the system is gaining or losing a particular nutrient. Hubbard Brook is accumulating N, S, and P and losing Ca, Na, Mg, and K". Notice that the cations (K, Na, Ca, Mg) rank in strict order of atomic size and charge, which determines mobility (the so-called 'lyotropic series'). Of these seven elements, N and P are probably limiting, K, Ca, Mg and S are essential but nonlimiting, while Na is neither essential nor limiting (for plants). It is therefore a bit surprising that Na is recycled to such an extent at Hubbard Brook: Finn¹⁰ has shown that Na cycling indices can be very small, in other forest systems.

For Edmisten's11 model of a tropical rain forest, Finn1 finds the cycling index for N to be 1.78. This value, more than

double the 0.76 for N in the temperate Hubbard Brook forest, accords with the conventional wisdom about greater recycling of nutrients in tropical forests.

It is tempting to use Finn's cycling index in pursuit of other ecological generalities. We may, for example, expect to find that the cycling index typically increases as succession advances. Conversely, given that many kinds of pollution and other maninduced disturbances of mature ecosystems have effects akin to precipitating the system back into early successional patterns12, it may be that such perturbations are characterized by decreases in cycling indices. These are interesting speculations. They may even be taken to suggest that the cycling index could be a useful diagnostic in the design of environmental impact statements.

As Finn² himself has emphasized, however, "Flow measures do not take into account residence times or storages, but only the amounts of nutrients actually moving and the way in which they move. . . . all the different processes involved in nutrient cycling cannot be captured and quantified in a single index". The cycling index is an elegant construct, having both theoretical and practical significance; but when it comes to evaluating the impact of disturbances upon ecosystems, I would be wary of attempting to distill complex arrays of information about flow patterns into any such single number 13 .

^{1.} Finn, J.T. J. theor. Biol. 56, 363 (1976).

Finn, J.T. Ecology 61, 562 (1980).
 Hannon, B. J. theor. Biol. 41, 535 (1973).
 Patten, B.C., Bosserman, R.W., Finn, J.T. & Cale, W.G. in Systems Analysis and Simulation in Ecology (ed. Patten, B.C.) 457 (Academic, New York, 1976).

^{5.} Haven, D.S. & Morales-Alamo, R. Limnol. Oceanogr. 11, 487 (1966)

^{6.} Frankenberg, D. & Smith, K.L. Limnol. Oceanogr. 12, 443

 ^{(1967).} Tilley, L.T. Ecol. Monogr. 38, 169 (1968).
 Likens, G.E., Bormann, F.H., Pierce, R.S., Eaton, J.S. & Johnson, N.M. Biogeochemistry of a Forested Ecosystem (Springer, Berlin, 1977).

Whittaker, R.H., Likens, G.E., Bormann, F.H., Eaton, J.S. & Siccama, T.G. Ecology 60, 203 (1979).

Finn, J.T. thesis, Univ. Georgia (1977).
 Edmisten, J. in A Tropical Rain Forest (eds Odum, H.T. & Pigeon, R.F.) H211 (US Atomic Energy Commission, 1970).

^{12.} May, R.M. in Theoretical Ecology: Principles and Applications 2nd edn, Ch.9 (Blackwell, Oxford, 1981). 13. May, R.M. Nature, News & Views 258, 285 (1975).

Robert M. May is Class of 1877 Professor of. Zoology at Princeton University.

Plasma lipoproteins and cellular metabolism

from J. S. Owen

PLASMA LIPOPROTEINS are macromolecular complexes in which lipids are bound to a variety of polypeptides (the apoproteins) through non-covalent forces. Four main classes of lipoprotein are recognized, each defined by the density at which it floats in the ultracentrifuge chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Although these classes vary widely in size, and in lipid and apoprotein composition, they appear to have a similar general structure: they are spherical particles with a hydrophobic core of cholesteryl ester and triglyceride surrounded by a 2 nm-thick monolayer of apoprotein, cholesterol and phospholipid

Chylomicrons transport triglyceride and other fat-soluble substances from the intestine while VLDL transports endogenous triglyceride from the liver. The triglyceride contained in these particles is delivered to extrahepatic tissues, thereby providing cells with an important energy source. Although almost all cells can synthesize cholesterol, several clinical and biochemical studies suggest that most cellular cholesterol is derived from uptake and degradation of LDL. By contrast, one function of HDL may be to transport cholesterol from peripheral tissues to its principal site of catabolism and excretion in the liver.

These lipid transport processes are complex; they depend on an interchange of constituent lipids and apoproteins amongst the various lipoproteins, whether by exchange collision or by specific transfer proteins, and the activities of lipoprotein lipase, hepatic lipase and lecithincholesterol acyltransferase (LCAT). Other aspects of intravascular lipoprotein metabolism being investigated include the relationship between the various HDL subclasses and the mechanism by which specific HDL particles remove cellular cholesterol (Tall and Small Adv. Lipid Res. 17; 1, 1980), the origin of the lipoproteins producing cholesteryl ester deposition in the macrophage-like cells of the arterial wall (Goldstein et al. J. biol. Chem. 255; 1839, 1980, and Schechter et al. J. Lipid Res. 22; 63, 1981), and the involvement of the liver in LDL and HDL clearance (Pittman et al. Proc. natn. Acad. Sci. U.S.A. 76; 5345, 1979). In contrast, one aspect of lipoprotein metabolism has been relatively neglected: the influence of lipoproteins on cellular function by their regulation of selected cellular biochemical reactions and by their role in maintaining a normal cell membrane lipid composition.

The interaction of circulating hormones and certain non-hormonal molecules with

specific cell-surface membrane receptors is an important method of regulating cellular metabolism. Plasma lipoproteins also appear to possess this regulatory capacity. The elegant work of J. Goldstein and M. Brown at the University of Texas, Dallas has established that cultured cells have high-affinity receptor sites which recognize the apoprotein moiety (ApoB) of LDL. Subsequent studies, principally by R Mahley and colleagues at the National Heart and Blood Institute, Bethesda, have shown that ApoE-containing lipoproteins, including both HDL, (the HDL sub-class through which cholesterol may be delivered to the liver) and chylomicron remnants (the particles remaining after partial hydrolysis of core triglyceride), also bind to the same cell-surface receptor. After binding, these various lipoproteins regulate intracellular cholesterol metabolism and receptor activity, processes dependent on cellular uptake and degradation of lipoproteins.

Current evidence suggests that cells may also have additional lipoprotein receptors, specific for the apoprotein moieties and distinct from the classical 'LDL receptor'. and that lipoprotein occupancy of such receptors is sufficient to modulate selected cellular functions without requiring internalization of the intact particles. These include the activity of membranebound enzymes such as adipocyte adenylate cyclase and erythrocyte Mg2+-ATPase which are stimulated by VLDL and LDL, the enhancement of lipogenesis in fat cells by VLDL and the apparent LDL-induced reduction of prostacyclin production in endothelial cells. However, the type of regulation most extensively studied has been the potent inhibition of mitogen-induced lymphocyte proliferation by the ApoB- and ApoE-containing lipoproteins (Hui et al. J. biol. Chem. 255; 11755, 1980). J. Harmony and associates at Indiana University have shown that several primary mitogen-induced membrane events in lymphocyte transformation, such as calcium ion and cyclic GMP accumulation and phosphatidylinositol turnover, are markedly suppressed by membranereceptor binding of these apoproteins.

The membrane lipid composition of cells may be influenced by lipoprotein lipid composition as a consequence of the ready exchange between lipoprotein cholesterol and phospholipid (mainly phosphatidylcholine and sphingomyelin) and the corresponding lipids in cellular plasma membranes. Although cell membrane

transport, receptor and enzymatic functions are primarily determined by the protein constituents, there is considerable evidence that such membrane protein functions may be influenced by the properties of the fluid lipid bilayer matrix. A shift in the dynamic equilibrium between membrane and lipoprotein lipids, through abnormalities in lipoprotein lipid pattern, would therefore be expected to lead to changes in membrane lipid composition and so indirectly to membrane and cellular dysfunction. For example, the ratio of cholesterol to phospholipid is an important determinant of membrane fluidity, many membrane-bound enzymes depend on specific phospholipids for full activity, and phospholipid fatty acid composition may be a critical factor in secondary modulation of fluidity and in synthesis of prostaglandins.

Is there experimental evidence that this potential influence of plasma lipoproteins on cellular metabolism, through their effects on either membrane lipid composition or regulation of selected biochemical reactions, is physiologically important? Support has largely been obtained by study of those dyslipoproteinaemias, associated with either genetic, pathological, hormonal or dietary disturbances, in which substantial changes in apoprotein and lipid patterns have occurred. In this context, reports by N. McIntyre and his group at the Royal Free Hospital, London on the cellular abnormalities associated with the acquired LCAT deficiency of liver disease are of interest. Lipid changes were found in erythrocytes and platelets and the abonormalities correlated with cellular dysfunction (Owen et al. J. Lipid Res. 22: 423, 1981); there was also evidence in patients with liver disease that their abnormal HDL, enriched in ApoE, could effect cellular regulation by reducing LDL uptake, by altering erythrocyte morphology and by suppressing lymphocyte proliferation. However, the majority of cells studied in such cases of abnormal lipoprotein composition have been isolated from the lipoprotein-rich environment of the plasma compartment and clearly there is a need to extend these observations to cells which have access only to the lipoproteins of the extravascular fluid. That such investigations will be fruitful is suggested by the interactions of abnormal lipoproteins with cultured cells and by the poorly understood metabolic and cellular disturbances frequently associated with those dyslipoproteinaemias, such as primary and secondary LCAT deficiency and abetalipoproteinaemia, in which marked changes in lipid and apoprotein composition occur.

J. S. Owen is in the Academic Department of Medicine, The Royal Free Hospital, London.

MST radars: advanced tools for gravity wave studies

from Jürgen Klostermeyer

In 1974, Woodman and Guillén1 published observations of winds, gravity waves and turbulence in the neutral atmosphere obtained by the powerful Jicamarca radar in Peru. The radar operates in the very high frequency (VHF) band at a frequency of 50 MHz (wavelength = 6 m) and was originally built for investigations in ionospheric heights. Their work stimulated the construction of several VHF Doppler radars which were specifically designed for studies in the mesosphere, stratosphere and troposphere²⁻⁴ (MST radars). Their ability to measure simultaneously height profiles of background winds, atmospheric gravity waves and turbulence makes them unique instruments for studying not only each process separately but also their nonlinear interactions.

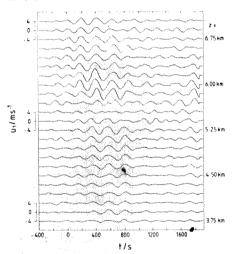


Fig. 1. Line-of-sight velocity oscillations at heights between 3.75 and 7.05 km produced by a Kelvin — Helmholtz instability during a jet stream passage. Sliding scales on the left indicate velocity amplitudes up to 3 m s⁻¹. Parameters on the right denote the heights at which the velocities were measured. The measurements were made by the German SOUSY MST radar with height and time resolutions of 150 m and 10 s. (From Klostermeyer and Rüster¹⁴.)

The radar echoes are produced by both scattering from refractive index structures with scales equal to half the radar wavelength and partial reflections from refractive index gradients. Refractive index variations are due to changes in temperature, pressure, humidity and concentration of free electrons. Humidity changes are most important in the troposphere whereas free electrons play a major part only during daylight hours at mesospheric heights above some 50 km. In the troposphere and stratosphere, the radar echo power decreases strongly with height due to the exponential decrease of

the air density whereas in the mesosphere, the free electrons give rise to a small local power maximum. The small radar scattering cross-section of the turbulent fluctuations requires extremely sensitive radars with operating frequencies preferably below 50 MHz⁵. However, depending on daytime and mode of radar operation, there is always a more or less extended height range around the stratopause refusing the onslaughts of even the most potent MST radars.

MST radars are phase coherent so that transmitted and received signals can be compared to get both amplitude and phase of the signals returned from any height. The usual procedure is to find the echo power density as a function of the Doppler shift from the transmitter frequency. This Doppler spectrum contains the necessary information about the intensity of turbulent refractive index fluctuations at scales equal to half the radar wavelength, the mean velocity of the scattering volume in the direction of the antenna beam (lineof-sight velocity) and the distribution of random velocities within the scattering volume1. The total velocity vector can be obtained by changing the direction of the antenna beam. The temporal and spatial resolutions depend primarily on the signalto-noise ratio of the radar echoes, optimum resolutions are a few seconds and a few tens of metreo6.

Due to their excellent range and time resolutions, MST radars offer unique opportunities for studying the role of gravity waves in various atmospheric processes. Radar records show that gravity waves with periods between a few minutes and several tens of minutes are generally present at all heights below 100 km. At Jicamarca, several experiments using two or three antenna pointing directions were performed to measure both wave periods and horizontal and vertical phase velocities⁷⁻⁹. The results show dominant periods of between 5 and 10 min, the range of the Väisälä-Brunt resonance period in the atmosphere below 100 km. The majority of the waves propagates horizontally indicating that there is no vertical energy transport. This suggests wave sources within the observed height range rather than sources far below or above it.

A few radar experiments indicate that there are height levels at which the horizontal phase speed of observed gravity waves matches the wind speed. A wave encountering such a 'critical' level

Jürgen Klostermeyer is at the Max-Planck-Institut für Aeronomie, Germany. transfers momentum and energy to the wind or vice versa depending on the strength of the vertical wind shear. In the presence of strong wind shears, a gravity wave reflected from a critical level may be stronger than the incident one. Within the

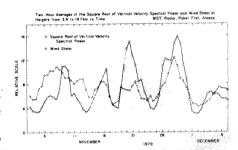


Fig. 2. Time variation of square root of gravity wave spectral power and vertical wind shear. The gravity wave spectrum was obtained by the Poker Flat MST radar in Alaska with height and time resolutions of 2.25 km and 1.5 min in the height range from 4 to 20 km. The wind shear data were derived from radiosonde ascents at Fiarbanks, Alaska. (From Ecklund et al. 15.)

limits of available information, this process of 'over-reflection' could be observed by the Jicamarca radar 10. Another process closely related to overreflection is the Kelvin-Helmholtz (K-H) instability: in regions of strong wind shear, small perturbations in the meteorological noise may grow exponentially with time and rise far above the initial noise level. The horizontal phase velocity of the resulting wave matches the wind speed somewhere within the shear region so that there is again a critical level providing energy transfer from the wind to the wave. In the upper troposphere, polar front jet streams have strong shears and thus act as gravity wave sources via the K-H mechanism11-13. Oscillations of the line-ofsight velocity at several heights which were generated by the K-H mechanism at the bottom side of a jet stream are shown in Fig 1. The amplitude varies strongly with height and has two maxima near 5 and 6 km, whereas the phase is almost constant in the lower and upper regions. The critical level is at 5.55 km and is characterized by a marked amplitude minimum and a rapid phase change with height.

Gravity waves have horizontal wavelengths from a few kilometres up to a few 100 km and thus cannot be resolved on the grid scales of forecasting and general circulation models. Since they play an essential part in the redistribution of momentum and energy from large-scale to small-scale atmospheric motions, proper methods have to be developed to parameterize their effects in terms of large-

scale motions. This task requires, among other things, research on the synoptic variability of gravity waves. An important step in this direction has recently been taken by Ecklund et al. 15 who used part of the yet unfinished Poker Flat MST radar in Alaska to demonstrate that gravity wave activity is strongly controlled by propagating planetary waves. Whenever intense baroclinic zones moved across the radar site, the power of the gravity wave spectrum at tropospheric heights increased significantly. Intense baroclinic zones in turn are associated with strong winds and wind shears pointing towards K-H instability as a major source mechanism. Figure 2 shows the variation of the square root of the gravity wave spectral power along with the mean vertical wind shear for a period of 34 days indicating a good correlation between both quantities. This strongly suggests that K-H instabilities affect the life expectation of synoptic disturbances by continuously borrowing from their energy and momentum budgets.

Future applications of MST radars will

be directed towards more intensive studies of sources and sinks of gravity waves and their interaction with large-scale and smallscale atmospheric motions. Preliminary investigations during thunderstorms indicate, for example, that fast-rising air parcels in cumulus clouds can penetrate through the tropopause into the convectively stable stratosphere, thereby exciting a broad spectrum of gravity waves¹³. At heights below 100 km, genesis of turbulence is probably the most important wave sink besides absorption at critical levels. Radar observations show that strong bursts of turbulence arise

1. Woodman, R.F. & Guillén, A. J. atmos. Sci. 31, 493

- 2. Green, J.L., Warnock, J.M., Winkler, R.H. & VanZandt,
- Green, J.L., warnock, J.M., winkier, K.H. & vanzangi, T.E. Geophys. Res. Lett. 2, 19 (1975).
 Röitger, J., Klostermeyer, J., Czechowsky, P., Rüster, R. & Schmidt, G. Naturwissenschaften. 65, 285 (1978).
 Balsley, B.B., Ecklund, W.L., Carter, D.A. & Johnston, P.E. Radio Sci. 15, 213 (1980).
- 5. Gordon, W.E., Bowhill, S.A., Evans, J.V. & VanZandi, T.E. Atmospheric Dynamics in the 1980s, Position Pap. for the 1978 Summer Study Programs of the National Academy of Science Committee on Solar-Terrestrial Research, Rice University, Texas (1978).
- Röttger, J. & Schmidt, G. IEEE Trans. Geosci. Electr. GE-17, 182 (1979).

whenever a gravity wave generates convectively unstable regions¹⁴. Such bursts are probably identical with clear air turbulence that occasionally threatens aircrafts. In considering the stability of gravity waves we arrive at the problem of parametric instabilities. Waves with finite amplitudes so small that shear or convective instabilities do not occur should enhance a spectrum of disturbance waves by their rocking motions (like the excitation of a swing by a child). This is one of a variety of nonlinear gravity wave processes awaiting their discovery by MST radar experiments.

- 7. Rastogi, P.K. & Bowhill, S.A. J. atmos. terr. Phys. 38,
- Rüster, R., Röttger, J. & Woodman, R.F. Geophys. Res. Lett. 5, 555 (1978).
- 9. Fukao, S., et al. J. geophys. Res. 84, 4379 (1979).
 10. Klostermeyer, J. & Liu, C.H. Geophys. Res. Lett. 6, 507
- 11. VanZandt, T.E., Green, J.L., Clark, W.L. & Grant, J.R. Geophys. Res. Lett. 6, 429 (1979).

 12. Klostermeyer, J. & Rüster, R.J. Geophys. Res. 85, 2841
- (1980)
- 13. Röttger, J. Pageoph 118, 494 (1980).
- Kodiger, J. Fugeoph List, 474 (1909).
 Klostermeyer, J. & Rüster, R.J. Geophys. Res. in the press).
 Eckland, W.L., Gage, K.S. & Riddle, A.C. Geophys. Res. Lett. 8, 285 (1981).

X-ray laser obtained by pumping with a nuclear bomb?

from Peter Knight

RUMOURS have been buzzing around the laser community for some months that an X-ray laser has been made to work by a group led by George Chapline of Lawrence Livermore National Laboratory, Initial reports in the February 23 issue of Aviation Week and Space Technology (stressing military applications) have been followed by notes in the April and May issues of Laser Focus that the Livermore group tested a device pumped by a small nuclear bomb at the DoE Nevada Test Site and produced a pulse of several hundred terawatts of X rays at 1.4 nanometres. So far no official comment or confirmation has been made by the Livermore. As the correspondent of Laser Focus points out, 1.4 nanometres corresponds to the Ka line of neon. Early reports that nanosecond pulses were produced seem unlikely since such a device would probably operate as a singlepass self-terminating amplified spontaneous emission system limited at least by the picosecond upper state lifetimes. Picosecond or femtosecond pulse lengths would be more reasonable. The rumoured experiment has attracted much interest and a full account is eagerly awaited. Past efforts in this field have not survived careful scrutiny. Since a nuclear bomb is necessary in the present case, it seems difficult to check by independent experiment.

It is very difficult to create the right conditions for X-ray laser action. No mirrors are envisaged and single-pass gain

must be high enough that spontaneous fluorescence is efficiently amplified by stimulated emission during its transit down a rod-shaped inverted medium. A gain of about 100 dB is required according to G. Chapline and L. Wood (Physics Today June 1975, p.40) and this requires inversion densities $N^* > (\Delta \nu / L) \times 10^{18} \text{cm}^{-3}$ where $\Delta \nu$ is the bandwidth in electron volts and L is the length of the inverted medium in centimetres. 'Cold matter' has an Auger bandwidth $\Delta \nu \sim 1$ eV and enormous pump intensities are required to reach such large values of N^* . In a plasma, Δv is governed by Stark widths and then $N^* > 5 \times 10^{19}/L$ cm⁻³ which requires fractional inversion $N^*/N \simeq 10^{-3}$. If pump intensities are such that $N^*/N < 10^{-3}$, then one way out is to use material of more than solid density obtained through some compression scheme. It is interesting to note in this context that the Livermore experiment was supposed to be pumped by a nuclear bomb.

Many schemes for producing X-ray population inversion have been studied. Most use very high-power laser excitation of plasma targets. Promising results using this route have been reported by workers at the University of Hull (D. Jacoby et al. Opt. Commun. 37; 193, 1981) with the Balmer α line of Carbon VI at 182Å. An early proposal due to M. Duguay and P.

Peter Knight is in the Optics Section of the Blackett Laboratory, Imperial College, London,

Rentzepis (Appl. Phys. Lett. 10; 350, 1967) suggested exploiting the frequency dependence of photoionization crosssections to produce inversion. Tightly bound electrons are actually more easily photoionized by X rays than weakly bound electrons, so that an incident high-intensity flash of X rays could produce population inversion by leaving ions in excited states. Conventional X-ray sources are not bright enough to achieve the required gain. It is possible that nuclear explosions can produce the required rapid inversion and one might guess that this was used in the Livermore experiment.

The properties of coherent X-ray sources are so valuable that great efforts are being made to construct a useful X-ray laser device. If one were available, phasecontrast microscopy would make atomicscale resolution of the dynamics of biological systems in situ possible, could solve the phase problem in X-ray crystallography and lead to the study of atomic dvnamics with $\sim 10^{-15}$ second resolution. The nuclear bomb-pumped single-shot X-ray laser would be of value in scientific diagnostics but would not seem to be a route to a useful device. Nevertheless, it does represent an invigorating advance which might help in obtaining funds to continue the expensive investigation of potential X-ray laser systems. We will have to wait for more details than the leaked rumours before a proper assessment can be made.

More surprises from mitochondria

from Thomas D. Fox

MITOCHONDRIAL GENETICS began in the 1940s with the work of Boris Ephrussi on cytoplasmic petite mutants in yeast (Saccharomyces cerevisiae); these petites (which have extensive deletions of mitochondrial (mt)DNA) still figured in many reports at a recent Cold Spring Harbor meeting on 'Mitochondrial Genes' held in his memory. However, the study of mitochondrial genes, with or without genetics, has now been extended to a wide range of organisms. Some patterns are beginning to emerge from the comparisons, and some are not. To sum up, one might say that the functional proteins produced by mitochondrial genetic systems are generally highly conserved, while the structure, coding and modes of expression of the genes that specify them are astonishingly different in the various major groups of eukaryotic species.

Although yeast has long been the favourite organism for mitochondrial genetic studies, the best characterized mitochondrial genomes are now those of mammals. The complete human and bovine mtDNA sequences (S. Anderson, MRC Cambridge) and an analysis of transcripts from the human organelle (G. Attardi, California Institute of Technology) were reviewed. These studies and a comment on them recently appeared in Nature 290, April 9, 1981 and little new was left to report at the meeting. In brief, these geonomes are highly compact, containing virtually no intergenic DNA and no intervening sequences within genes. Discrete transcripts appear to be cut from long precursors, generally at sites 'marked' by tRNAs, and have little or no untranslated sequence at either end. There is probably only a single promoter for transcription of each strand. The genome codes for five proteins of known function and up to eight unknown proteins appear to be coded by unidentified reading frames, or URFs. The identity and function of the URF polypeptides remains one of the major unanswered questions in this system.

A comparison of the genomes of yeast and mammals reveals very little similarity except for the deduced amino acid sequences of the five identified proteins. The yeast genome is anything but compact, with large A+T-rich regions between genes. Unlike the orderly process of mtDNA replication in animals, involving well defined start and even stop signals (D. Clayton, California Institute of Technology), there appear to be multiple origins of replication for yeast mtDNA (G. Bernardi, University of Paris; H. Blanc, Stanford University). Duplication of these origins may have helped generate the

A+T-rich DNA since Bernardi finds that origin-like sequences occur in these regions more often than expected randomly. Like replication, transcription is also more complex in yeast than in mammals, involving not only processing but multiple promoters as well. Independent primary transcripts, retaining di- or tri-phosphate 5' ends, can be detected for the regions coding the large and small rRNAs, and from at least four other sites around the genome (D. Levens, University of Chicago).

A yeast mitochondrial gene required for the synthesis of mitochondrial tRNAs has been identified and mapped (N.C. Martin, University of Texas, Dallas). The gene product is unknown, but is active in petites (which lack mitochondrial protein synthesis) and thus cannot be a protein. A plausible hypothesis here is that a mitochondrially coded RNA interacts with a cytoplasmically made protein, imported into the mitochondria, to form an enzyme (similar to RNaseP of E. coli) that processes mitochondrial tRNA precursors. A converse problem, that of a gene product in search of a gene, also arises in yeast, namely, where is the gene for the var-1 polypeptide (see Nature 289; 119, 1981)? Although mitochondrially inherited genetic determinants cause polymorphic variations in this mitochondrially synthesized ribosomal protein, no structural gene can be found in yeast mtDNA (R.A. Butow, University of Texas, Dallas).

Several yeast mitochondrial genes contain introns, and intron-2 of the cytochrome b gene (cob) appears to code for a protein, termed maturase, which is required to splice out the intron itself (see Nature 289; 439, 1981). Studies on intron-4 of cob have revealed a similar, but rather more complex picture. First, mutations in this intron can block the processing not only of cob transcripts, but also those of the mitochondrial gene coding cytochrome oxidase subunit I. Second, several new polypeptides appear in intron-4 mutants. although only one seems to be directly affected by a mutation in the open reading frame (H. Mahler, Indiana University). Finally, there are cis-dominant mutations both in the intron's open reading frame and in the non-translatable region preceding the next exon (C. Jacq, CNRS Gif). However, one can avoid dealing with these complications if one simply makes them disappear. P. Pajot (CNRS Gif) has

Thomas D. Fox is at the Biocenter, Basel, Switzerland, but will be moving shortly to the Section of Genetics and Development, Cornell University. done just that by isolating a spontaneous mutant in which *cob* introns 4 and 5 were both deleted, but which still made functional cytochrome b. This indicates that both introns were very precisely excised; where they went is unknown. Furthermore, owing to a nuclear mutation that occurred simultaneously with the intron deletions, cytochrome oxidase subunit I is made despite the absence of *cob* intron-4!

Two features of the Aspergillus nidulans mitochondrial genome are particularly revealing when compared with those of yeast and mammals (R.W. Davies, University of Essex). First, the cytochrome b gene of Aspergillus has a single intron, whose position corresponds almost exactly to intron-3 of the yeast cob gene, and whose anatomy closely resembles that of the 'maturase-coding' intron-2 of cob: it has an open reading frame in register with the preceding exon, followed by a short untranslatable region. Thus mitochondrial intron splicing mechanisms may be conserved, at least in fungi. Of even more interest to humans is the fact that Aspergillus mtDNA has two open reading frames which would code for proteins homologous to the URF-1 and URF-4 proteins coded by the mammalian genome. This finding indicates that these polypeptides are conserved beyond the animal kingdom, and raises the possibility of examining their function by genetic analysis in fungi. It also begs the question, where are these genes in yeast? Extensive sequence studies in yeast by A. Tzagoloff and co-workers (Columbia University) have not located them, and the prospects of doing so appear bleak. Are they in the yeast nucleus?

That genes have moved between the mitochondria and the nucleus is well established by the case of the ATPase subunit 9 gene, which is mitochondrial in yeast but nuclear in Neurospora crassa (see Tzagoloff et al. A. Rev. Biochem. 48; 419, 1979). Tha tmoving genes may leave tracks has now been demonstrated by the finding that the Neurospora mitochondrial genome nevertheless contains what appears to be a pseudo-gene for ATPase subunit 9: a homologous sequence with an in-frame stop codon and a region of gibberish in the middle (van den Boogaart, State University, Groningen).

The mitochondria of higher plants have large genomes (greater than 100 kilobases), synthesize more proteins than their fungal counterparts, and in some cases also contain linear plasmid molecules whose presence is associated with cytoplasmically inherited male sterility of the plants. Detailed analysis of plant mtDNA will be

accelerated by the fact that defined probes for yeast genes can be used to pick out homologous sequences by crosshybridization (C.J. Leaver, University of Edinburgh). Protozoan parasites, yet more distant relatives of yeast, must also have homologous mitochondrial genes because yeast mtDNA coding for three cytochrome oxidase subunits and for cytochrome b gave specific signals when hybridized at low stringency to fragments of the kinetoplast maxicircle DNA from Leishmania tarantolae (L. Simpson, University of California, Los Angeles).

One aspect of genetic systems that is not supposed to evolve, the genetic code, apparently does so in mitochondria. Several differences exist between the codes used in mitochondrial genes of mammals and yeast (see Nature 287; 9, 1980). The only feature common to them (and to Neurospora and Aspergillus) but not to nuclear genes is the coding of tryptophan by UGA. However, it now appears that the use of UGA for tryptophan may not even be 'universal' for mitochondria, as the gene coding maize cytochrome oxidase subunit II does not contain UGA codons (T.D. Fox, Biocenter, Basel). Instead, the maize gene appears to use the codon CGG for tryptophan, in addition to the standard UGG.

Another established coding novelty in mitochondria is the ability of a number of single tRNA species with U in the wobble position to read all four codons in a family (Nature 287; 9, 1980). Several tRNAs, however, whose gene sequence predicts U at the wobble position, read only codons ending in A or G. In Neurospora, this dilemma is apparently resolved by a modification of the U in those tRNAs with restricted pairing (Heckman et al. Proc. natn. Acad. Sci. U.S.A. 77; 3159, 1980). While similar modified U residues have now been found in the corresponding tRNAs of yeast (R.P. Martin, CNRS Strasbourg), the mammalian systems must use another method to restrict pairing as direct tRNA sequencing has revealed no modified U residues at the wobble position of mammalian mitochondrial tRNAs (S. Anderson, MRC Cambridge).

Not only the evolution of mitochondria themselves was discussed at the meeting, but also the use of mitochondrial studies to trace the evolution of the organisms in which they reside. Since the evolutionary rate of base pair substitutions in the mtDNA of vertebrates is approximately ten times that in nuclear DNA (Brown et al. Proc. natn. Acad. Sci. U.S.A. 76; 1967, 1979), mtDNA provides a unique highresolution tool for examining relationships among animals whose divergence times are relatively short. For example, a comparison of the DNA sequences of homologous regions of mtDNA from humans and four great apes allows the derivation of an evolutionary tree more firmly rooted in data (over 300 base pair substitutions) than has hitherto been

possible (W.M. Brown, University of Michigan). Studies of mtDNA restriction map polymorphisms within our own species indicate that *Homo sapiens* probably arose from a small group of individuals as recently as 130,000 years ago, although an anomalous restriction pattern from a single individual has led to speculation that 'non-sapiens' mitochondrial genomes could still be present in modern human populations (R.L. Cann, University of California, Berkeley).

Somewhat lost in the discussion of mitochondrial genes was the fact that their

expression must be largely under the control of nuclear genes. This point was brought home forcefully by the description of several nuclear mutants of *Neurospora* which fail to splice the intron from the large mitochondrial rRNA precursor (A.M. Lambowitz, St Louis University), and a nuclear mutant of yeast that specifically lacks any detectable transcripts of the *cob* gene (C. Dieckmann, Columbia University). Much more activity in this area can be expected in the future, and the fungi will again probably lead the way — at least until the complete sequence of the human nuclear genome becomes available.

A golden age of astronomy

from Ben Zuckerman

THE latter half of the 20th century is one of the two golden ages of astronomy. This has primarily been a result of the opening up of new wave bands that extend over the entire electromagnetic spectrum. Excitement should continue with major technological advances anticipated in construction of new telescopes with larger collecting areas and improved spatial resolutions. The latter was emphasized at a meeting* entitled the "Scientific Importance of High Angular Resolution at Infrared and Optical Wavelengths".

The fundamental problem in achieving high angular resolution with ground-based telescopes is, of course, posed by the atmosphere of the Earth which distorts and distends the diffraction-limited images of all large telescopes at visual and nearinfrared wavelengths. Currently, there are three main techniques for achieving high angular resolution. A. Labeyrie (Centre d'Etudes et de Recherches Géodynamiques et Astronomiques) reviewed the field of speckle interferometry. The application of this technique to optical and infrared astronomy has been pioneered by himself and his French colleagues. C. H. Townes (University of California, Berkeley) multiple telescope discussed interferometry in the infrared emphasizing the heterodyne technique that he and his research group have developed. The third technique. Michelson interferometry, has been utilized, in the optical, by D. Currie (University of Maryland) and, in the infrared, by D. McCarthy, R. Howell and F. Low (University of Arizona).

The best angular resolutions achieved to date are due to the CERGA group who have been operating a 35-m baseline interferometer in southern France and observing fringes with a photon-counting TV camera which integrates every 20 milliseconds. This system has measured stellar angular diameters and binary star

separations as small as 1 milli-arcsecond. A new 350-m baseline optical interferometer with novel concrete support structures for damping flexures may be operating at CERGA within a year's time. Baseline flexures at a level of only a few microns can obliterate the fringes. Because of phase jitter due to the atmosphere, the faintest object measureable with such an interferometer is about 10⁵ times brighter than could be studied with a space interferometer of equal size. D. Dravins (Lund Observatory) described a proposed ground-based optical interferometer that would have substantially longer baselines - up to 100 km - capable, in principle at least, of achieving micro-arcsecond resolution. This would be a digital version of Hanbury Brown's intensity interferometer which has successfully measured angular diameters of bright, hot stars at the milli-arcsecond level. Here the signals from separate telescopes are mixed after detection rather than before as in all the other techniques mentioned above.

Speckle interferometry attempts to compensate for distortions due to atmospheric fluctuations by very short exposures that 'freeze' the image in a time interval that is shorter than the characteristic time scale of these fluctuations. An alternative approach to that of speckle interferometry is active optics. J.W. Hardy (ITEK) described how the figure of the primary or secondary mirror is actively controlled on a time scale that matches atmospheric changes and, also, distortions in the telescope figure induced by variations in gravity or temperature. J. E. Nelson (University of California, Berkeley) described a University of California design for a 10-m diameter segmented hexagonal primary mirror. The 36 segments, each 1.8-m in diameter, would be controlled by 168 displacement sensors, 3 tilt sensors and 108

Ben Zuckerman is in the Institute for Astronomy, University of Hawaii at Manoa.

^{*}The meeting was held at the new headquarters of the European Southern Observatory in Munich from March 24 to 27.

displacement actuators. Townes pointed out that modern telescopes are already so complicated that these actuators do not represent a great increase in complexity.

Among the highlights of the meeting were discussions of unusual galaxies such as quasars and Seyferts by M. J. Rees (University of Cambridge) and by the chair-person of the scientific organizing committee, M. H. Ulrich (European Southern Observatory). Rees considered how measurements of the shape, location and fine structure of a quasar imaged by a foreground gravitational lens could be used to deduce the distribution of mass in the lens. In the most likely case where the lens is a massive galaxy, angular fine structure in the quasar image on a scale ranging from one arc second down to one micro-arcsecond could be used to distinguish mass contributions in the lens that range between entire galaxies and single stars. Since there is, at present, roughly 70 orders of magnitude uncertainty in the mass of the paramount contributor to the unseen mass in galaxies (ranging all the way from massive neutrinos to million solar mass black holes), such measurements are of considerable interest.

Finally, there was discussion of the future of large telescopes and, specifically, whether large single apertures (up to 10- or 15-m diameter) are preferable to somewhat smaller telescopes operating as an interferometer. As might be expected, no consensus was achieved. R. Angel (University of Arizona) lamented that the Multiple Mirror Telescope on Mt Hopkins in Arizona is the first real technological innovation in 50 years in optical astronomy. But it seems clear, from the impressive results already achieved by the CERGA group and others and by the range of novel proposals presented at the conference, that high angular resolution ground-based and space-based optical and infrared astronomy has a bright future.

From embryo to teratocarcinoma in tissue culture

from Brigid Hogan

Mouse teratocarcinomas are well established as a versatile model system for the study of gene expression and cell interaction during early mammalian embryogenesis. In culture, aggregates of the pluripotent stem cells, known as embryonal carcinoma (EC) cells, differentiate into embryoid bodies resembling the inner cell mass of the early mouse embryo. At first, these structures consist simply of an outer layer of endoderm surrounding a solid core of epiblast (or primitive ectoderm) cells, but many subsequently develop a quite complex organization, with various tissues derived from endoderm, ectoderm and mesoderm. However, the most dramatic evidence for the ability of EC cells to differentiate in an orderly and coherent sequence is undoubtedly their behaviour when injected into normal mouse blastocysts. Here, the cells integrate with the host inner cell mass and in the course of development may contribute towards most tissues of the chimaeric offspring.

Impressive as these results are, the degree of chimaerism obtained after injecting EC cells is unpredictable, to say the least. With some cell lines, the probability of an EC cell differentiating into normal tissues is very low, and cells which fail to do so may give rise to tumours. With other cell lines, the frequency and extent of chimaerism is much higher, although still unpredictable, and only in few tissues is the contribution more than 50 per cent. One of the most

Brigid Hogan is in the Mammalian Development Laboratory, Imperial Cancer Research Fund Laboratories, Mill Hill, London. celebrated chimaeras was "Terry Tom", a mouse in which nearly all tissues, including sperm, were populated by derivatives of EC cells of the OTT 6050 line, which had been maintained in the laboratory for more than 8 years as an ascites tumour 1.2. The prospect of generating more mice like Terry Tom, carrying germ cells derived not only from standard EC lines but also from teratocarcinoma cells selected *in vitro* to carry specific mutations, or new DNA sequences, ideally inserted into specific chromosomes, has inspired an immense amount of effort

One of the ultimate goals of this work is to engineer strains of mutant mice which can act as models for human genetic diseases3, so male mice, which can transmit chromosomes to large numbers of offspring, are particularly prized. But, in spite of the fact that EC cells carrying specific nuclear or mitochondial mutations4, human5 or rat6 chromosomes, or foreign DNA6 have been generated and in some cases injected into blastocysts and incorporated into chimaeric offspring, the colonization of the male germ line remains elusive. One reason for this is undoubtedly the fact that all the established EC lines so far used for genetic manipulation have an XO karyotype — that is, they have lost the Y chromosome during prolonged culture. For this reason, it would be very useful to be able quickly grow new teratocarcinoma cell lines from normal male embryos, insert genetic material or mutations into them, and make chimeras, either by blastocyst injection or by the simpler technique of aggregation with monula-stage embryos7. Recent successes with nuclear transplantation in mouse eggs8 may



100 years ago

STUDENTS of Cretaceous geology will regret to hear that Griffiths, the well-known "fossil man" of Folkestone, has been disabled for many months by rheumatism, brought on by constant exposure during the past twenty-five years, in which he has daily extracted from the wet and slippery tract of Gault clay in Eastweir Bay the remarkable series of mollusca with their pearly nacre preserved, plants, corals, crustacea, and reptilian remains that ornament not only the private collection of those who make the Gault a subject of special study, but the national museums both of this country and of the New World. In addition to collecting by far the most perfect specimens of the

Gault fauna and flora hitherto obtained, Griffiths has rendered an important service to science in carefully noting the bed or horizon from which each specimen was procured, which identification has formed the groundwork of the divisions which English geologists have been able to make in the Gault, and the correlation of these zones by M. Barrois and others with deposits occurring on the Continent. In consideration of these results, carried out by a working man under the difficulties of a struggle for life with circumstances, and the rigorous weather of the English Channel coast, it has been thought advisable to appeal to English geologists to raise a small fund which should render it unnecessary for work to be carried on when dangerous to health, and to tide him over present difficulties.

The evening *fête* of the Royal Horticultural Society was held on the 28th ult. in the Gardens at South Kensington. Coloured

lamps were disposed about the lawn, and here and there the cool plash of fountains was to be heard. The Siemens and Maxim electric lights were placed in the upper part of the Gardens, and in the lower part were two tents illuminated by the Brush electric light, and containing the plants of a flower-show, which continued next day. Brilliant effects were obtained with coloured fixes behind the trees and the spray of the fountains.

There was recently landed at Marseilles a magnificent zebra which the King of Choa, Menelick II, has sent as a present to the President of the French Republic. This zebra, called the Semephore, has been brought from Abyssinia by two Marseillais. The Société de Géographie, to which it was addressed from Aden, has intrusted it to the Marseilles Zoological Garden.

From Nature 24, 224-226. July 7, 1881.

eventually make it possible to obtain mice in which all the genetic information comes from one teratocarcinoma nucleus.

Until now, however, isolation of new teratocarcinoma cell lines has been a lengthy procedure involving transplantation of genital ridges or early embryos into extra-uterine sites such as the kidney or testis. In this environment pluripotent cells in the graft continue to grow and may give rise to tumours containing populations of EC cells which can be maintained during serial transplantation and then tissue culture. These techniques restrict work to inbred strains of mice since, for unknown reasons, tumours containing EC cells fail to develop from grafts into nude or immunosuppressed animals9.

Over the years, many attempts have been made to circumvent these restrictions by generating EC cell lines from embryos cultured in vitro. Now, Martin Evans and Mat Kaufman in Cambridge have at last been succesful (see this issue of Nature p.154). Studies in several laboratories suggest that EC cells have more biochemical properties in common with epiblast cells in the inner cell mass of 51/2-day blastocysts around the time of implantation, than with pluripotent cells present earlier or later. Normally, the number of epiblast cells is small but this increases when implantation is delayed by treating the mother with hormones. Evans and Kaufman collected batches of delayedimplantation blastocysts, allowed them to attach and spread in vitro, harvested the embryonic knobs containing the epiblast cells, dissociated them in trypsin and plated the cells on a feeder layer of non-dividing fibroblasts. After a few days clumps of EClike cells were located, dissociated and reseeded, and several cycles of such treatment yielded cultures containing predominantly undifferentiated EC cells.

The trick seems to be to provide conditions in which the pluripotent cells in the embryos are both encouraged to proliferate, either in response to growth factors made by themselves or the feeder fibroblasts, and discouraged from differentiation by disrupting cell-cell interactions. Using this technique Evans and Kaufman have isolated at least 15 new pluripotent teratocarcinoma cell lines. some from outbred mouse stocks. Those which have been analysed have a completely normal karvotype, including two with an XY genotype. It remains to be seen whether these new teratocarcinoma lines are particularly efficient at colonizing normal embryos and, in particular, the male germ line. Meanwhile, the technique opens up many exciting possibilities for exploiting the teratocarcinoma system more fully. For example, EC cells could be isolated from mouse strains carrying electrophoretic variants of X-linked enzymes, or from T/t mutants blocked in postimplantation development, and the biochemical properties of these cells followed during the in vitro development of mass cultures as embryoid bodies.

One criterion used by Evans and Kaufman to identify their teratocarcinoma stem cells is expression of a specific, cellsurface carbohydrate sequence recognized by one of the monoclonal antibodies made in humans suffering from a rare haemolytic

disease known as 'cold agglutinin disease'. These people make antibodies against different, chemically defined, carbohyrate domains of the Ii blood group antigens. The antibodies have been used by Ten Feizi and her colleagues at the Clinical Research Centre in London and Martin Evans in Cambridge as highly specific probes with which to follow the transient expression of carbohydrate sequences on the surface of different cell population during early mouse development¹⁰. These studies have now been extended by Gooi and the above workers (see this issue of Nature p.156) to define the carbohydrate sequence reconized by a monoclonal antibody SSEA-1 raised in mice immunized with teratocarcinoma EC cells11 and to show that simple glycosylation steps involving addition of branch points or extra fucose or sialic acid groups occur at different stages of development. The significance of these subtle changes in terms of cell interactions, binding of hormones and growth factors, or morphogenesis remains to be seen.

The ultimate computer

from Paul Davies

BLACK HOLES, it seems, get into everything these days. Jacob Bekenstein who, along with Stephen Hawking. invented quantum black holes, turns his attention to the more practical issue of telegraphy in a recent paper (Phys. Rev. Lett. 46; 623, 1981). In the spirit of Shannon's celebrated analysis of the limits on information transfer through a communication channel, Bekenstein deploys some remarkable arguments gleaned from his experience with more astronomical matters.

The basis of this new departure is an unexpected relation, recently published by Bekenstein himself, connecting the ratio of entropy to energy for any physical system, with its physical size. The ratio is bounded by the dimension of the system, whether it be a black hole (an extreme case) or a box of photons. Using the fact that information is the negative of entropy, Bekenstein turns his new relation inside out to address the problem of energy limitations on information

The transmission of information is expensive, as every telephone subscriber knows. The pressing question is whether the energy dissipation can, in principle, be arbitrarily reduced by some future technological innovations. Bekenstein says no, even if you try to get the theory of relativity to help. His result is disarmingly

information rate = $(2\pi^2/h \ln 2) \times \text{energy}$ where h is Planck's constant.

Turning to an obvious application, Bekenstein considers the maximum conceivable speed of a digital computer. These days computers are so fast that designers have to worry about the speed of light being a limiting factor. We are already in the era of the relativistic computer. To beat the speed of light one can try to make the device smaller, but then quantum theory threatens. Ultimately an over-compact computer risks imploding under gravity down its own black hole.

In a somewhat rule-of-thumb analysis Bekenstein considers the problem of computer overheating. There is an inevitable entropy generation involved in shunting any information around, and in the end this will melt the system unless it is efficiently cooled. The author considers various cooling mechanisms and their inherent limitations, and eventually comes up with the best performance criterion for the ideal computer: 1015 operations per second.

That is pretty fast by contemporary standards, so Bekenstein's limit will not cause too many sleepless nights in the computer industry just yet. Nevertheless, an ultimate bound on any form of technology is intriguing in its own right, and it would be interesting to see whether the idea is followed up.

Paul Davies is in the School of Physics, University of Newcastle upon Tyne.

^{1.} Mintz & Illmensee Proc. natn. Acad. Sci. U.S.A. 72, 3585 (1975).

Cronmiller & Mintz Devl. Biol. 67, 465 (1978).

Mintz Harvey Lect. 71, 193 (1978).
Devey et al. Proc. natn. Acad. Sci. U.S.A. 74, 5564 (1977); Watanabe et al. Proc. natn. Acad. Sci. U.S.A. 75, 5113 (1978); Goldstein et al. Proc. natn. Acad. Sci. U.S.A. 75, 1914 (1978).
5. Illmensee et al. Proc. natn. Acad. Sci. U.S.A. 75, 1914

^{(1978).}

^{6.} Pellicer et al. Proc. natn. Acad. Sci. U.S.A. 77, 2098 (1980)

Fujii & Martin Devl. Biol. 74, 239 (1980); Stewart J.

Embryol, exp. Morph. 58, 289 (1980). Illmensee & Hoppe Cell 23, 9 (1981).

Solter & Damjanov Nature 278, 554 (1979)

Kapadia, Feizi & Evans Expl Cell Res. 131, 185 (1981). Solter & Knowles Proc. natn. Acad. Sci. U.S.A. 75, 5565 (1978).

REVIEW ARTICLE

Tempo and mode in hominid evolution

J. E. Cronin', N. T. Boaz', C. B. Stringer & Y. Rak

- * Department of Anthropology, Harvard University, Cambridge, Massachusetts 02138, USA
- † Department of Anthropology, New York University, New York, New York 10003, USA ‡ Department of Palaeontology, British Museum (Natural History), London SW7 5BD, UK
- § Department of Anatomy and Anthropology, Sackler School of Medicine, Tel-Aviv University, Ramat-Aviv, Israel

The nature of human evolution has been viewed recently as a specific example of a more general model of evolution termed 'punctuated equilibrium'. The characteristics of this model are long periods of little or no evolutionary change (stasis) interspersed with periods of rapid (punctuated) morphological change. Careful analysis of the hominid fossil record over the past 4.0 million years, however, suggests no well documented examples of either stasis or punctuation. The evidence for the evolution of the hominid lineage is most reasonably interpreted by a model of more gradual change with periods of varying rates of evolution.

THE evolution of populations through time occurs by changes in the constitution of the gene pool. The traditional viewpoint has been that most of the change along lineages is cumulative and gradual. This hypothesis of 'phyletic gradualism', proposes that if a complete fossil record of an evolving lineage were to be found, it would consist of a finely graded series of forms progressively more similar through time to descendent species and less and less similar to ancestral species. The temporally intermediate forms would be intermediate morphologically. The fact that morphological gaps appear between ancestral and descendant populations is a function of the imperfect nature of the fossil record.

This view of evolution has recently been questioned. It has instead been suggested that a substantial fraction of evolutionary change is concentrated in rapid bursts of speciation events. In the intervening periods most species exhibit slow, little, or no change at the morphological and genomic levels. The proponents of this theory suggest that phyletic gradualism plays very little part in the evolution of character states. The gaps in the fossil record are real and are the outcome of very rapid change from one form to another, thus leaving few or no intermediates in the fossil record. Speciation events occur in a short period of time, predominantly in geographically marginal populations with small effective breeding sizes. Thus the geological record is unlikely to preserve such saltations. This view of evolution has been termed punctuated equilibrium, or rectangular evolution.

Recently Gould and Eldredge¹ have argued that "a punctuational view of change may have wide validity at all levels of evolutionary processes" and have cited human evolution as a particularly good example of punctuated equilibrium. They state that "no gradualism has been detected within any hominid taxon, and many are long ranging".

It is our intention in this review to show that with more fossil evidence and better dating techniques than in the past, one can clearly demonstrate directional morphological change. There are fossils that appear clearly intermediate in form and time between recognized fossil hominid taxa. We review all the examples put forward by Gould and Eldredge in support of punctuated evolution in hominid evolution and conclude that a hypothesis of phyletic gradualism is more parsimonious. Furthermore we question the degree to which genetic studies support punctuation; changes at regulatory loci or chromosomal reorganization may have rapid and pronounced effects on

phenotype but the commonly observed allozymic changes cannot clearly be related to punctuational events.

Major stages of hominid evolution

Largely because of increased sample sizes and better dating (Fig. 1), there is better consensus than ever before on the major stages of hominid evolution through the Pliocene and Pleistocene. We will confine ourselves to examining the Plio-Pleistocene hominid fossil record for evidence of punctuational change and not try to argue for or against the various possible phylogenetic trees presented in Fig. 2.

The origin of the hominid family is a topic of continuing research. The hominid fossil record is largely nonexistent before 4 million years ago (4 Myr) and it is therefore uncertain how Pliocene hominids and Miocene hominoids are related²⁻⁴. The lack of Upper Miocene to Pliocene fossils precludes detailed discussion of absolute rates of evolutionary change. Biomolecular results indicate a date of 4-6 Myr for the pongid-hominid split⁵⁻⁷.

The earliest good sample of undoubted hominids comes from the Laetolil Beds of Tanzania^{8,9}, dated at between 3.5 and 3.8 Myr, close to the putative hominid-pongid separation predicted by macromolecular evidence. These and later samples from Hadar^{8,9} and early parts of the Omo sequence¹⁰ in Ethiopia, and from Makapansgat, Sterkfontein and perhaps Taung in South Africa¹⁰, dating from approximately 3 to over 2 Myr, comprise the known samples of early gracile australopithecines (Figs 1, 3).

The earliest appearance of the genus *Homo* is generally considered to have been between 2.2 and 1.8 Myr^{2,9,10-12}. Specimens attributable to *Homo* are known from the Lower Member of the Koobi Fora Formation¹³, Olduvai Bed I¹⁴, Omo Shungura Members E, F and G¹⁵, Swartkrans Member 1^{16,17} and Sterkfontein Member 5¹¹ in Africa and the Djetis levels (Putjangan Formation) in Java^{18,19}. The African sample has generally been referred to as *Homo habilis* L. Leakey, Tobias and Napier 1964, or simply *Homo* sp. ²⁰, while the earliest Javan hominids have been referred to as *Homo modjokertensis* von Koenigswald 1936. These two may be morphologically and temporally co-terminous ^{2,10,21,22}.

Based on the close congruence in the last appearance of A. africanus and the first appearance of Homo, and on the morphological continuities seen in the two taxa (Figs 1,3), an ancestral-descendant relationship between them has been widely, but not unanimously accepted 11.23-25. Opposition stems

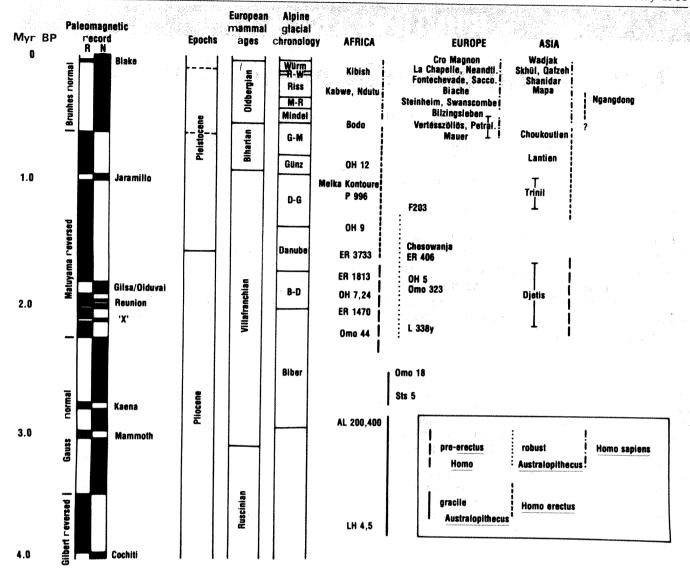


Fig. 1 Time scale and hominid lineages during the Plio-Pleistocene. Hominid fossils documenting stages along lineages are positioned according to their temporal locations. Large degrees of temporal uncertainty are indicated by error bars. The alpine glacial chronology is presented because of its historical importance rather than its accuracy. Some of the Choukoutien material may be considerably younger than shown here and in Fig. 3.

from those who believe that late surviving Australopithecus africanus or early occurring Homo²⁶⁻²⁸ preclude a simple ancestral-descendant relationship. Others divide the gracile australopithecines⁹.

Robust australopithecines were first recovered from the South African sites of Kromdraai and Swartkrans. This South African group is now referred to as Australopithecus robustus (Broom), 1938. The peculiar facial and dental morphology of A. robustus led Broom to believe that this hominid represented a side branch of human evolution.

In 1959 the type specimen of another robust hominid was discovered at Olduvai Gorge. This hominid, Australopithecus boisei, has since been found at other East African localities: Beds I, II and IV at Olduvai Gorge²⁹, Peninj (Humbu Formation)³⁰, Chesowanja (Chemoigut Formation)³¹, Lower and Upper Members of the Koobi Fora Formation²⁰ and Omo Shungura Members E, F, G and possibly H and L¹⁵.

The earliest appearances of the robust australopithecines in both East and South Africa seem to be at 2 Myr, and they persisted as late as 1.0 Myr^{2,32}.

The actual phylogenetic relationship of the robust lineage is widely debated. Some believe that the taxon A. africanus is directly ancestral to robust australopithecines (Fig. 2), with the former species showing shared derived morphological features

with the latter taxa. Others believe that this relationship has not been clearly demonstrated and that A. africanus provides a suitable candidate for a form that gives rise subsequently to the contemporaneous lineages Homo habilis and robust australopithecines².

The first remains of Homo erectus were discovered by Dubois in 1891. Assigned by him to the genus Pithecanthropus, these and subsequent discoveries from Java, China and Africa are now classified within the genus $Homo^{33}$. The morphology of the H. erectus material from Choukoutien, China (Fig. 3) was described by Weidenreich and this sample has provided the main data on the characteristics of this species³⁴⁻³⁷. Further important material has been recovered from Early and Middle Pleistocene deposits in Java, Middle Pleistocene deposits in Algeria and Early Pleistocene deposits at Olduvai and Koobi Fora³³ for the occurrence of this species in the Middle Pleistocene of Europe are overly dependent on the parts preserved in the various fossils and the characters which are utilized for classification—see discussion on the Petralona cranium below.) Recent definitions of this species have been provided by Le Gros Clark²³ and, in greater detail, by Howell¹⁰. Most anthropologists accept that H. erectus was ancestral to Homo sapiens. Specimens often quoted as displaying 'intermediate' or 'mosaic' characters between H. erectus and H. sapiens include Broken Hill and Omo

(Kibish) in Africa, Ngandong in Java, and Arago, Vertésszöllös and Petralona in Europe³³ (Fig. 3). Other recently discovered fossils which may belong to this intermediate category are the African Middle Pleistocene specimens from Sale³⁸, Ndutu^{29,39} and Bodo⁴⁰. However, not all palaeoanthropologists are convinced that *H. erectus* is ancestral to *H. sapiens*^{41,42,133}.

The term H. sapiens has recently been used to include various Middle and Late Pleistocene fossils which can be distinguished from H. erectus. Campbell introduced a system utilizing subspecific names for geographical or temporal sub-groups within *H. sapiens* ⁴³ and, rightly or wrongly, the subspecies name H.s. sapiens has become synonymous with anatomically modern humans in general. Some palaeoanthropologists have preferred the term 'archaic H. sapiens' for fossils considered more 'advanced' than H. erectus but still morphologically distinguishable from 'modern' H. sapiens44 (Fig. 3). Within the former category are the 'Neandertals', placed by Campbell⁴³ in the category H. sapiens neanderthalensis. Others have defined 'Neandertal' more broadly to include fossils from the Old World which they consider to be ancestral to modern humans throughout the Late Pleistocene range of H. sapiens^{25,45}. Yet others have attempted to subdivide the Late Pleistocene material using morphological criteria and have delineated 'Neandertals' as a group supposedly restricted to the earlier Late Pleistocene of Europe and western Asia, which can apparently be differentiated from penecontemporaneous fossils from Africa and eastern Asia 46-48.93. The origin of anatomically modern *H. sapiens* (whether from one restricted area or more widely) is still in dispute and lack of good fossil evidence from many parts of the Old World makes resolution of this problem more difficult.

Hominid fossil evidence and rates of evolution

Gould and Eldredge¹ suggested that the gaps in the fossil record should be treated as data, as though they were 'real' and not artefacts of the fossilization process. Thus the observation that intermediate forms between taxa are frequently not known is explained on the basis that evolution from one to another species occurred so rapidly that no transitional forms were preserved. The basic assumption here is that the fossil record is relatively complete.

Available data do not support Gould and Eldredge's assumption of a complete fossil record for the Hominidae.

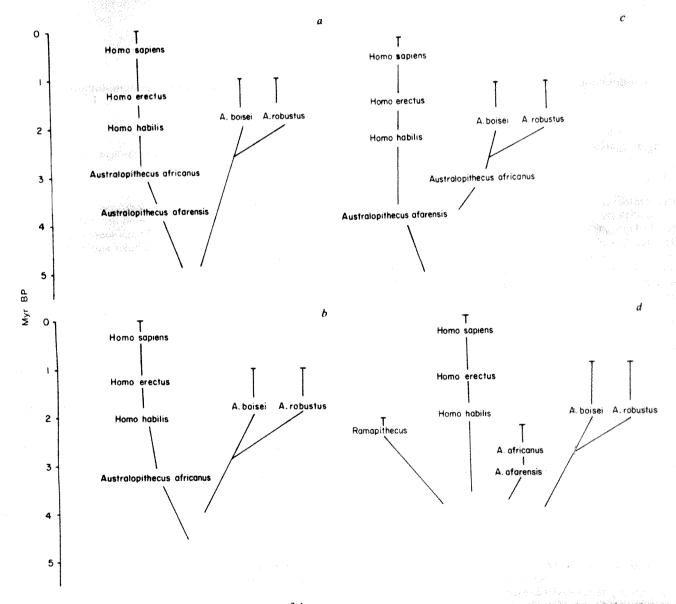


Fig. 2 Alternative phylogenies of the major hominid taxa²⁻⁴. Although not encompassing all proposed schemes, these four phylogenies are representative of the current most likely hypotheses. One of us (N.T.B.) favours phylogeny b, whereas another (Y.R.) favours phylogeny c. All of us concur, however, that A. africanus is not far removed from a basal australopithecine morphotype. The phylogeny sepresented in d is a polyphyletic hypothesis favoured by R.E.F. Leakey²⁷.

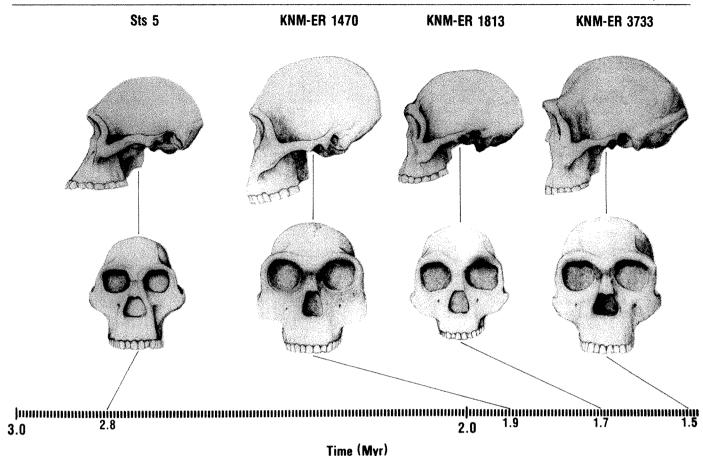


Fig. 3 Certain key hominid fossil crania in the 'gracile' hominid lineage through time, plotted along the bottom of the figure from left to right.

Behrensmever⁴⁹ estimates that only 4×10^{-5} of the original hominid population over a span of 1.5 Myr is represented at Koobi Fora. At Omo Shungura, with the longest sequence of hominid-bearing sediments yet known⁵⁰, 35 lithological units accounting for only 38.3% of the total vertical stratigraphical thickness have yielded hominid remains. If the total time represented by the Shungura Formation is almost 2.6 Myr¹⁰, this implies a loss of 1.6 Myr of time. Using an average of empirically derived population density estimates for Omo Hominidae (1.05 1, an area of 500 km² and a generation time for early hominids of 20 yr, the 215 known fossil hominid individuals from Omo Shungura derived from populations totalling $6.8 \times$ 10^7 individuals. Thus, the fossil sample represents a mere $3.2 \times$ 10⁻⁶ of the original population. In terms of both time represented and numbers of the original populations, the hominid fossil sample seems to be a very imperfect indication of the actual palaeobiology. In addition, the fragmentary nature of the preserved remains frequently does not allow phylogenetic interpretation. Thus there is almost no support for the assumption that the fossil record of hominids is complete, although a general overview of the major stages is possible while we await new fossil material.

Meanwhile recent sophisticated geochronological studies have provided sufficiently precise dates for existing fossil material to test the hypothesis of punctuated equilibrium. Two such tests are presented in Fig. 4. Means of body weight estimates and cranial capacity of the 'gracile' hominid lineage are plotted through time. The trends seem to show no jumps or discontinuities. Any impulse to draw a step diagram through the points should be resisted while the most parsimonious approach is to interpret the trends with a best-fit line.

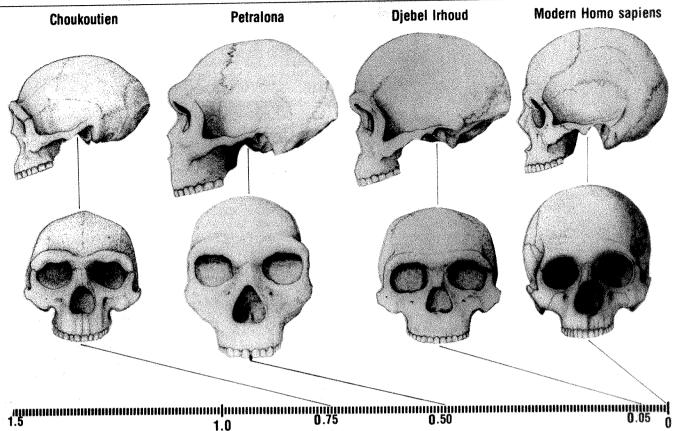
Gould⁵² has recently suggested that the supposed long temporal range of A. afarensis, based on a 3.7-3.8 Myr date on Laetoli and a 2.8-3.2 Myr date on Hadar, indicates morpholo-

gical stasis over time. Two considerations argue against this claim. The fossil remains from Laetoli are not abundant, and there are no diagnostic crania. Although some specimens in this sample do show dental and mandibular similarities to Hadar specimens, it is unknown to what degree the crania, which are taxonomically paramount for Hominidae, would correspond. Thus, a morphological argument for stasis cannot be sustained.

Second, while the K-Ar dating of tuffs at Laetoli and Hadar remain to be confirmed by other dating techniques, such as palaeomagnetism, there are preliminary faunal indications that Hadar and Laetoli may be closer in time than the absolute dates would suggest. Thus, a temporal argument for stasis within the proposed taxon A. afarensis is unconvincing.

A good case for phyletic gradualism within the gracile australopithecine lineage could be made from a comparison of South African A. africanus, with the more primitive and probably older A. afarensis. On the basis of the present samples, Hadar and Laetoli specimens evince generalized hominoid traits in having P3 mesiobucally set in the tooth row, large canines with distal longitudinal wear facets, I2-C diastemata and a relatively small cranial capacity9. They also possess certain derived traits in common with A. africanus from South Africa, particularly in the innominate and femur⁵³, face and cranial vault⁵⁴, and mandibular and dental morphology⁵⁵. Further fossil and geochronological evidence is needed to document the proposed transitional period from Laetoli and Hadar to classic A. africanus, but the balance of evidence does not support stasis within the Laetoli-Hadar samples through time nor punctuational change from these to A. africanus.

Two specimens of cardinal importance to the punctuated equilibrium argument come from the Koobi Fora Formation, Kenya (KNM-ER 1470 and KNM-ER 3733, ref. 20; Fig. 3; Table 1). The dating of these specimens is of paramount



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Time (Myr)

Fig. 3 (cont.)

importance but faunal and geochronological dating of East Lake Turkana had been controversial until a recent solution was reached 56,63.

KMN-ER 1470 comes from the Lower Member of the Koobi Fora Formation, 37 m below the KBS Tuff in Area 131 (refs 20, 57, 58), and at an unknown distance above the Tulu Bor Tuff. The specimen has been dated on the basis of 40 Ar/ 39 Ar determinations on the overlying KBS Tuff, initially reported to be $2.6 \pm .26$ Myr BP⁵⁹. Leakey⁵⁷ claimed an age of 2.9 Myr BP for the find, adding $\sim 300,000$ for deposition of the 37 m of sediment separating ER 1470 and the KBS Tuff (12.3 cm per 1,000 yr). However, faunal studies^{60,61}, K-Ar redating of the KBS Tuff^{62,63}, tuff chemistry studies⁵⁶ and fission track dating⁶⁴ have now demonstrated that the KBS-131 Tuff is only ~ 1.8 Myr BP and is correlative with Tuff H-2 in the nearby Omo sequence at the same date. The sub-KBS fauna, which includes ER 1470, correlates with Omo levels about 2.0 Myr BP (refs 61, 65) and this is also the most likely date of KNM-ER 1470.

KNM-ER 3733 comes from the lower part of the Upper Member of the Koobi Fora Formation⁶⁶ in Area 104 (ref. 28), having been found in a sand infilling ~10 m above the post-KBS erosion surface⁶⁷. Another specimen, KNM-ER 1813, to be discussed below, comes from the upper Lower Member of Koobi Fora Formation in Area 123 (refs 13, 20), 8–10 m below the lateral correlative of the post-KBS erosion surface⁶⁷. ER 1813 is thus stratigraphically 18–20 m lower in the sequence than ER 3733. Assuming a sedimentation rate of 10–15 cm per 1,000 yr (60 m between the KBS Tuff and the overlying Chari Tuff 1.8–1.25 Myr), the difference in time between ER 3733 and ER 1813 is between 200,000 and 120,000 yr. Probable absolute dates are about 1.8 Myr BP for ER 1813 and 1.6 Myr BP for ER 3733.

Morphological and chronological arguments have been advanced to demonstrate the evolution of the earliest *Homo* from gracile australopithecines^{2,10}. Opponents of this view have held that *Homo* co-existed with gracile *Australopithecus*^{27,68}.

KMN-ER 1470, which has now been shown to have a spuriously old date, was a key part of this argument. It is a cranium lacking teeth but showing the large cranial vault, slight mid-facial prognathism, post-orbital expansion, relatively small temporal fossae and unpronounced supraorbital tori generally associated with the genus Homo. However, its relatively robustly constructed face, flattish naso-alveolar clivus (recalling australopithecine dished faces), low maximum cranial width (on the temporals), strong canine juga and large molars (as indicated by remaining roots) are all relatively primitive traits which ally the specimen with members of the taxon A. africanus. The specimen probably represents a member of the earliest species of the genus *Homo*, *H. habilis* (or *H. modjokertensis*^{2,22}), also known in Africa from Olduvai¹⁴, Omo⁶⁹, Sterkfontein⁷⁰ and Swartkrans¹⁶. KNM-ER 1470, like other early Homo specimens, shows many morphological characteristics in common with gracile australopithecines that are not shared with later specimens of the genus *Homo* (Table 1); in fact, Walker^{26,28} has classified ER 1470 as australopithecine. When accurately dated, and considering the morphology, ER 1470 supports the graduated evolution of Homo from a gracile australopithecine species in the later Pliocene.

Various authors^{8,9,55} have recently discussed these ancestral australopithecine populations. The Pliocene specimens, whether referable to the new taxon A. afarensis⁸ or to A. africanus² (Fig. 2), and undeniably primitive in their morphology, do show many characters which link them with later Homo (Table 1); M. D. Leakey⁶⁸ has in fact, classified them as Homo sp.

The gap between *H. habilis/modjokertensis* and *H. erectus* has now been filled by several important specimens, including KNM-ER 1813⁷¹. ER 1813 shows incipient supraorbital tori depressed in the glabellar region, a post-toral sulcus, a post-toral temporal ridge (similar to OH 9), a slightly more anteroposteriorly elongated cranium than specimens such as OH 13, a slight torus occipitalis and a mid-facial region slightly more

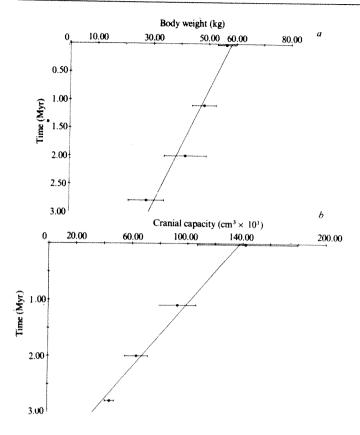


Fig. 4 a, Means of body weight estimates of hominid taxa in the 'gracile' lineage through time. The mean of A. africanus body weight estimates (27.7 kg) is plotted at 2.8 Myr BP and is an average of (in kg): 18.65, 25, 32 and 35.3 (refs 139, 140, 4, 141 respectively). The mean for H. habilits (41.8 kg) is plotted at 2.0 Myr BP and is an average of (in kg): 31.3, 40, 43 and 52.8 (refs 139, 140, 4, 141 respectively). The mean for H. erectus (48.5 kg) is plotted at 1.1 Myr BP and is an average of (in kg): 42.5, 50 and 53 (refs 139, 140, 4 respectively). The mean for H. sapiens (56.4 kg) is plotted at 0.0 Myr BP and is an average of (in kg): 52.2, 57.0 and 60 (refs 139, 140, 4 respectively). Bars represent 1 s.d. on each side of the mean. Regression line is BW = 58.25 - 9.9, r = -0.97, where BW is body weight and t is time in Myr. The trend appears to be gradual from A. africanus to H. sapiens.

b, Means of cranial capacity estimates of hominid taxa in the 'gracile' hominid lineage through time. Times for taxa are as in Fig. 4 legend. The mean of A. africanus (443 cm³) is from (in cm³): 450 for AL 288-1 and 385 for AL 166-9 (ref. 54); 485 for Sts 5, 436 for Sts 19/58, 428 for Sts 60 and Sts 71, 500 for MLD 1 and 435 for MLD 37/38 (ref. 139). The H. habilis mean is from '13° (in cm³): 687 for OH 7, 650 for OH 13, 590 for OH 24, 752 for ER 1470 and 509 for ER 1813. The H. erectus mean is from (in cm³): 1067 for OH 9, 727 for OH 12 (ref. 139) and 850 for ER 3733 (ref. 79); 850 for Sangiran 1, 775 for Sangiran 2, 890 for Sangiran 3, 750 for Sangiran 4, 975 for Sangiran 6, 915 for Sangiran 7, 1,030 for Ckt 2, 915 for Ckt 3, 1,225 for Ckt 10, 1,015 for Ckt 11 and 1,030 for Ckt 21 (ref. 140). The H. sapiens mean is estimated at 1,420 cm³ as midway between means for H.s. neander-thalensis 140 and H.s. sapiens 140. Bars for H. sapiens represent observed range 140 and for other taxa represent 1 s.d. on each side. Regression line is CC = 1378.6 - 352.1t, r = -0.99, where CC = cranial capacity and r = time in Myr. Similar results have been obtained by other authors 142.143.

prognathous than ER 1470. It nevertheless retains many early *Homo* characteristics such as lack of ectocranial crests, relatively straight nasals and a small temporal fossa. Its intermediate temporal position, as discussed above, and intermediate morphology can be interpreted as linking the earlier *Homo* populations and later *H. erectus*.

One *H. erectus* specimen from Koobi Fora (KNM-ER 3733) is claimed¹ to be earlier than non-African *H. erectus*, but morphologically within the range of Choukoutien *H. erectus*. Thus similarities between KNM-ER 3733 and Choukoutien *H. erectus* are said to provide an example of stasis after a rapid evolutionary speciation event. As discussed above, ER 3733 comes from the lower portion of the Upper Member of the

Koobi Fora Formation, with a probable date of about 1.6 Myr. If we exclude the Indonesian Putjangan specimens, it is older than other Old World H. erectus: OH 9, Bed II, Olduvai at 1.3 Myr BP (ref. 72); P996-17, Member K at Omo at 1.4 Myr (ref. 15). Trinil at ~ 1.3 Myr and later¹⁹; and Melka Kontoure at ~ 1.0 Myr BP (ref. 73). Choukoutien is not well dated radiometrically, but has been ascribed on faunal grounds to ages ranging from Early to Late Pleistocene 74. Fauna from the main hominid levels (such as Locus L, layers 8-9) is typically Middle Pleistocene and correlative with the European 'Cromerian'. Kahlke and Chou75 have reported Ailuropoda, Macaca robustus and Elephas cf. namadicus, taxa characteristic of the late Early Pleistocene and early Middle Pleistocene⁷⁶, from the lower hominid-bearing levels at Choukoutien. Kahlke⁷⁷ states that E. namadicus is part of the Asian Middle Pleistocene faunas which correlate indirectly with Süssenborn ('Mindel'-'Elster') faunas of eastern Europe. The Early/Middle Pleistocene boundary is now conventionally taken⁷⁸ as 0.69 Myr BP and on faunal grounds most of the Choukoutien hominids would post-date this datum. Although the entire assemblage spans a considerable time range, the main finds of Choukoutien H. erectus do in fact appear to be at least 0.8 Myr younger than ER 3733. On present evidence specimens attributed to H. erectus extend from about 1.6 to 0.6 Myr, or younger.

The available evidence does not support the hypothesis of morphological stasis in H. erectus through time. The peak in robusticity in the evolution of H. erectus did not occur with the earliest appearance of the species. The earliest examples of H. erectus possess a relatively longer and flatter face, shorter, thinner and less buttressed cranial vault, smaller cranial capacity, and a thinner and narrower supraorbital torus than later H. erectus. ER 3733, which is the earliest relatively complete example of H. erectus, falls outside and below the range for Choukoutien H. erectus fossils in four of the five measurements provided by Leakey and Walker⁶⁶. Its cranial capacity of about 850 cm3 (estimated by Holloway, quoted in ref. 79) is outside the Peking range (915-1,225 cm³)³³ and is closer to Javanese H. modjokertensis specimens. The supraorbital torus appears thinner than that of later H. erectus specimens such as OH 9, Sangiran 17 and the Choukoutien crania and recalls that of earlier fossil hominids ER 1470, ER 1813 and OH 24. The long, flat, vertically oriented face with a deep sub-nasal area is perhaps a retained primitive feature from an ancestor similar to ER 1470. Table 1 summarizes the morphological continuities to be seen from early to late H. erectus, thus vitiating Gould and Eldredge's contention of stasis in this clade¹

In the case of *H. sapiens*, Gould and Eldredge¹ imply that modern *Homo sapiens* is morphologically similar to the earliest examples of this species. This interpretation would be more defensible if they were referring only to anatomically modern man as *H. sapiens* because, for example, the earliest anatomically modern populations of Europe and Australia over 20,000 yr ago do closely resemble their present-day aboriginal counterparts ^{46,80}. But even here it can be argued that noticeable evolutionary change has occurred ^{81,82}. However, most anthropologists would extend the term *H. sapiens* to include various fossils from the Middle Pleistocene (such as Swanscombe, Steinheim, and Broken Hill) and the earlier Late Pleistocene (the Neandertals).

To test the alternative models of phyletic gradualism or punctuated equilibrium in the evolution of *H. sapiens* we require (as Gould and Eldredge state) reasonable samples with good stratigraphical control. The only area which approaches these conditions in terms of samples with good stratigraphical control is Europe. If phyletic gradualism is a reality and *H. erectus* was gradually transformed into 'archaic' H. sapiens and thence into 'modern' *H. sapiens*, then the earliest examples of *H. sapiens* should show *erectus*-like characteristics. The fossil record should progressively document the appearance of 'modern' features. Indeed, we might even expect there to be fossils with intermediate characteristics.

Table 1 Comparison of morphological traits, chosen on the basis of ability to differentiate hominid taxa, among four key specimens discussed in the text

	A. africanus	Intermediate	H. habilis	Intermediate	H. erectus	Intermediate	H. sapiens
One and a combatter	•		*†	‡		\$	
Cranial capacity	*			t			
Naso-alveolar clivus	*		‡		†		
Incisive alveolar margin		*	‡		Ť		
Canine juga		*					
Subnasal prognathism		*		†	‡		
Midfacial prognathism		*	t		‡		
Size of temporal fossa			*				
Angle of zygomatic process of temporal		*		†‡		VII	
Bizygomatic breadth relative to prosthion-nasion height			*	+			
Hafting of zygomatic process of maxillary			*+1				
Frontal plane concavity of nasals			*+				
Frontal plane concavity of lateral orbital rim			*+	‡		\$	
Frontal angle		*	†	*			
Cranial length relative to biporionic width		*	,				
Facial height relative to cranial length			t		‡		
Thickness of parietal and temporal			,		\$		
Thickness of occipital				†‡	٥		
Anterior protrusion of supraorbital torus			‡	* *	8		
Lateral thickening of supraorbital torus			+		•		Š
Retraction of lateral supraorbital torus							§.
Midline depression of supraorbital torus				†			* 3 - *
Deep post-toral sulcus				1			
Post-orbital constriction				1	†‡		
Anterior temporal line robusticity				‡	1 12	8	
Parasagittal flattening of frontals/sagittal keel			†	+		ā	
Supramastoid crest larger than suprameatal crest			†‡				
Frontal process of zygomatic angled anteriorly downwards	5		†	+	8		
Occipital angle				† ‡	8	8	
Torus occipitalis				Ŧ	‡	8 .	
Torus angularis					+		ğ
Low parietal angle					4		8
Euryon at torus angularis					+		
Deep and wide zygomatics					}		
Mesio-distal diameter of molar row					¥	ė.	
Upper facial breadth relative to orbital height						§	§
Supraorbital and maxillary pneumatization						e	8
Cranial vault height						8	g.
Relatively large diagonal dimensions of parietal							9
Temporal squama short and arched		戈					8
Axis of tympanic plate							9
Nuchal area					§		
Lambda-inion and inion-opisthion chords sub-equal						9	
Broadness of cranial base						§.	
Palate size						§.	

This chart shows the mosaic pattern of evolutionary change in hominids, the clear directionality to be seen in a number of morphological traits, such as cranial capacity, and the fact that there are hominid fossils showing morphology intermediate between accepted hominid species.

* KMN-ER 1470, classified as H. habilis, shows nine traits which are either shared with A. africanus (Sts5, AL 288-1, MLD 37/38) or intermediate between this taxon and other H. habilis (OH 7, OH 13, OH 24, L 894-1, SK 847).

† KNM-ER 1813, a later H. habilis, shows 11 traits characteristic of H. erectus or intermediate between H. erectus and H. habilis.

‡ KNM-ER 3733, classified as early H. erectus, shows 11 traits shared with H. habilis or intermediate between this taxon and other (Asian) H. erectus.

§ The Petralona cranium classified here as an early H. sapiens shows 13 traits either shared with H. erectus or intermediate in morphology.

The European Middle Pleistocene record does provide us with such examples. The fossils from Mauer, Arago, Vertésszöllös, Petralona and Bilzingsleben have been regarded by various workers as belonging to either H. erectus or H. sapiens $^{33,43,45,46,83-85}$. The most complete of the specimens is the Petralona cranium, apparently associated with a Biharian (Cromerian) fauna which would place it as broadly con-temporaneous with the Mauer, Vertésszöllös and perhaps Choukoutien fossils, and earlier than the Bilzingsleben, Swanscombe and Steinheim specimens⁸⁴. If phyletic gradualism operated to transform H. erectus into H. sapiens, a specimen from the middle part of the Middle Pleistocene would be expected to show intermediate or mosaic characteristics when compared with the two species. Table 1 shows that the Petralona cranium displays characteristics typical of each species (if we can accept that Neandertals do in fact belong in the species H. sapiens). In features associated with cranial expansion and dental reduction, it is apparently intermediate. All the earlier Middle Pleistocene European specimens may belong in the species H. sapiens, but placing them within H. erectus would not alter their evolutionary significance. They differ as much from early H. erectus fossils as they do from late H. sapiens

Vlček⁸⁵ has claimed that during the Holsteinian of Europe, *H. erectus* (as at Bilzingsleben) co-existed with *H. sapiens* (as at Swanscombe and Steinheim). Apart from the important ques-

tion of morphological variability to be expected in the Middle Pleistocene, correlations in Europe are now so uncertain that 'Holsteinian' faunas may, in fact, span two or more distinct interglacials covering a period of some 200,000 yr (ref. 86), and a recent study of the Bilzingsleben site suggests that it dates from the penultimate interglacial in Europe rather than the Holsteinian⁸⁷. Similarly, Swanscombe may be separated by a considerable period of time from other sites in the 'Holsteinian' complex. Certainly the morphology of the Bilzingsleben specimens suggests that present concepts of variation within the species *H. erectus* and *H. sapiens* need to be re-examined ⁸⁸⁻⁹⁰.

In the fossil record of the later Pleistocene of Europe we meet the continuing problem of the place of 'Neandertals' in human evolution 91.144,155. Stringer 46 has discussed the European evidence for a gradual emergence of a Neandertal morphology during the later Middle Pleistocene and early Late Pleistocene. Fossils such as Swanscombe and Fontéchevade, previously regarded as part of a 'pre-sapiens' evolutionary lineage separate from that of the Neandertals, are probably part of an early Neandertal lineage. However, European Neandertals may not be the best candidates for the ancestors of anatomically modern H. sapiens in Europe during the middle part of the last glaciation 46.47, notwithstanding much support for the opposite view that there is an evolutionary continuity between these European populations 45.81.83. Until some of the critical specimens in this debate are conclusively dated and the functional significance of

some of the relevant morphological differences better understood, resolution of this problem may lie in the analysis of character states present in Neandertals and other contemporaneous populations 48,90-95.

Thus the evolution of anatomically modern man can at present support either the model of evolutionary gradualism or that of punctuated equilibrium, although the present evidence may favour discontinuity between Neandertal and 'modern' populations in Europe and Southwest Asia. Had Europe been a relatively peripheral area, gradualism in other areas could have led to the evolution of populations of anatomically modern H. sapiens whose genes could have infiltrated into Europe relatively rapidly, producing an apparent example of punctuated equilibrium in the European record.

Punctuated evolutionary change would imply a high variance in the durations or lifetimes of hominid taxa, as the appearance of new taxa is supposedly a function of a rapid and random event. If the taxa A. africanus extended from 3.2 to 2.2 Myr (1.0 Myr), H. habilis from 2.2 to 1.6 Myr (0.6 Myr), H. erectus from 1.6 to 0.6 Myr (1.0 Myr), and H. sapiens appeared at 0.6 Myr, then change does not seem to be dissimilar from one species to the next. If Laetoli A. afarensis at 3.7 Myr is accepted as a forerunner of A. africanus, this species further supports this pattern, with a known species lifetime of 0.5 Myr. For these five taxa, whatever their phylogenetic relationship, there is a mean duration of 0.74 ± 0.24 Myr, indicating similar species lifetimes, as may be expected in a gradualistic model. For an alternative interpretation see Stanley⁹⁶.

Possible cases of punctuated equilibrium in the hominid fossil record

The geologically sudden appearance of robust australopithecines in South and East Africa between 2.1 and 2.0 Myr seems to represent an obvious example of punctuation. The fact that their morphology appears so specialized supports this. However, on closer examination the robust lineage reveals a distinctly gradual trend of evolution. It is the highly specialized and hence accountable morphology of the 'final product' that enables one to recognize the direction of the evolutionary trend and to define the position of each fossil within the sequence.

Rak⁹⁷ explains this trend as related to the forward movement of the origin of the masticatory muscles away from the fulcrum (the articular eminence), on the one hand, and the tucking in of the dental arcade, on the other. Australopithecus boisei is the most evolved species of the sequence. However, some of the A. boisei specimens, whether studied through actual measurements or through observations of their topography, are found to fall within the range of the less specialized species, A. robustus. The direction of evolution of the masticatory system is also detected in the known sample of A. robustus. The topography observed on many fragments (mainly the infraorbital region) again indicates the existence of a morphological sequence. A. robustus and A. boisei are ordered in this sequence, although sufficient differences exist to justify their recognition as distinct taxa. The claims1 that 'there is no direct evidence for gradualism within any hominid taxon' and 'each species disappears looking much as it did at its origin' probably result from a familiarity with only the most well known specimens.

The known sample of A. robustus is probably contemporary with at least part of the time span of A. boisei. Morphologically, however, A. robustus would be expected to be an intermediate stage between the more general australopithecines and the more specialized, hyper-robust A. boisei. One hypothesis to explain the relationship between A. robustus and A. boisei is that the former represents a relic population that continued to exist in South Africa, representing the prototype from which A. boisei evolved.

Some of the specimens which make up the morphological range of species of Australopithecus, that is A. africanus, that existed 0.5 Myr earlier, resemble A. robustus very closely in topographical features of the face. The fairly complete fossil Sts

71 can be considered representative of the robust end of the morphological range, although fragments and isolated teeth of other specimens hint at the possiblity that even more robust individuals existed. Specimens from the other end of the range, such as Sts 52 and TM 1512, exhibit a quite generalized primitive morphology. Rak⁹⁷ suggests that A. africanus may have a greater morphological spread than the sample of A. robustus, not because the former is represented by a larger sample (it is not), but because a longer time span is covered by its sample.

Robust australopithecines probably evolved from earlier, more gracile hominids similar in morphology to A. africanus. Whether the known fossils of this taxon represent the actual ancestral population of later robust australopithecines is debatable (Fig. 2) but A. africanus is clearly morphologically closer to a common australopithecine ancestor than are robust australopithecines. Continuities to be seen from gracile to robust australopithecines therefore do not support a punctuational view of australopithecine evolution.

Other parts of the hominid fossil record suggest that stasis may have predominated over evolutionary change in certain peripheral areas. The Ngandong crania are supposedly Late Pleistocene in age⁷⁴ but show fundamental resemblances to much earlier *H. erectus* specimens^{46,98}. As with the Broken Hill specimen⁹⁹, this apparent Late Pleistocene anomaly may eventually be removed by evidence that the Ngandong fossils are, in fact, of Middle Pleistocene age; but at the moment these fossils provide one of the strongest examples of relative stasis in a peripheral and relict human popoulation.

Evidence from genetic studies

Genetic study of current populations is a major focus of attempts to elucidate patterns and mechanisms of speciation 100-103. The traditional view—that there are large genetic changes, or 'a

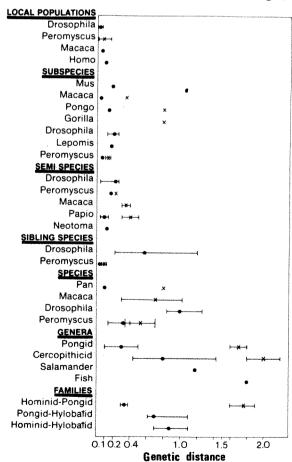


Fig. 5 Range of genetic distances (bottom axis) for the varying taxonomic levels as referenced in Table 2. ×, Fast loci; ●, typical mix of loci.

Table 2 Genetic distance values for taxa compared at different 'levels' of speciation

Addic 2			-		
		D typical	Genetic distance	D	fast
Taxon	Drosophila	Vertebrates	Primates	Peromyscus	Primates
Populations Sub-species Semi-species Species Genera	$0.02 \pm 0.012 \\ 0.186 \pm 0.057 \\ 0.226 \\ 0.451 \pm 0.329$	0.028 ± 0.02 0.163 ± 0.02 0.168 ± 0.01 0.319 ± 0.194 0.706 ± 0.319	0.011 ± 0.012 0.084 ± 0.048 0.163 ± 0.14 0.318 ± 0.233 0.631 ± 0.31	0.066 ± 0.06 0.139 ± 0.009 0.224 0.305 ± 0.257	0.30 ± 0.235 0.477 ± 0.239 0.4 ± 0.086 0.67 ± 0.276 1.36 ± 0.512

The term semi-species is defined by Mayr¹⁰² as species that were recently conspecific and subsequently speciated through geographical isolation and remain mostly allopatric. Hybridization does occur to differing extents (refs 7, 101, 115, 123, 124, 126, 127, 134–138, and J. E. C. and V. M. Sarich, unpublished observations).

genetic revolution', often accompanying speciation events—would be supported if the process occurred in small, peripheral or isolated populations 104-109. Various papers have reviewed the extent of genetic variation in natural populations 110,111 and genomic distances can be quantified between taxa 112,113. Gould and Eldredge have cited structural gene studies of allozymic differences between taxa of differing levels as the major criterion of their model. Ayala 114 and Avise 115 (Table 2) have indeed observed a gap between genetic distances as measured at both the local population level and at the level of sub- and semi-species but Lewontin 116 and White 101 find little genetic distance accumulating during speciation. Many species are less different genetically than populations within the same species, and morphological diversity may not correlate with genic divergence 101,117,118.

The apparent gap between populations and measures between sub- and semi-species is due to a differential weighing of loci with different function 119,120 and different rates of evolution 7,121. Work on primates 121-123 and on rodents 124 demonstrate that this gap disappears when fast-evolving loci are included in the study (Table 2, Fig. 5). In fact allozymic differences between taxa may be highly time-dependent and useful for divergence date estimates 6,7,121,122, an observation not compatible with suggestions of punctuation or genetic revolution.

Hence there is no obvious correlation between the degree of genetic differentiation and speciation; the development of reproductive isolation, by whatever mode, may be accompanied by little, some, or substantial allozymic change.

Electrophoresis of gene products may well be too insensitive a method to detect the genetic changes accompanying speciation. Nucleic acid hybridization shows that species differ by quantitatively small values of base-pair difference, much less than 1% in unique sequence DNA. Sub- and semi-species differ by even less 125.

So what is the genetic basis of speciation? Microgenic changes may result cumulatively in reproductive isolation. Single gene mutations at structural loci may have a profound effect on the phenotype and correspondingly to speciation events. Alternatively, not all genes may be involved in a genomic reorganization. A model of genetic 'transiliance' in which only a few polygenic systems each with major and minor genes undergo change is also possible. Thus a change in only a small fraction of the genes could contribute to a genomic revolution.

Also, true revolution may be found at the chromosomal level. Chromosomal rates of evolution seem to correlate with the degree of morphological evolution and rates of speciation ¹⁰⁷. Such change would be macrogenic and would best be sought at the level of satellite DNA, quantity of DNA per locus, chromosomal rearrangements and regulatory loci mutations ¹⁰¹.

Conclusions

We should not be surprised to find specific instances of explosive and gradual evolution at both the molecular and organismal level. The questions at hand are whether punctuation or saltation is the predominant mode of evolution as Gould and Eldredge assert¹, and whether human evolution is a case which confirms the general model of punctuated equilibrium at either the genetic or morphological level.

The specific course of genomic evolution along the hominid lineage can be conjectured. Since the time of divergence, humans and chimps have come to differ by about 1% at each gene¹²⁶, a value characteristic of differences generally found between sibling species^{122,126,127}. On the other hand, humans and chimps differ by some nine pericentric inversions¹²⁸. Fixation of such inversions is facilitated by low effective breeding size of populations or inbreeding ^{101,129}. Conditions for fixation of such inversions would be optimal during speciation by bottlenecking. Thus, it is highly probable that a few bottlenecks in effective breeding size, some resulting in speciation, without apparent major effects on the phenotype may have occurred along the hominid lineage.

Perhaps the most interesting area of evolution—the origins of the human lineage—can only be cautiously dealt with due to incomplete evidence. It may be the most likely period of time when rapid or explosive evolution may have occurred. The earliest undoubted hominids in the fossil record, from Hadar and Laetoli9, are quite primitive and date to somewhat less than 4.0 Myr. The molecular clock suggests a hominid-pongid divergence somewhat before this 6.7. Thus, the hominid transition could have been characterized by extremely rapid morphological change (the evolution of bipedality), and by a period of low population numbers, or bottlenecks, with most of chromosomal evolution, genic reorganization, or allelic substitutions occurring in rapid sequence. It is here that the evidence fails us in being particularly incomplete, only partially suggestive and open to many different interpretations 130,131, and it may be that as more evidence becomes available even this possible case of punctuation may be like the cheshire cat and disappear until only its smile is left.

In summary, the evidence that we, unlike some others 96,132,133, have adduced from the fossil record does not support the model of punctuated equilibrium for hominid evolution. Apparent cases of punctuated equilibrium in the fossil record of hominids are due to one of three causes. Specimens are certainly misdated, as in the case of KNM-ER 1470, possibly or probably misdated, such as the Ngandong specimens, or open to very different morphological interpretations, for example, regarding KNM-ER 1813 as a gracile australopithecine versus a member of the genus Homo. A review of the morphology of hominid fossils with their most probable dates strongly suggests a closer approximation to a gradualistic model. There is demonstrable change within hominid taxa through time as well as continuities from one species to its supposed descendent species. There is little or no support for the belief that the hominid fossil record is complete. Thus gaps in the record are most likely artefacts of the processes of fossilization and discovery and not true palaeobiological discontinuities. Further assessment of the model of punctuated equilibrium must await a more rigorous definition of such parameters as the temporal difference between punctuation and fast gradualism and the degree of morphological change considered to constitute either a gap or stasis. If a finer, continuous analysis were possible, it

would probably reveal that gradualism is, itself, an average of periods of horotely (fast change) and bradytely (slow change). Evolution of the Hominidae is still most reasonably interpreted by a model of phyletic gradualism with varying rates.

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- Gould, S. J. & Eldredge, N. Paleobiology 3, 115-151 (1977).
 Boaz, N. T. A. Rev. Anthrop. 8, 71-85 (1979).
 Pilbeam, D. et al. Nature 270, 689-695 (1977).
 Pilbeam, D. R. & Gould, S. J. Science 186, 892-901 (1974).
 Sarich, V. M. & Cronin, J. E. in Molecular Anthropology (eds Goodman, M. & Tashian, R.)
 Sarich, V. M. & Cronin, J. E. in Molecular Anthropology (eds Goodman, M. & Tashian, R.)
 Sarich, V. M. & Cronin, J. E. in Molecular Anthropology (eds Goodman, M. & Tashian, R.)
 Johanson, D. C., White, T. D. & Coppens, Y. Kirilandia 28, 1-14 (1978).
 Johanson, D. C. & White, T. D. Science 202, 321-330 (1979).
 Howell, F. C. in Evolution of African Mammals (eds Maglio, V. M. & Cooke, H. B. S.)
 154-258 (Harvard University Press, Cambridge, Massachusetts, 1979).
 Hughes, A. R. & Tobias, P. V. Nature 265, 310-312 (1977).
 Howell, F. C. & Coppens, Y. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y. et al.) 522-532 (University of Chicago Press, Chicago, 1976).
 Leakey, R. E. F. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y. et al.)

- (eds Coppens, Y. et al.) 522-532 (University of Chicago Press, Chicago, 1976).
 Leakey, R. E. F. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y et al.) 476-483 (University of Chicago Press, Chicago, 1976).
 Leakey, L. S. B., Tobias, P. V. & Napier, J. R. Nature 202, 7-9 (1964).
 Boaz, N. T., Howell, F. C., Coppens, Y. & Senut, B. (in preparation).
 Clarke, R. J. & Howell, F. C. Am. J. phys. Anthrop. 37, 319-336 (1972).
 Clarke, R. J. & Afr. J. Sci. 72, 46-49 (1977).
 von Koenigswald, G. H. R. Proc. 8th int. Congr. anthrop. ethnol. Sci. 1, 104-105 (1970).
 Ninkovich, E. & Burckle, L. H. Nature 275, 306-308 (1978).
 Leakey, M. G. & Leakey, R. F. Kaohi Fora Research Project Vol. 1 (Clarendon, Oxford).

- 20. Leakey, M. G. & Leakey, R. F. Koobi Fora Research Project Vol. 1 (Clarendon, Oxford,

- Leakey, M. G. & Leakey, R. J. Holland, 1978.
 Tobias, P. V. & von Koenigswald, G. H. R. Nature 204, 515-518 (1964).
 Howell, F. C. Curr. Anthrop. 6, 399-401 (1965).
 Le Gros Clark, W. E. The Fossil Evidence for Human Evolution (University of Chicago
- 24. Robinson, J. T. in Evolution und Hominisation (ed. G. Kurth) 120-140 (Fischer, Stuttgart,

- RODINSON, J. I. IN EVOLUTION and FROMINISMIND (CG. G. RUILI) 120-1-10 (Society, Guilley, 1962).
 Brace, C. L. The Stages of Human Evolution (Prentice-Hall, New Jersey, 1965).
 Walker, A. C. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y. et al.) 484-489 (University of Chicago Press, Chicago, 1976).
 Leakey, R. E. F. Am. Sci. 64, 174-178 (1977).
 Walker, A. C. & Leakey, R. E. F. Scient. Am. 239, 54-66 (1978).
 Oakley, K. P., Campbell, B. G. & Molleson, T. I. Catalogue of Fossil Hominids. Part I: Africa, 2nd edn (British Museum Natural History, London, 1977).
 Leakey, L. S. B. & Leakey, M. D. Nature 202, 3-9 (1964).
 Bishop, W. W., Pickford, M. & Hill, A. Nature 258, 204-208 (1975).
 Vrba, E. S. Nature 254, 301-304 (1975).
 Vrba, E. S. Nature 254, 301-304 (1975).
 Weidenreich, F. Palaeont. Sinica D7, 3, 1-163 (1936).
 Weidenreich, F. Palaeont. Sinica D1, 1-180 (1937).
 Weidenreich, F. Palaeont. Sinica D5, 1-150 (1941).
 Weidenreich, F. Palaeont. Sinica D10, 1-291 (1943).
 Jaeger, J. J. in Afler the Australopithecines (eds Butzer, K. W. & Isaac, G. L.) 399-418

- Jaeger, J. J. in After the Australopithecines (eds Butzer, K. W. & Isaac, G. L.) 399-418 (Mouton, The Hague, 1975).
 Mturi, A. A. Nature 262, 484-494 (1976).
 Conroy, G., Jolly, C. J., Cramer, D. & Kalb, J. Nature 276, 67-70 (1978).
 Leakey, L. S. B. in The Origin of Homo supiens (ed. Bordes, F.) (UNESCO, Paris, 1972).

- Delson, E., Eldredge, N. & Tattersall, I. J. hum. Evol. 6, 263-278 (1977).
 Campbell, B. G. in Classification and Human Evolution (ed. Washburn, S. L.) (Methuen, London, 1964).
- Pilbeam, D. R. in After the Australopithecines (eds Butzer, K. W. & Isaac, G. L.) 809-856 (Mouton, The Hague, 1975).
 Wolpoff, M. H. in Determinants of Mandibular Form and Growth (ed. McNamara, J. A.)
- 1-64 (Center for Human Growth and Development, Ann Arbor, 1975).

 Stringer, C. B. in Recent Advances in Primatology Vol. 3 (eds Chivers, D. J. & Joysey, K. A.) 393-418 (Academic, London, 1978).

 Howells, W. W. in Paleoanthropology: Morphology and Paleoecology (ed. Tuttle, R.)
- Howells, W. W. in Paleoanthropology: Morphology and Paleoecology (ed. 1uttle, R.) 389-408 (Mouton, The Hague, 1975).
 Santa Luca, A. P. J. hum. Evol. 7, 619-636 (1978).
 Behrensmeyer, A. K. Yb. phys. Anthrop. 19, 36-50 (1976).
 de Heinzelin, J. (ed.) Sedimentary Formations of Pliocene and Early Pleistocene Age in the

- Omo Basin, Ethiopia (Tervuren, Mus. R. Afr. Cent., in the press). Boaz, N. T. Science 206, 592-595 (1979). Gould, S. J. Nat. Hist. 88, 40-44 (1979).

- Gould, S. J. Nat. Hist. 88, 40-44 (1979).
 Lovejoy, C. O. Am. J. phys. Anthrop. 50, 460 (1979).
 Kimbel, W. H. Am. J. phys. Anthrop. 50, 454 (1979).
 Leakey, M. D. et al. Nature 262, 460-466 (1976).
 Cerling, T. E. et al. Nature 279, 118-121 (1979).
 Leakey, R. E. F. Nature 242, 447-450 (1973).
 Findlater, I. thesis, Univ. London (1977).

- Fitch, F. J. & Miller, J. A. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y. et al.) 123-147 (University of Chicago Press, Chicago, 1976).
 Cooke, H. B. S. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y. et al.) 251-263 (University of Chicago Press, Chicago, 1976).
 White, T. D. & Harris, J. M. Science 198, 13-21 (1977).
 Curtis, G. H. Nature 258, 395-398 (1975).

- Drake, R. E. et al. Nature 283, 368-372 (1980). Gleadow, A. J. W. Nature 284, 225 (1980).
- Shuey, R. T. et al. in Geological Background to Fossil Man (ed. Bishop, W. W.) 103-124 (Scottish Academic, Edinburgh, 1978).
 Leakey, R. E. F. & Walker, A. C. Nature 261, 572-574 (1976).
- Findlater, I. (in preparation).
- Findater, I. (in preparation).
 Leakey, M. D. Natn. Geogr. 155, 446-457 (1979).
 Boaz, N. T. & Howell, F. C. Am. J. phys. Anthrop. 46, 93-108 (1977).
 Tobias, P. V. Z. Morph. Anthrop. 69, 225-265 (1978).
 Day, M. H. et al. Am. J. phys. Anthrop. 45, 369-436 (1976).

- 72. Hay, R. L. Geology of the Olduvai Gorge (University of California Press, Berkeley, 1976).

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- Chavaillon, J. & Coppens, Y. Bull. Mem. Soc. Anthrop. Paris 8, 125-128 (1975).
 Oakley, K. P., Campbell, B. G. & Molleson, T. I. Catalogue of Fossil Hominids, Part III: Americas, Asia and Australia (British Museum Natural History, London, 1975).
 Kahlke, H. D. & Chou, B. S. Vertebr. Palasiat. 5, 212-240 (1961).
 Pope, G. G. Pap. Kroeber anthrop. Soc. 50, 63-73 (1977).
 Kahlke, H. D. in After the Australopithecines (eds Butzer, K. W. & Isaac, G. Ll.) 309-374 (Mouton, The Hague, 1975).
- Butzer, K. W. & Isaac, G. Ll. (eds.) After the Australopithecines (Mouton, The Hague, 1975). Rightmire, G. P. in Current Argument on Early Man (ed. Königsson, L.-K.) 70-85 (Pergamon, Oxford, 1980).
- (Pergamon, Oxford, 1980).
 Thorne, A. G. in The Origin of the Australians (eds Kirk, R. L. & Thorne, A. G.) 95-112 (Humanities, New York, 1976).
 Frayer, D. W. Evolution of the Dentition in Upper Paleolithic and Mesolithic Europe (University of Kansas Press, Lawrence, 1978).
 Brace, C. L. Cur. Anthrop. 21, 141-164 (1979).
 Brace, C. L. Cur. Anthrop. 5, 3-43 (1964).
 Stringer, C. B., Howell, F. C. & Melentis, J. K. J. archaeol. Sci. 6, 235-253 (1979).
 Vlček, E. J. hum. Evol. 7, 239-251 (1978).
 Kukla, G. I. Earth Sci. Rem. 13, 307-374 (1977).

- Vicek, E. J. hum. Evol. 7, 239-251 (1978).
 Kukla, G. J. Earth Sci. Rev. 13, 307-374 (1977).
 Harmon, R. S., Glazek, J. & Howak, N. Nature 284, 132-135.
 Wolpoff, M. H. J. hum. Evol. 9, 338-358 (1980).
 Stringer, C. B. Anthropologie (Brno, in the press).
 Stringer, C. B. & Trinkaus; E. in Aspects of Human Evolution (ed. Stringer, C. B.) 129-165
 (Taylor and Francis, London, 1981).

- (Taylor and Francis, London, 1981).

 91. Trinkaus, E. Am. J. phys. Anthrop. 44, 95-104 (1976).

 92. Trinkaus, E. J. hum. Evol. 6, 231-235 (1977).

 93. Trinkaus, E. & Howelis, W. W. Scient. Am. 241, 118-133 (1979).

 94. Smith F. H. Am. J. phys. Anthrop. 48, 523-532 (1978).

 95. Hublin, J.-J. Cr. hebd. Séanc. Acad. Sci., Paris 287, 923-926 (1978).

 96. Stanley, S. M. Macroevolution (Freeman, San Francisco, 1979).

 97. Rak, Y. thesis, Univ. California, Berkeley (1981).

 98. Santa Luca, A. P. The Ngandong Fossil Hominids (Yale University Publications in Anthropology no. 78, 1980).

 99. Klein, R. G. Nature 244, 311-312 (1973).

 100. Bush, G. A. Rev. ecol. Syst. 6, 339-364 (1975).

- Bush, G. A. Rev. ecol. Syst. 6, 339-364 (1975).
 White, M. J. D. Modes of Speciation (Freeman, San Francisco, 1978).
- Mayr, E. Animal Species and Evolution (Belknap, Cambridge, 1978).

 Mayr, E. Populations, Species and Evolution (Belknap, Cambridge, 1970).

 Wright, S. Genetics 16, 97-159 (1931).

 Wright, S. Am. Nat. 74, 232-248 (1940).

- Dobzhansky, T. The Genetics of the Evolutionary Process (Columbia, New York, 1970). Wilson, A. C., Bush, G. L., Case, S. M. & King, M. C. Proc. natn. Acad. Sci. U.S.A. 72, 5061–5065 (1975).
- Carson, H. L. Stadler gen. Symp. 3, 51-70 (1973).
- 109. Carson, H. L. Populat. Genet. Monogr. no. 3 (ed. Morton, N. E.) (University of Hawaii,
- 110. Powell, J. R. Evol. Biol. 8, 79–119 (1975).
 111. Nevo, E. Theor. Populat. Biol. 13, 121–177 (1978).
- 112. Selander, R. K. in Molecular Evolution (ed. Ayala, F. J.) 21-45 (Sinauer, Sunderland,
- 113. Nei, M. Am. Nat. 106, 385-398 (1972)
- Ayala, F. J. in Evolutionary Biology (eds Dobzhansky, T., Hecht, M. K. & Steere, W. C.)
 1-78 (Plenum, New York, 1975). 115. Avise, J. in Molecular Evolution (ed. Ayala, F. J.) 106-122 (Sinauer, Sunderland, 1976).
- Lewontin, R. C. The Genetic Basis of Evolutionary Change (Columbia, New York, 1974).
 Avise, J. Proc. natn. Acad. Sci. U.S.A. 74, 5083-5087 (1977).
 Templeton, A. R. Evolution 34, 719-729 (1980).
 Gillespie, J. H. & Langley, C. H. Genetics 76, 837-848 (1974).

- 120. Johnson, G. B. in Molecular Evolution (ed. Ayala, F. J.) 46-59 (Sinauer, Sunderland,
- 1974). Sarich, V. M. Nature 265, 24-28 (1977)
- Sarich, V. M. Nature 265, 24-28 (1977).
 Cronin, J. E., & Meikle, E. Syst. Zool. 28, 259-269 (1979).
 Cronin, J. E., Cann, R. & Sarich, V. M. in The Macaques: Studies in Ecology, Behavior and Evolution (ed. Lindburg, D.) 31-51 (Van Nostrand Reinhold, New York, 1980).
 Zimmerman, E. G., Kilpatrick, C. W. & Hart, B. J. Evolution 32, 565-579 (1978).
 Benveniste, R. E. & Todaro, G. J. Proc. natn. Acad. Sci. U.S.A. 71, 4513-4518 (1974).
 King, M. C. & Wilson, A. C. Science 188, 107-116 (1975).
 Bruce, E. J. & Ayala, F. J. Evolution 33, 1040-1056 (1979).
 Yunis, J. J., Sawyer, J. R. & Dunham, K. Science 208, 1145-1148 (1980).
 Bush, G. L., Case, S. M. Wilson, A. C. & Patton, L. J. Proc. natn. Acad. Sci. U.S. A. 74, 148 (1980).

- 129. Bush, G. L., Case, S. M., Wilson, A. C. & Patton, J. L. Proc. natn. Acad. Sci. U.S.A. 74, 3942-3946 (1977).
- Zihlman, A., Cronin, J. E., Cramer, D. & Sarich, V. M. Nature 275, 744-746 (1978).
 Lovejoy, C. O. Science 211, 341-350 (1981).
 Vrba, E. S. Afr. J. Sci. 76, 61-84 (1980).
 Eldredge, N. & Tattersall, I. Contrib. Primat. 5, 218-242 (1975).

- Anderson, J. E. & Giblett, E. R. Biochem. Genet. 13, 189-212 (1975).
 Nozawa, K., Shotake, T., Ohkura, Y., Kitajima, M.& Tanabe, Y. Contemp. Primat. 5, 75-89 (1975).
- Nozawa, K., Shotake, T., Otkuro, Y. & Tanabe, Y. Jap. J. Genet. 52, 15-30 (1977).
- Shotake, T., Nozawa, K. & Tanabe, Y. Jap. J. Genet. 52, 223-237 (1977) Bruce, E. J. thesis, Univ. California.
- 139. Holloway, R. L. in Earliest Hominids of Africa (ed. Jolly, C. J.) 397-402 (Duckworth, London, 1978).

 140. Tobias, P. V. The Brain in Hominid Evolution (Columbia University Press, New York,
- 1971).
- 141. McHenry, H. M. Am. J. phys. Anthrop. 45, 77-84 (1976).
 142. Lestrel, P. E. & Read, D. W. J. hum. Evol. 2, 405-411 (1973).
 143. Lestrel. P. E. J. hum. Evol. 5, 207-212 (1975).
- Wolpoff, M. H. Nature 289, 823 (1981). 145. Stringer, C. B., Kruszynski, R. G. & Jacobi, R. M. Nature 289, 823-824 (1981).

ARTICLES

Depth of geological contrast across the West African craton margin

J. C. Briden, D. N. Whitcombe, G. W. Stuart & J. D. Fairhead

Department of Earth Sciences, The University of Leeds, Leeds LS2 9JT, UK

C. Dorbath & L. Dorbath

Office de la Récherche Scientifique et Technique Outre-Mer, 24 Rue Bayard, 75008 Paris, France

Anomalies in teleseismic arrivals at stations astride the West African Craton margin in Senegal are large and systematic for rays which have passed beneath the craton margin. Lateral variation in seismic velocity structure beneath the margin persists to several hundred kilometres depth. The major relative delay time and slowness—azimuth anomalies fit a simple model of a steep boundary in the mantle aligned with the major gravity anomaly associated with the craton margin. The mantle beneath the Mauritanide orogenic belt has lower P-velocity than the adjacent craton between ~80 and 220 km, but higher average velocity both above and below that depth range.

IF mantle structure could simply be categorized as subcontinental and sub-oceanic, there would be no consensus over whether lateral differences persist to depths greater than ~ 220 km (refs 1-5). Seismological and other evidence indicate a more complex regional characterization of mantle structure. Significant lateral differences occur down to at least 500 km in the vicinity of many active subduction zones^{6,7}. Theory of formation of orogenic belts at convergent plate margins requires the persistence of subduction, and hence of lateral variations to great depths, throughout the period of orogenesis. Surface wave data from Tertiary⁸ and older orogenic belts⁹ suggest that shear wave velocity structure beneath such belts differs from that beneath both ancient cratons and modern oceans. Global and regional teleseismic delay times 10,11 and heat flow 12,13 have been taken to indicate lithospheric thicknesses > 200 km in places. Thus differences which arose at the time of orogenesis have persisted for hundreds of millions of years after the cessation of orogenic activity so that determination of present-day seismic structure of ancient orogenic belts may clarify the geodynamics of orogenesis.

We report here preliminary results for temporary teleseismic arrays laid out across the junction of a Precambrian craton with a Phanerozoic orogenic belt to detect lateral differences in mantle seismic velocity structure and to determine whether a boundary between these two regions is detectable to mantle depths.

Location and design of field experiment

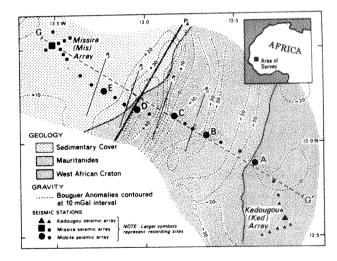
Figure 1 shows the site of the experiment in Eastern Senegal, where the western margin of the West African craton ($\sim 2,000$ Myr and older) abuts the Mauritanide orogenic belt of late Palaeozoic age (~ 250 Myr).

There are differences of up to 1.0 s in teleseismic delay times between seismic stations on the craton (Kedougou, Fig. 1) and on the orogenic belt¹⁴. The major long-wavelength Bouguer gravity anomaly¹⁵ parallel to the craton margin is characteristic of many craton margins elsewhere ^{16,17}. These data suggest that lateral differences across the margin may persist at least into the uppermost mantle.

Two short period vertical (SPz) seismic arrays, each of 20 km aperture, were operated for 6 months: the L-shaped nine station Kedougou (Ked) array on the craton and the cross-shaped eight station Missira (Mis) array centred 180 km to the north-west on the orogenic belt provided travel-time and slowness-data necessary for three-dimensional interpretation of the structure beneath and between them. A mobile linear array of three or four SPz seismometers was placed for 1 month in each of five

successive positions A-E (Fig. 1) to provide records at 10-km spacing along the whole profile between the Ked and Mis arrays. A multichannel analogue tape recorder at the central station of each array recorded an adjacent three component set of short-period seismometers and a single vertical long-period seismometer together with the radio-telemetered signals from the outstations of each array. The internal time base of each recorder was made absolute by recording time signals from BBC World Service transmissions.

All recorded events were digitized at 50 samples per s, and relative arrival times were picked by waveform matching producing overall accuracy of better than 0.05 s. Epicentral



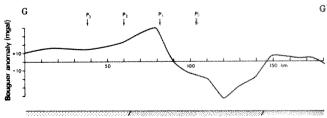


Fig. 1 Part of Senegal, showing the seismic arrays, the Bouguer gravity contours 15, and the Bouguer anomaly profile along the line GG'. Po to P4 are examined positions of a postulated boundary separating cratonic and orogenic velocity structures.

information was taken from USGS PDE bulletins and predicted arrival times were computed utilizing the programme GEDESS¹⁸ with the Jeffreys-Bullen travel-time tables¹⁹. To date 550 seismic events have been identified on the analogue tapes, of which 330 originated in the teleseismic P window, 120 were core phases and the remainder were of local origin. This report considers a subset of the SPz records at the main Ked and Mis arrays, of teleseismic events for which delay times and apparent velocities have been determined, together with some delay time data from the mobile array.

Results

The delay time differences between the central stations of the Mis and Ked arrays are plotted as a function of epicentral distance and azimuth in Fig. 2a. The main features are: rays into the Mis array on the orogenic belt from almost all azimuths and teleseismic distances are slower (positive delays) relative to those into the Ked array on the craton. However, the magnitude of the delays is sensitive to distance and azimuth; between azimuths of $\sim 40^\circ$ and 70° there is a rapid decrease in relative delay time from +0.8 to -0.2 s. The maximum relative delays (+0.8 s) occur for events from the north-east. For westerly azimuths, the delay times decrease with increasing distance from 0.7 s at $\Delta = 60^\circ$ to 0.3 s at $\Delta = 80^\circ$. The relative delays have also been determined for a set of PKIKP core phases in the distance ranges $174^\circ < \Delta < 178^\circ$ and show a similar constant delay of +0.3 s.

Variations of relative delay time along the whole profile are shown in Fig. 3. Each nuclear test site (Fig. 3a-c) can be regarded as a single repeated source. The Kazakh data (Fig.

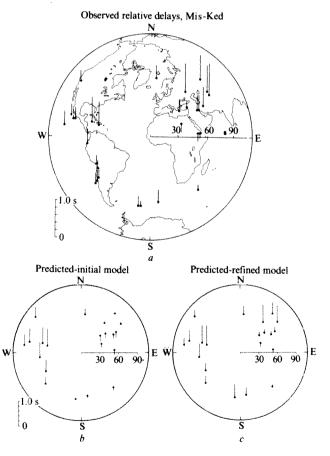
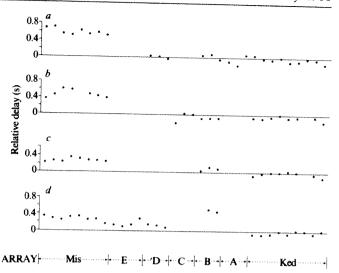


Fig. 2 Teleseismic delay time differences Mis-Ked between the Missira and Kedougou arrays as a function of the position of the source; a, observed; b, predicted by ray tracing through the starting model of Fig. 6a in which the craton boundary is at position P_3 and vertical; c, predicted by ray tracing through the refined model of Fig. 6b in which the craton boundary is at position P_2 and dips at 85° to the west.



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Fig. 3 Teleseismic delay time profiles for underground nuclear tests at a, East Kazakh $\Delta=80^\circ$, $\phi=41^\circ$; b, West Kazakh $\Delta=61^\circ$, $\phi=42^\circ$; c, Southern Nevada $\Delta=92^\circ$, $\phi=309^\circ$; and d for PKIKP phases in the range $174-178^\circ$.

3a, b) show a sharp westerly increase in relative delay within array E, ~ 80 km west of the outcropping margin. The PKIKP phases (Fig. 3d) have all travelled by similar paths through the receiver structure; they do not show the same pattern of relative delay, though they do show an anomalous (though poorly defined) delay within array B.

Slowness-azimuth anomalies at the Ked array (Fig. 4b) are small and non-systematic from all azimuths except the west; for westerly paths (for rays which have passed beneath the craton-orogenic margin) slownesses are anomalously high and seem to have been refracted across a structure striking NNE. At the Mis array the dominant feature (Fig. 4a) is southerly slowness-azimuth anomalies which are thought to be due to a northerly dipping sedimentary-basement interface.

When the effect of this interface is 'stripped-off' rays from only two sectors appear anomalous (Fig. 4c). Northeasterly arrivals, which have traversed beneath the craton margin, seem to have been perturbed by a NNW-striking structure—slowness being anomalously low for the steepest arrivals but anomalously high for arrivals at larger incidence. Anomalies which persist for azimuths close to 280° could be due to lateral variation in the deep structure of the orogenic belt.

Two-dimensional velocity models

Because the regional strike of the gravity anomaly, the craton margin and the orogenic structure are generally constant and the slowness anomalies follow their local variation, we can treat the seismic data two-dimensionally on a regional scale. In our velocity models we adopt a compromise strike of our models (P_0-P_3) between the trends of the segments of the line P_4 in Fig. 5.

Models of crustal structure which would produce the observed anomalies all have unacceptable features, notably large lateral velocity contrasts and/or Moho topography which are incompatible with the observed gravity field across the arrays. Hence velocity differences must extend into the upper mantle. The craton has predominantly the higher velocity structure because vertically travelling core phases through the sub-orogenic structure are delayed by 0.3 s. We treat the structure beneath the Mis and Ked arrays as essentially horizontally layered because these arrays are clear of the craton margin and its associated gravity anomaly, and except for ray paths which pass beneath the craton margin, the slowness-azimuth anomaly patterns do not suggest inclined layering at depths²⁰. For such models the azimuth-distance dependence of relative delay times and the drastic change in direction of the slowness-azimuth anomaly vectors for northeasterly arrivals into Mis, both require

Ked array Mis array Mis array stripped Mis array Ked array modelled modelled

Fig. 4 Slowness anomalies for a. Missira; and b, Kedougou arrays²⁰ Expected slowness. Anomalies generated at a single interface are orthogonal to the strike of that interface. In c the effect of basement topography dipping at 348° azimuth is stripped-off by subtraction of a vector 1.7 s deg^{-1} in that direction. In d and e the observations in c and b are modelled by ray tracthrough the velocity structure of Fig. 6c in the position indicated in Fig. 5.

that between certain depth ranges the orogenic belt has the higher seismic velocity structure. The abrupt change in relative delay times along the profile (Fig. 3) and its marked variation with azimuth (Fig. 2a) require that the transition between the sub-cratonic and sub-orogenic velocity structure is both sharp and steep even at mantle depths.

Two-layer models which account for these anomalous features cannot also account for the negative relative delay times for easterly ray paths (Fig. 2a); these can be accommodated if the orogenic crust is thinner and/or has higher velocity than the cratonic crust. Thus to explain all the main features of the data, we need a model with a minimum of three 'layers' in the crust and upper mantle in which the laterally uniform sub-craton and sub-orogen velocity structures are separated by a near vertical boundary; the top and bottom layers have the higher average velocities beneath the orogenic belt and the middle layer has the higher velocity beneath the craton.

Each layer i delays an arrival travelling through the suborogenic structure by a time a, relative to an arrival travelling through the same layer in the sub-cratonic structure: thus a_2 will be positive while a_1 and a_3 are negative. Vertically-travelling core phases travel entirely in either sub-orogenic and subcratonic velocity structures, so that $a_1 + a_2 + a_3 = 0.3$ s. The same relative delay terms a, apply to inclined teleseismic paths, errors due to non-verticality being negligible. The relative delays for these inclined paths will depend on the amount of mixing of the rays through the two structures. For example, the high relative delay of +0.8 s from the north-east is interpreted as being due to rays into Mis crossing the model boundary near the bottom of the middle layer: hence $a_1 + a_2 = 0.8$ s. Similarly, negative relative delays for rays from the east which cross the boundary within the top layer, make $a_1 = -0.2$ s. Thus $a_2 =$ 1.0 s and $a_3 = -0.5$ s.

If h_i is the thickness of the *i*th layer then a_i is related to the

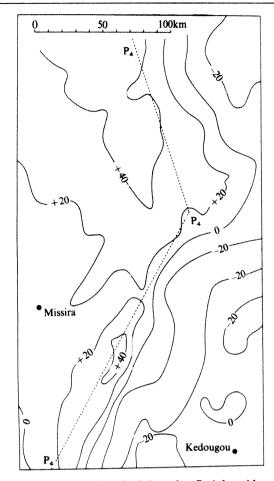


Fig. 5 The position of the seismic boundary P_4 , inferred from the slowness models (Fig. 4d, e) superimposed on the Bouguer anomaly map¹⁵.

average velocities V_{oi} and V_{ci} within that layer beneath the craton and orogen respectively by

$$a_i = h_i \left(\frac{1}{V_{oi}} - \frac{1}{V_{ci}} \right)$$

hence

$$\frac{\delta V_i}{V_i} \simeq \frac{V_i a}{h_i}$$

where V_i is the average velocity within layer i and $\delta V_i = V_{ci} - V_{oi}$.

Interpretation of the data requires the thickness of the layers and the position of the boundary to be determined. To satisfy the slowness anomalies and delay time variations from westerly azimuths, rays to Ked from the west must cross the boundary across a negative velocity contrast (within the bottom layer). The largest positive delay times for rays into Mis from easterly azimuths must cross the boundary near the base of layer 2. These two constraints can be met if the boundary at depth occurs west of its outcropping position—a conclusion supported by the nuclear explosion profile data (Fig. 3). More easterly estimates of the position of this boundary lead to contradictory depth estimates for westerly and northeasterly ray-path data. With the boundary at P3 (Fig. 1) the thickness of the top layer is estimated at 80 km to account for the ray paths which show a relative delay of -0.2 s, assuming that they cross the boundary at the base of layer 1. Arrivals at Ked from the west, with anomalously large slowness, cross such a boundary in the depth range 220-390 km while those to Mis with relative delays of 0.8 s cross the boundary at ~240 km. Hence the base of layer 2 is taken at 230 km and that of layer 3 at 400 km for our starting model. This definition of model layer thicknesses h_i constrains the velocity contrasts in each layer to be -1.7%, +5.4% and -2.8% respectively.

This starting model (Fig. 6a) may now be refined to reconcile all the relative delay time data and to allow for refraction across the boundary, using a 'shooting' ray tracing technique²¹. The fit of predicted and observed delay times for arrivals from the east is further improved by dividing the top layer into two (Fig. 6b). An inadequacy of the starting model is that signals from East Kazakhstan would be internally reflected at the boundary before reaching Mis. This problem would not exist with a lower velocity contrast in the bottom layer, or if the strike of the boundary were bent as in Fig. 5, or if the boundary were inclined at a small angle to the vertical so that it is dipping beneath the orogen. The teleseismic delay times are sensitive to the inclination of the boundary, as rays are travelling steeply through the model. It is difficult to satisfy the large relative delays from the north-east with the smaller relative delays from the west unless the dip of the boundary deviates from the vertical. The refined model (Fig. 6b), which has the surface expression of the boundary at P2, and the dip of the contact as 85° towards the west, displays the main features of the observed relative delay time pattern: compare Fig. 2c with Fig. 2a.

To investigate further the interdependence of model parameters, the relative delay times have been directly inverted by a modification of the least squares technique of Aki et al. ²². This modification divides each layer of the model into two to simulate sub-cratonic and sub-orogenic structure. The block boundary, which need not be vertical, thus simulates the actual lateral velocity discontinuity within each layer. The travel time of each ray through each block is calculated using three-dimensional ray tracing ²¹. This inversion method mimics the tectonic regionalizations common for surface wave dispersion studies ²³. The trial models allowed for velocity differences down to 400 km, either side of a vertical or near vertical boundary in positions P_0 , P_1 , P_2 and P_3 (Fig. 1).

The lowest solution variance occurred with the boundary at a position P_2 , and dipping beneath the orogenic belt between 85° and 80°. This solution accommodated $\sim 80\%$ of the observed data variance. Ray tracing through this inversion model yields predicted delay times similar to those of Fig. 2c. The most consistent feature of all the solutions with variance close to the minimum is the region in which the sub-cratonic structure has the higher velocity: this occurs at a depth $80-200 \, \mathrm{km}$ if the boundary is at the preferred position P_2 consistent with the

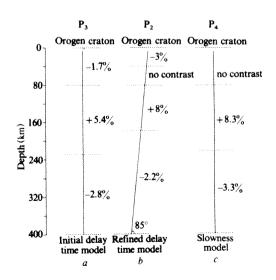


Fig. 6 Velocity contrast models obtained by forward modelling using three-dimensional ray tracing. For all these models the boundary between the craton and orogenic velocity structure is assumed to be planar and positioned at P2, P3 or P4 (see Fig. 1). The dip of the boundary is represented to true scale. The contrasts in the slowness model c must be greater to explain the magnitude of the observed slowness anomalies in Fig. 4.

deductions from forward ray tracing. If the boundary were only 20 km to the east (P1) the equivalent layer would be displaced downwards by 40 km; a comparable upward displacement of this layer would be inferred if the boundary lay 20 km to the west (P₃).

All solutions tend to show higher average velocities beneath the orogenic belt below this layer down to 400 km (layer 3 in the ray tracing modelling). However, neither this 'layer' nor the detailed velocity structure within it is well defined, probably because the velocity perturbations are small and comparable with their standard errors.

Although the inversions do not define a top layer as clearly as the ray tracing approach they do suggest a faster velocity structure within the top 80 km beneath the orogenic belt as inferred from ray tracings. The velocity perturbations determined by the inversions are all smaller than the equivalent velocity perturbations obtained by ray tracing. Simulated data have demonstrated that this inversion method can underestimate the magnitude of velocity anomalies.

Aspects of three-dimensional modelling

The slowness-azimuth anomaly patterns (Fig. 4b, c) require that their causative structure should not be precisely linear. A seismic boundary positioned along the line P4 in Fig. 5 gives a 'best' fit to the data in conjunction with the velocity contrast model in Fig. 6c in that it reproduces the magnitude and direction of the slowness anomalies for westerly paths into Kedougou and the antiparallel slowness anomaly for the NNW event (Fig. 4e) and reproduces the size, orientation and (to some extent) azimuth dependence of the slowness anomalies from the north-east quadrant into Missira (Fig. 4d). Because the line P4 was chosen on the basis of the seismic data, the concordance of its strike with that of the Bouguer anomaly (Fig. 5) is noteworthy. (The coincidence of its position in Fig. 5 is of less significance because position and dip of the model boundary and depths of the layers are to some extent mutually traded-off in the search for acceptable models.)

Although the teleseismic P and PKIKP data recorded by the end-arrays are fitted well by the models, the isolated observation of large relative delays for a PKIKP event recorded within subarray B (Fig. 3d) is not predicted, and the more complex model required must await more data, notably from the mobile

Tectonic interpretation of the seismic models

The contrast in background Bouguer anomaly between the craton and the orogenic belt (Fig. 1) is consistent with the cratonic crust being either thicker or having lower average density (and seismic velocity) than the orogenic crust, and hence is consistent with the uppermost part of the seismic model (Fig. 6).

The seismically-determined craton margin at depth lies $\sim \! 80$ km west of the outcropping margin, implying at least that amount of thrusting and/or depositional overlap of the rocks of the orogenic belt onto the craton. This intervening terrain is composed of a metamorphic belt (correlating with the linear positive Bouguer anomaly) and a zone of unmetamorphosed sediments and volcanics (correlating with the negative Bouguer anomaly, and pinching out with it in the north of Senegal); these correlations suggest that the causes of the linear Bouguer anomalies are within the overlap-overthrust rocks of the upper crust.

The major seismic velocity contrasts along the profile occur within the depth range ~80-200 km where the sub-cratonic structure is the faster by ~6%. This result is consistent with delay time inversion studies of lateral differences between older and younger orogenic regions in North America^{3,24} and in Europe²⁵ with the older structures having the higher velocities. The depth range 80-200 km also correlates well with the S-wave low velocity zone determined by the inversion of surface waves beneath aseismic continents²⁶. We thus prefer to ascribe the 6% P velocity contrast to a low velocity sub-orogenic structure rather than to an anomalously high velocity subcratonic structure.

At depths ≥ 200 km the younger, sub-orogenic, structure has the higher velocity, although the contrast is not as marked $(\sim 3\%)$. It may be argued that such a small contrast may be a random fluctuation in velocity, and not correlatable with the tectonics. However, studies in North America and Europe 3,24,25 have revealed similar features: cratonic North America has 2-3% lower velocity in the depth range 250-400 km than both the Appalachians and Western Cordillera. Similarly the Precambrian Grenville Province has lower velocities than the Palaeozoic Appalachian Province for the depth range 200-350 km (ref. 24). Within Europe in the depth range 250-400 km the P-wave velocity beneath the Alps is up to 2% higher than beneath adjacent areas²⁵. Furthermore the interpretation of NORSAR travel-time data using the Herglotz-Wiechert inversion technique indicates that the Baltic craton has higher velocities than orogenic Europe down to 300 km, while in the depth range 300-420 km it has the lower velocities²⁷

Because of the difficulties²⁹ of determining the depth of origin of teleseismic travel time anomalies, it is worth emphasizing that the principal grounds of our identification of deep lateral variations are: (1) the large epicentral dependence of relative delay times of ~1.0 s require lateral variations in upper mantle structure; (2) slowness anomaly vectors orthogonal to the local strike of the long wavelength gravity anomaly and craton margin imply that the seismic anomalies are inherently associated with that margin. Furthermore a reversal of the anomaly vectors at each array provides direct evidence for a velocity contrast reversal with depth. Such a model will accurately predict the major delay time anomalies.

If density contrasts at depth alternate then isostatic compensation depths may be much deeper (~400 km) than hitherto suspected. The gravity anomaly observed at surface may be regarded as the sum of several individual anomalies of alternate sign, generated in alternate depth zones: because these contributions will partially cancel each other out, the observed anomaly will not fully reflect the magnitude and extent of sub-surface contrasts. Relative teleseismic delay times for vertical paths to cratonic and orogenic stations show a similar effect if the boundary is near vertical: if we had taken delay times for vertical paths only or calculated a mean delay time for each station we would have inferred much less contrast and our models would not have extended to such great depths. It is the larger differential delay times from inclined ray paths which allow this deeper structure to be recognized.

It is beyond the scope of this article to speculate on the possible origin of a mantle boundary such as that inferred here, extending essentially vertically to 400 km into the mantle, but some preliminary points are worth noting. For example, where the lithospheric thickness exceeds ~300 km most sources of continental magmatism must be travelling with the lithospheric plate and must evolve with time, with or without replenishing mechanisms. If lithospheric thickness is variable, then the asthenosphere has considerable topography and variable thickness which would act as controls on plate motion. The near vertical attitude of a boundary within the upper mantle is not obviously compatible with an origin either at a compressional plate margin or an extensional margin; explanations may have to be sought in relation to transcurrent tectonics³⁰ (as has been suggested for the north-west margin of the West African craton on palaeomagnetic grounds³¹) or ideas about the deep thermal structure of plate margins may need revising.

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- Jordan, T. H. Rev. Geophys. 13, 1 (1975).
 Powell, C. Nature 254, 40 (1975).
- 3. Romanowicz, B. A. Geophys. J. R. astr. Soc. 57, 479 (1979).

- Anderson, D. L. J. geophys. Res. 84, 7555 (1979).
- Okai, E. A. & Anderson, D. L. Geophys. Res. Lett. 2, 313 (1975). Engdahl, E. R. Geophys. Res. Lett. 2, 420 (1975).
- Jordan, T. H. J. Geophys. 43, 473 (1977).
- Menke, W. H. Bull. seism. Soc. Am. 67, 725 (1977)
- 9. Haddon, R. A. W. & Husebye, E. S. Geophys. J. R. astr. Soc. 55, 19 (1978).
- Sengupta, M. K. & Julian, B. R. Bull. sepism. Soc. Am. 66, 1555 (1976).
 Fairhead, J. D. & Reeves, C. V. Earth planet. Sci. Lett. 36, 63 (1977).
- 13. Gass, I. G., Chapman, D. S., Pollack, H. N. & Thorpe, R. S. Phil. Trans. R. Soc. A288, 581
- 14. Dorbath, C. & Dorbath, L. Cah, Géophys: ORSTOM No. 16, 27 (1979).
- Cr. an, Y. & Rechenmann, J. Cah. Géophys. ORSTOM No. 6 (1965). Gibbs, R. A. Earth planet. Sci. Lett. 27, 378 (1975).
- 17. Mathur, S. P. Tectonophysics 24, 151 (1974).

- 18. Young, J. B. & Gibbs, P. G. AWRE Rep. No. 0 54/68 (HMSO, London, 1968)
- 19. Jeffreys, H. & Bullen, K. E. Gray Milne Trust (reprinted by Smith & Ritchie, Edinburgh,
- Briden, J. C., Mereu, R. F. & Whitcombe, D. N. Geophys, J. R. astr. Soc. (submitted).
- Whitcombe, D. N. Geophys. J. R. astr. Soc. (submitted).
 Aki, K., Christoffersson, A. & Husebye, E. S. J. geophys. Res. 82, 277 (1977).
 Léveque, J. J. Geophys. J. R. astr. Soc. 63, 23 (1980).
 Taylor, S. R. & Toksöz, M. N. J. Geophys. Res. 84, 7627 (1979).

- Romanowicz, B. A. Geophys. J. R. astr. Soc. 63, 217 (1980)
 Knopoff, L. Tectonophysics 13, 497 (1972).
- 27. England, P. C., Worthington, M. H. & King, D. W. Geophys. J. R. astr. Soc. 48, 71 (1977).
- Smith, M. L., Julian, B. R., Engdahl, E. R., Gubbins, D. & Gross, R. EOS 59, 1130 (1978). Christoffersson, A. & Husebye, E. S. J. geophys. Res. 84, 6168 (1979)
 Sutton, J. & Watson, J. V. Nature 274, 433 (1974).
- 31. Onsott, T. C. & Hargraves, R. B. Nature 289, 131 (1981).

Purified λ regulatory protein cII positively activates promoters for lysogenic development

Hiroyuki Shimatake & Martin Rosenberg

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The bacteriophage λ regulatory protein, cII, has been purified and shown to activate positively RNA transcription from the two phage promoters which coordinately regulate phage lysogenic development. To obtain this protein, the cII gene was cloned into a plasmid vector carrying the strong, regulatable λ phage promoter P_L such that it was overproduced to levels approaching 5% of cellular protein.

WHEN bacteriophage λ infects Escherichia coli cells, the phage can either grow lytically, resulting in the formation of new progeny virus particles which are released on lysis of the host cell, or the phage may enter a state of lysogeny, in which the viral DNA integrates into the host genome and the expression of lytic functions is repressed. In normal physiological conditions, a balance between lytic and lysogenic growth is achieved and appreciable numbers of cells enter both developmental path-

The establishment of the lysogenic state requires the synthesis of two phage functions—repressor (cI), a protein which inhibits transcription of lytic functions, and integrase (int), a protein which catalyses the integrative recombination between the viral DNA and the host genome. The production of both proteins is positively regulated by the phage gene products cII and cIII and also affected by various host-encoded functions 5,6. The synthesis of repressor and integrase is coordinately controlled at the level of transcription from the two λ promoters P_E and P_I (Fig. 1). The DNA structure of these two sites has been determined⁷⁻¹⁰ and their location precisely defined 11,12. Little is known, however, about the precise roles of the cII and cIII gene products in this activation process. In particular, it is not known whether cII works alone or in combination with other factors or whether cII works directly or indirectly to activate transcription from PE and P₁. Only by purifying the components and reconstituting the positive activation system in vitro can we hope to elucidate the role of cII and understand positive activation at these two promoters.

Cloning a 'lethal' gene function

During a normal λ infection, cII protein is synthesized for only a short time and seems to be rapidly turned-over¹³. Thus we set out to clone the \(\lambda \) cII gene into a plasmid vector in such a way that the gene would be efficiently and continually expressed. The general approach involved inserting a 1,300-base pair (bp) DNA fragment carrying the cII gene into a plasmid vector, in such a way that the cII coding region was positioned in proper orientation downstream from an efficient promoter signal for RNA transcription. We reasoned that efficient transcriptional expression of cII from the multicopy vector would result in high-level cII production. The purified 1,300-bp DNA fragment^{7,14} was inserted into several different derivatives of pBR322, each of which contained a known promoter signal of

either phage or bacterial origin positioned upstream from the site of insertion. Recombinants (Amp') were screened by size and restriction analysis for the presence of the fragment. Among multiple independent isolates (>10) which contained the insert, all had the fragment positioned with the cII coding region in opposite orientation to the direction of transcription. It appeared that transcriptional expression of the fragment gave rise to some lethal function.

This contention was supported by the facts that cleavage of the fragment at the single HincII restriction site within the cII gene now allowed each subfragment to be cloned downstream from the promoter signal, and that the entire fragment was readily inserted in both orientations into a pBR322 derivative lacking the promoter.

To circumvent these problems we reasoned that the cII gene (as well as any other lethal function) would have to be cloned such that its expression could be regulated. Thus we utilized a pBR322 derivative, pKC30, which contained a \(\lambda \) DNA fragment carrying the highly efficient promoter signal P_L (Fig. 1). This promoter can be regulated by the λ repressor protein (cI), a product which is synthesized continually and is regulated autogenously in an E. coli & lysogen15. A HpaI restriction site unique to pKC30 (Fig. 1) is located 321bp downstream from the startsite of P_L transcription. The 1,300-bp DNA fragment carrying the cII gene was inserted into this site and transformants (Amp^r) were selected in an E. coli λ lysogen. In contrast to our initial results, recombinants were now obtained carrying the intact 1,300-bp DNA fragment positioned in both possible orientations relative to P_L-directed transcription. The desired recombinant, pKC30cII, carries the cII gene oriented correctly with respect to P_L transcription, whereas the recombinant pKC30IIc carries the same fragment inserted in reverse orientation. When these plasmids were used to retransform both lysogenic and non-lysogenic E. coli strains, pKC30IIc transformed both strains with high efficiency, whereas pKC30cII only transformed the lysogen with high efficiency and was unable to transform the non-lysogen. Apparently, the amount of repressor being synthesized in the single lysogen is sufficient to reduce cII expression in the pKC30cII derivative to a non-lethal level.

To express the cII gene product in the lysogen, it was necessary to induce (derepress) the P_L promoter, by using a lysogen which carried a temperature-sensitive mutation in the phage repressor gene $(c1857)^{16}$. Thus, raising the temperature of these

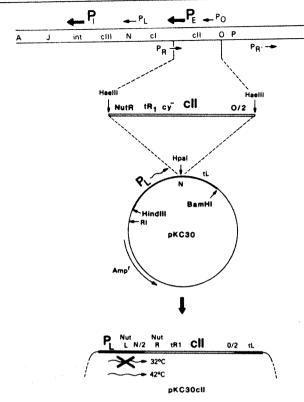


Fig. 1 Top: partial genetic map of bacteriophage λ . The locations of the positively regulated promoters, PE and PI, are indicated. These promoters are responsible for the coordinate expression of the phage lysogenic functions, repressor (cI) and integrase (int), respectively. Other major A promoters are also shown (P_R, P_L, P_O, P_R) . Expanded below is the region encompassed by a 1,300-bp HaeII DNA restriction fragment, which contains the cll gene and several other previously characterized phage regulatory sites (see below). Bottom: diagram of the construction of the plasmid vector which overproduces cII protein (pKC30cII). The HaelII restriction fragment was inserted into the single Hpal restriction site which occurs on the plasmid pKC30. This fragment contains the proposed site of recognition for the antitermination function N (NutR), the rho-dependent transcription termination site (tR1), The P_E promoter mutation cy3048 (cy⁻), the cII coding region and the amino-terminal half of the O gene $(0/2)^{7.18.24.25}$. The pKC30 plasmid is a derivative of plasmid pBR322 which contains a HindIII-BamHI restriction fragment derived from phage \(\lambda \) inserted between the HindIII and BamHI restriction sites within the tetracycline gene of pBR322 (R. N. Rao, unpublished data). The \(\lambda\) insert contains the promoter signal, PL, another proposed site of N recognition (NutL), the N gene and the strong rho-dependent transcription termination signal, tL^2 The HpaI restriction site occurs within the N gene coding region²⁰. Purific . Purified HaeIII fragment (0.1 pmol) and HpaI-cleaved pKC30 plasmid (0.1 pmol) were blunt-end ligated36 at 15 °C for 14 h and recleaved with HpaI after ligation. This DNA was used to transform a \(\lambda\) lysogen carrying a temperature-sensitive mutation (cI857) in its repressor gene. Ampr recombinants were obtained and screened by size and restriction for the presence of the insert. Recombinants were obtained carrying the insert in both possible orientations (described in text). Note that the final pKC30cII construction contains the transcription regulatory sites. NutL. NutR and tR1, preceding the cII gene. Preliminary studies indicate that high-level protein expression requires that the lysogen carry a functional N gene which is induced by temperature (A. Shatzman and M.R., unpublished). This N gene product presumably functions at the Nut sites to antiterminate transcription at tR1.

cells (from 32 to 41 °C) inactivates the repressor, thereby turning on transcription from the P_L promoter (Fig. 1). We had now achieved the desired construction: the cII gene had been inserted into a high-copy number plasmid vector and was positioned downstream from a strong, regulated promoter signal.

High-level production and purification of the cII gene product

Lysogens carrying either the pKC30cII plasmid or the control plasmid pKC30IIc were examined for their ability to synthesize proteins in response to temperature induction. Cells were initially grown at 32 °C and then pulse-labelled with ³⁵S-Met before and at various times after induction at 42 °C (Fig. 2). Total cellular protein from each pulse was then characterized by electrophoresis on SDS-polyacrylamide slab gels. The results

(Fig. 2A) indicate that two major polypeptides (of molecular weights (M,s) 10,500 and 32,000 respectively) are synthesized selectively in the pKC30cII lysogen in response to the temperature shift (pKC30cII lanes g-l). Neither of these polypeptides are made in the lysogen carrying pKC30IIc.

The molecular weight of the smaller peptide is appropriate to that expected for the cII gene product, as predicted by the DNA sequence of the cII coding region¹⁷. Moreover, this protein migrates identically with a polypeptide produced in a cell-free protein-synthesizing system (Fig. 2A, lane a) previously identified as cII (ref. 18 and C. Queen, unpublished results). The fact that the appearance of this polypeptide also depends on the correct orientation of the cII coding region and the temperature induction identifies this protein as the cII gene product.

The other polypeptide induced in the pKC30cII lysogen is probably a fusion peptide which derives from the O gene region of phage λ . The 1,300-bp DNA fragment inserted into the plasmid also contains information for the N-terminal 286 amino acids of the λ O gene¹⁹. The DNA sequence surrounding the HpaI fusion site²⁰ predicts that 14 additional in-frame translatable codons will be added to the 286 derived from the O gene before a translation termination codon occurs in frame. Thus, the P_L transcript from pKC30cII encodes a 300-amino acid O gene fusion product (32,000- M_T band) in addition to the cII gene product (the 10,500 band).

The intensities of the cII and O fusion products indicate that they are being synthesized at high rates relative to the other cellular products (Fig. 2A, lanes g, h). As cII is thought to be a relatively unstable protein¹³, we next examined whether the high rate of synthesis would lead to an accumulation of the cII protein in the cell. Again, lysogens carrying either pKC30cII or pKC30IIc were grown at 32 °C and temperature induced. After induction, total cellular protein was characterized on SDSpolyacrylamide gels and the major accumulated proteins detected by staining (Fig. 2B, lanes c, d). The cII and O fusion products were readily identified and found as major accumulated products in the pKC30cII lysogen (Fig. 2B, lane c). In these conditions cII protein was estimated to represent some 4-5% of total cellular protein. The cII protein was purified to ~98% homogeneity using standard procedures (Fig. 2B, lanes a, b). A detailed description of the isolation procedure and physical characterization of the protein will be given elsewhere (Y. Ho, H. S. and M. R., in preparation).

The procedure used here to clone, overproduce and obtain the cII protein (as well as the O gene fusion peptide) should have general application to the production and isolation of other proteins in bacterial cells. Of particular interest might be those products whose overproduction proves lethal to the cell21. The fact that the multiple copies of the highly efficient P_L promotor can be regulated in the single-copy lysogen allows cells to be grown initially without expression of the cloned function and then subsequently induced to produce the gene product. The rapidity of the induction procedure (1-1.5 h for maximum cII production) ensures maximum expression of the function within a relatively short time period. The system is readily adaptable to the production of almost any gene product (prokaryotic, eukaryotic or synthetic) provided the DNA insert contains all the necessary information for translating the product in the bacterial cell. A similar cloning vehicle has been used to overproduce the bacterial trp A gene product²².

cII-dependent transcription from promoters P_E and P_I

We next examined the ability of RNA polymerase, in the presence and absence of pure cII protein, to interact with and initiate transcription from the P_E and P_I promoters. We initially used a nitrocellulose filter binding assay to monitor the interaction, because RNA polymerase binds to DNA fragments containing promoters and forms heparin-resistant complexes which can be trapped on the filter 23 . Our assay results (Table 1) indicated that RNA polymerase alone cannot form a stable initiation complex at the P_E site. In fact, the DNA fragment

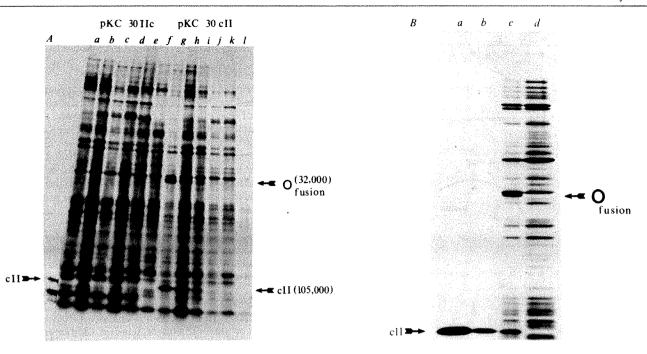


Fig. 2 A, autoradiogram of an SDS-polyacrylamide gel electrophoretic analysis of pulse-labelled proteins made in a λcl857 lysogen carrying either pKC30cII (lanes g-l) or pKC30IIc (lanes a-f) at various times after heat induction. Cells were grown at 32 °C in minimal media (M56 supplemented with 0.5% glycerol and 25 μg ml⁻¹ ampicillin)³⁷. At A₆₅₀ = 0.35 the temperature was raised to 42 °C. Aliquots (50 μl) of these cells were pulse-labelled for 45 s with ³⁵S-methionine (Amersham, 1,000 Ci mmol⁻¹) immediately before (lanes f, l) and at 5 (lanes e, k), 10 (lane e, i), 30 (lane b, h) and 40 (lanes a, g) min after heat induction. After pulse labelling, the cells were frozen (dry ice bath) and precipitated with trichloroacetic acid (final concentration 10%). The precipitate was resuspended in sample buffer (30 μl, 5.0 M urea, 0.1 M dithiothreitol, 1% SDS), boiled at 95 °C for 5 min, and subjected to electrophoresis on a polyacrylamide gradient slab gel (10% acrylamide, 0.25% bisacrylamide to 26% acrylamide, 0.325% bis-acrylamide) containing 0.1% SDS. The gels were dried and fluorographed³⁸. Lane a contains an ³⁵S-labelled marker polypeptide synthesized in a cell-free protein-synthesizing system and previously identified as the cll gene translation product (ref. 18 and C. Queen, unpublished data). Molecular weights are shown in parentheses. B, an SDS-polyacrylamide gel analysis of proteins accumulated in a cl857 lysogen carrying either pKC30flf (c) following a 40-min heat induction. Cells were initially grown at 32 °C as described above and at A₆₅₀ = 0.7, the temperature was raised to 42 °C for 40 min. Aliquots of these cells were analysed as above except that after electrophoresis the gel was stained with Coomassie brilliant blue R-250. The amount of clI was estimated by scanning the stained gel with a laser scanning densitometer (Zeineh). To purify the clI protein, cells transformed with pKC30clI (c) bluomided for 40 min at 42 °C. Cells were pelleted (8g), frozen and thawed, and then resuspended in

Table 1 Effect of cII pr	otein on ini	tiation com	plex form	ation
	P _E (cv ⁺)	P _E (cy3048)	P _E cy3008)	Po
	(% c.p.	m. retained	on nitroc	ellulose
DNA fragment		filte	r)	
RNA polymerase	8	6	9	87
RNA polymerase + cII protein	75	8	7	
cII protein	6	2	5	-

The 1,300-bp DNA fragment (Fig. 1) was cleaved with restriction endonuclease HincII and the products labelled at their 5' termini with T4 polynucleotide kinase (Boehringer-Mannheim) and $[\gamma^{-32}P]ATP$ (ICN, 2,000 Ci mmol⁻¹)³⁴. The resulting fragments of 400 and 900 bp, which contain the PE and Po promoters respectively, were separated by electrophoresis on a 4% polyacrylamide slab gel and recovered in pure form by electroelution. End-labelled fragments were prepared identically from either λ wild-type DNA (cy^+) or from mutant derivatives of λ carrying single base pair changes in the P_E promoter signal (cyL3048) and cyR3008, Fig. 5 and ref. 25). Transcription initiation complexes were formed by incubating the appropriate fragment (in the presence of 0.5 µg carrier DNA) with RNA polymerase (1.0 µg, Enzo Biochemicals) and/or cII protein (0.3 µg) in a 50 µl transcription reaction mixture (25 mM Tris-HCl pH 7.9, 80 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol) lacking the four nucleoside triphosphates. Reactions were incubated for 10 min at 37 °C and then heparin (final concentration $100~\mu g~ml^{-1})$ was added and after 2 min the mixture passed through a nitrocellulose filter (Schleicher and Schuell B6). The filter was washed with 500 µl of transcription buffer and counted, and the percentage of counts retained on the filter determined (100% = 5,000 c.p.m.).

containing P_E was not filter bound by either RNA polymerase or cII protein alone, whereas a control fragment carrying the phage promoter, P_O , was retained. When RNA polymerase and cII protein were combined, however, the P_E fragment was retained on the filter, suggesting that cII protein is required for RNA polymerase interaction at P_E . To prove that the binding was P_E specific, the experiment was repeated using the same DNA fragment obtained from two different phage derivatives carrying P_E point mutations 24,25 . One is a single base change in the -10 region of the promoter (cyL3048) and the other is a base change in the -35 region of the promoter (cyR3008). Each mutation eliminated filter binding (Table 1). Thus, the cII-dependent RNA polymerase interaction is specific for the wild-type P_E site.

We also examined the effect of cII on the ability of RNA polymerase to initiate transcription from either the PE or PI promoters in a purified in vitro transcription reaction. Transcriptions were carried out either in the presence or absence of purified cII protein using several different DNA templates. Two of these templates were constructed by inserting a \(\lambda \) DNA fragment carrying either P_E or P_I into the plasmid pBR322 (Fig. 3). Before transcription each template was cut with a restriction endonuclease such that RNA polymerase initiating at either PE or P₁ and proceeding to the end of the fragment would give rise to a discrete 'run-off' transcript. For each template a major RNA transcript of the expected size was obtained and its appearance depended completely on the presence of cII in the reaction (Fig. 4a, b). Several other discrete RNA products were obtained which apparently derive from other promoter signals contained on these plasmid derivatives (such as Pamp Ptet, Prep,

Table 2 5'-end analysis of P_E and P_I RNAs

	ρ α- ³² F	E Plabel	α^{-32} p	l label
	GTP	ATP	UTP	CTP
T ₂	pppAp* (0.6) ppp*Gp (0.4)	ND	ppp*Up* ppp*Up (1.8)	pppUp* (0.2)
\mathbf{P}_{1}	ppp*G	ND	ppp*U (1.0)	***
T_1	ND	(p)ppApGp	(p)ppUUCAG	ND

The PE and PI transcripts were labelled in vitro with the indicated ³²P]-nucleotides and gel-purified. After recovery from the gel, the following analyses were performed. RNA was digested in separate reactions with ribonucleases P1 and T2 and the products resolved by one-dimensional electrophoresis on DE81 paper at pH 1.7 (refs 27, 35). The products were identified by their relative mobilities using appropriate markers. The numbers given in parentheses indicate the relative molar yield of each product (determined by scintillation counting of the product after its detection by autoradiography). RNA was also digested with T₁ ribonuclease and the products separated by two-dimensional fingerprinting procedures²⁷. The first dimension was electrophoresis on strips of Cellogel (Kalex) at pH 3.5. The second dimension was homochromatography on TLC plates of DEAE cellulose (Analtech) using homosolvent B. The 5'-terminal oligonucleotide was identified after digestion of each T₁ oligonucleotide with pancreatic ribonuclease and electrophoretic separation of the products in one dimension on DE81 paper at pH 3.5 (ref. 27). ND, not determined.

* Indicates the labelled phosphate.

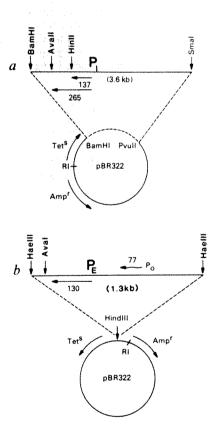
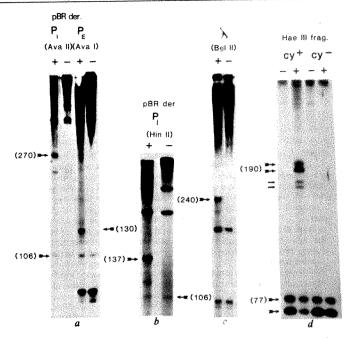


Fig. 3 Diagram of the plasmids used as DNA templates for in vitro RNA transcription. a, The 3,600-bp SmaI-BamHI λ DNA fragment containing P_I was inserted between the PvuII-BamHI site of pBR322. This plasmid was constructed in our laboratory by U. Schmeissner (unpublished data). b, The 1,300-bp HaeIII fragment (Fig. 1) containing P_E was inserted into the HindIII restriction site of pBR322 using HindII synthetic linkers (Collaborative Research). Note that this fragment also contains the P₀ promoter from which the 77 nucleotide-long RNA is transcribed (Fig. 4a, c and d)^{7,39}. Restriction enzyme cleavage sites are indicated and their respective distances from the P_I and P_E promoters noted when important to the description in the text.



Autoradiograms of polyacrylamide-gel electrophoretic analyses of ²P-labelled RNA transcripts synthesized in vitro from several different DNA templates either in the presence (+) or absence (-) of purified cII protein. Molecular weights ($\times 10^3$) are shown in parentheses. a, The pBR332 derivatives shown in Fig. 3a (carrying P1) and b (carrying PE) were cleaved with restriction endonucleases AvaII and AvaI, respectively, and used as templates. The cII-dependent 270 nucleotide-long P1 transcript and the 130-nucleotide-long PE transcript are indicated. Also indicated is the 106 nucleotide-long transcript which derives from the P_{rep} promoter located hear the replication origin of the plasmid⁴⁰. b, The pHR322 derivative shown in Fig. 3a (carrying P₁) was cleaved with HinII and used as template. The cII-dependent 137 nucleotide-long Pi transcript is indicated. The longer discrete transcripts seen in this gel are RNAs which presumably initiated at Pamp and Pter promoters. c, A DNA was cleaved with BglII and used as template. The cII-dependent 240 nucleotide-long Pg-initiated RNA is indicated. d, The purified 1,300-bp & HaeII restriction fragment (Fig. 1) obtained from either wild-type phage (cy*) or phage carrying the cy3048 point mutation in the P_E promoter (cy⁻) was used as template. The cIIdependent 190 nucleotide-long $P_{\rm E}$ transcript is indicated as well as the 77 nucleotide-long RNA. All DNA templates were transcribed and analysed on 4 or 5% polyacrylamide slab gels which contained 8 M urea as previously described7. The transcription reaction mixture (50 µl; as in Table 1 legend) contained the DNA template (0.5-2 µg), E. coli RNA polymerase holoenzyme (3-5 μ g; Enzo Biochemicals) the four nucleoside triphosphates (each at 200 μ M) and [α^{-3} P]ATP, -UTP or -GTP (Amersham, specific activity 300 Ci mmol⁻¹). When present, cII protein was added to a final concentration of 10-20 µg ml⁻¹ and all reactions were carried out at 37 °C for

Fig. 4). These transcripts were unaffected by the presence of cII in the reaction. Thus, cII selectively activates transcription from the P_I and P_E promoters.

The dependence of P_E transcription on cII was also demonstrated using a phage λ DNA template. Total λ DNA was cleaved with restriction endonuclease BgIII, which cleaves λ six times²⁶. One of these cleavage sites occurs 240bp downstream from P_E (ref. 14). In vitro transcription of the BgIII-cut template resulted in the appearance of a cII-dependent RNA product ~240 nucleotides long (Fig. 4c). Two other well characterized λ RNA transcripts were also resolved from these reactions: the 77 nucleotide-long P_O -initiated RNA and the 192 nucleotide-long P_R -initiated RNA. The transcription of both these RNAs was unaffected by cII. In an analogous experiment using λ DNA cut with restriction endonuclease AvaII (which cuts 265 bp downstream from P_I), we observed the predicted cII-dependent P_I -initiated transcript (data not shown).

We also examined the effect of a point mutation in the $P_{\rm E}$ promoter on the ability to synthesize the cII-dependent transcript. The 1,300-bp HaeIII DNA restriction fragment (Fig. 1) was obtained either from wild-type λ (cy⁺) or from a mutant derivative (cyL3048), and used as template. Transcription of the cy⁺ DNA resulted in a major cII-dependent transcript ~190 nucleotides long (actually a doublet, Fig. 4d). The size of this RNA is consistent with the distance between $P_{\rm E}$ and the HaeIII

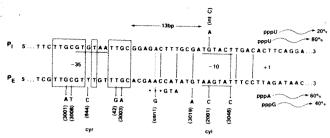


Fig. 5 Comparison of the DNA sequence of the $P_{\rm r}$ (refs 8-10) and $P_{\rm E}$ (refs , 11, 25) promoters. Only the non-template strands are shown cII-dependent transcription startsites used in vitro are indicated. Also shown (underlined) are the sequences in the -10 region of both promoters and in the -35 region of the P₁ promoter which exhibit some homology to the conserved sequence found in other promoters for E. coli RNA polymerase³². The boxed regions indicate 11 of 14 base pairs in the -35 regions of these two promoters which are identical. Note that the distance between the region of homology and the transcription startsites is exactly the same in each promoter. This region of homology has been proposed to be the site of positive activation 11,25. The various cyR and c-yL mutations which eliminate PE promoter function are indicated and also the can't mutation which has no effect on P_E promoter function but does not alter the second codon of the cII structural gene²⁵. The ATG initiation codon cII translation is also shown (on the opposite strand). The intC mutation^{8.9} which occurs in the -10 region of the P₁ promoter and confers cII-independent, constitutive expression of int gene is designated.

end of the fragment. Two-dimensional T₁-fingerprint analyses identified both of the RNAs in the doublet as transcripts which initiated at the P_E site. The cy point mutation eliminated all cII-dependent P_E transcription (Fig. 4d).

Note that the cII requirement for transcription from both the P_{I} and P_{E} sites seems absolute. This is in contrast to transcription observed at other promoters which are positively regulated (such as P_{lac} , P_{gal} , P_{RM})²⁷⁻²⁹. Even in the absence of the positive effector some level of transcription, albeit low, is usually observed. Moreover, this low-level transcription can be increased by including glycerol in the transcription reaction³⁰. This is not the case for P_E and P_I; addition of glycerol (up to 25% v/v) in the absence of cII protein resulted in no detectable P_I or P_E transcription (data not shown).

Fingerprint identification and 5'-terminal nucleotide analyses

The identification of the in vitro synthesized cII-dependent RNAs as transcripts derived from the PE and PI promoters was confirmed by two-dimensional T₁-oligonucleotide fingerprint analysis (data not shown, see Table 2 and refs 7, 31). The T_1 oligonucleotides obtained from these fingerprints were compared and found to be identical to those predicted from the known DNA sequence of the regions immediately downstream from the $P_{\rm E}$ and $P_{\rm I}$ promoters⁷⁻¹⁰. In addition, we precisely positioned and quantified the 5'-terminal transcription startsites used in vitro by RNA polymerase at P_E and P_I (Table 2). The results indicate that the cII-dependent \overline{P}_{E} transcript initiates in vitro from the same sites used in vivo¹¹, predominantly with pppA (60%) and less often with the adjacent pppG (40%) (Fig. 5). Analogously, the cII-dependent P₁ transcript also initiates in vitro from two adjacent sites, one major (80%) and one minor (20%), using in both cases a UTP starting nucleotide (Fig. 5). Again, the in vitro startsite coincides precisely with that demonstrated in vivo 12 and constitutes the first demonstration both in vitro and in vivo of the use by RNA polymerase of a uridine triphosphate as the initiating nucleotide.

Conclusions

We have described the cloning, overproduction and isolation of the λ cII gene product and demonstrated the general potential of this vector system for overproducing gene products which may be either lethal to the cell or rapidly turned-over. We have also shown that cII protein alone functions as the positive effector and turns on transcription from the two phage λ promoters, P_E and P_I, required for lysogenic development.

A variety of other phage and cellular factors have been

implicated in the transcriptional activation process of λ repressor and integrase expression^{2,3}. Speculations abound concerning the possible parts played by these various components in the PE and P₁ activation mechanism. Our present finding, that cII protein and RNA polymerase alone are both necessary and sufficient to turn on PE and P1 transcription in vitro, strongly implies that these other factors are likely to exert their effects only in an indirect fashion.

The DNA structure which encodes the P_E promoter site also encodes the ribosome binding site information and the Nterminal coding region of the cII protein (Fig. 5). We previously speculated that if cII protein were the positive activator of PE transcription, then its site of action would overlap with its own translational regulating and coding information7. This is now clearly the situation. We also proposed that the positive effector would probably interact at P_E in the -35 region^{11,25}, because of (1) the complete lack of any $P_{\rm E}$ -35 region homology with other known promoter sites³²; (2) the nature and positions of the -35 region mutations associated with $P_{\rm E}$ (cyR mutations); and (3) the extensive -35 region homologies exhibited between P_E and P_I (Fig. 5). Recent experiments designed to probe the ability of cII to protect the PE region from DNase cleavage (footprinting indicate that cII is a DNA binding protein which interacts specifically in the -35 region of the P_E site (Y. Ho and M. R., in preparation). Apparently our ability to obtain cII in large quantity and demonstrate its function as a positive effector molecule now allows us to study directly the potential interactions of this protein with itself, the DNA template and RNA polymerase.

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- Wollman, E. L. Annis Inst. Pasteur, Paris 84, 281-293 (1953).
- Weisberg, R. A., Gottesman, S. & Gottesman, M. E. Compreh. Virol. 8, 197-257 (1977).
 Herskowitz, I. & Hagen, D. A. Rev. Genet. 14, 399-445 (1981).
 Hershey, A. D. (ed.) The Bacteriophage Lambda (Cold Spring Harbor Laboratory, New York, 1971)
- Kaiser, A. D. Virology 3, 42-61 (1957).
 Eisen, H., da Silva, P. & Jacob, F. C. r. hebd. Séanc. Acad. Sci., Paris 266, 1176-1178
- 7. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. Nature 272, 414-423
- Abraham, J. et al. Proc. natn. Acad. Sci. U.S.A. 77, 2477-2481 (1980)
- Hoess, R. H., Foeller, C., Bidwell, K. & Landy, A. Proc. natn. Acad. Sci. U.S.A. 77, 2482-2486 (1980).
- 10. Davies, R. W. Nucleic Acids Res. 8, 1765-1782 (1980).
- Schmeissner, U., Court, D., Shimatake, H. & Rosenberg, M. Proc. natn. Acad. Sci. U.S.A. 77, 3191-3195 (1980).
- Schmeissner, U. et al. Nature 292, 173-175 (1981)
 Reichardt, L. J. molec. Biol. 93, 267-288 (1975).
- Roberts, T., Shimatake, H., Brady, C. & Rosenberg, M. Nature 270, 274-275 (1977).
 Ptashne, M. et al. Science 194, 156-161 (1976).
- Sussman, R. & Jacob, F. C. r. hebd. Acad. Séanc. Acad. Sci., Paris 254, 1517-1519 (1962).
- Schwarz, E., Scherer, G., Hobom, G. & Kossel, H. Nature 272, 410-414 (1978).
 Rosenberg, M. & Paterson, B. M. Nature 279, 696-701 (1979).
 Scherer, G. Nucleic Acids Res. 5, 3141-3156 (1978).

- Franklin, N. C. & Bennett, G. N. Gene 8, 197-219 (1979).
 Saito, H., Tabor, S., Tamanoi, F. & Richardson, C. C. Proc. natn. Acad. Sci. U.S.A. 77, 3917-3921 (1980).
- 22. Bernard, H.-U. et al. Gene 5, 59 (1979).
- Hinkle, D. & Chamberlin, M. J. molec. Biol. 70, 157-185 (1972).
 Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. in The Operon (eds Miller, J. H. & Reznikoff, W. S.) 304-324 (Cold Spring Harbor Laboratory, New York, 1972).
- 25. Wulff, D. L. et al. J. molec. Biol. 138, 209-230 (1980)
- Wultt, D. L. et al. J. moiec. Biol. 138, 209-230 (1980).
 Pirrota, V. Nucleic Acids Res. 3, 1747-1760 (1976).
 Reznikoff, W. S. & Abelson, J. N. in The Operon (eds Miller, J. H. & Reznikoff, W. S.) 221-243 (Cold Spring Harbor Laboratory, New York, 1978).
 Miller, Z., Varmus, H. E., Parks, J. S., Perlman, R. L. & Pastam, I. J. biol. Chem. 246, 2803 (2001).
- 2898-2903 (1971)
- Meyer, B. J. & Ptashne, M. J. molec, Biol. 139, 195-205 (1980)
- 30. Nakanishi, S., Adhya S., Gottesman, M. E. & Pastan, I. J. biol. Chem. 248, 5937-5924 31. Barrell, B. G. in Procedures in Nucleic Acids Research Vol. 2 (eds Cantoni, G. & Davies, D.)

- Barrell, B. G. in Procedures in Nucleic Acids Research Vol. 2 (eds Cantoni, G. & Davies, D.) 751-779 (Harper & Row, New York, 1971).
 Rosenberg, M. & Court, D. A. Rev. Genet. 13, 319-353 (1979).
 Galas, D. & Schmitz, A. Nucleic Acids Res. 5, 3157-3170 (1978).
 Maniatis, T., Jeffrey, A. & Kleid, D. Proc. natn. Acad. Sci. U.S.A 72, 1184-1188 (1975).
 Cashel, M., Lazzarini, R. A. & Kalbacher, B. J. Chromatogr. 40, 103-109 (1969).
 Ullrich, A. et al. Science 196, 1313-1319 (1977).
 Gottesman, M. E. & Yarmolinsky, M. B. J. molec. Biol. 31, 487-505 (1968).
 Paterson, B. & Bishop, J. O. Cell 12, 751-765 (1977).
 Rosenberg, M., deCrombrugghe, B. & Weissman, S. J. biol. Chem. 250, 4755-4764 (1975).
 Levine, A. & Rupp, W. in Microbiology 1978 (ed. Schlessinger, D.) 163-166 (American Society Microbiology, 1978). Society Microbiology, 1978).

LETTERS TO NATURE

Soft γ -ray emission from the region of MCG8-11-11

F. Perotti*, A. Della Ventura*, G. Villa*, G. Di Cocco†, R. C. Butler‡§, J. N. Carter‡ & A. J. Dean‡

* Istituto di Fisica Cosmica, C.N.R. Via Bassini, 15/A Milan, Italy † Istituto TE.S.R.E., C.N.R. Via De' Castagnoli, 1 Bologna, Italy

‡ Department of Physics, University of Southampton, Southampton SO9 5NH, UK

The galaxy MCG8-11-11 inside the 0.093 square degrees error-box of the X-ray source 2A0551+466 (ref. 1), was recently found to be a Type 1 Seyfert galaxy2. A 40-arcs error-box from the A3 experiment on HEAO 1 has confined the X-ray emission to the nucleus or bar of this 14-m, galaxy3. The redshift is z = 0.0205 (ref. 2), corresponding to a distance of 123 Mpc $(H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1})$. The 2-10 keV luminosity ranges from $5 \times 10^{43} \text{erg s}^{-1}$ to $1.2 \times 10^{44} \text{erg s}^{-1}$ (refs 1, 3). Hard X-ray emission above 20 keV from the region of MCG8-11-11 has been recently reported4, and the A2 experiment on HEAO 1 has provided for the first time the emission spectrum of this object up to 30 keV (ref. 5). During a balloon flight on 30 September 1979 from Palestine, Texas of the Milan/Southampton (MISO) low-energy γ -ray telescope, an excess at the 3.9σ level in the flux above 90 keV was detected from a region of the sky containing MCG8-11-11. The emission spectrum, evaluated in the energy range 0.02-19 MeV, has similar spectral characteristics to those observed with the same telescope and associated with the Seyfert galaxy NGC4151 (ref. 6). Both spectra show evidence for a high-energy cutoff close to 3 MeV and this feature could be interpreted on the basis of the 'Penrose-Compton scattering' process⁷. A large amount of the observed low-energy γ-ray diffuse background8 could be produced by a few per cent of the X-ray emitting Seyfert galaxies having a γ-ray luminosity comparable with that observed from the region of NGC4151 or MCG8-11-11.

The MISO telescope⁹ consists of a 'Compton-coincidence' detection system inside a semi-active shield. The sensitive area is about 560 cm² and the aperture is 3° FWHM in both the azimuthal and zenithal planes. For the September 1979 flight, the energy range was set to be 0.05–19 MeV. A passively shielded hard X-ray detector (20–280 keV) having an effective area of 600 cm² and the same field of view of 3° FWHM was mounted parallel with the main telescope. A two-axis orientation system was used to point the telescope with a precision of about 0.3°.

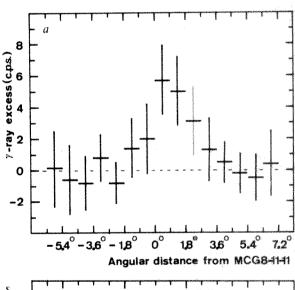
The region of the sky containing MCG8 -11-11 was studied on 30 September 1979 between 8 h 34 min UT and 14 h 59 min UT. Five drift-scans were performed to survey the region contained within the coordinate points RA 5 h 14 min and 6 h 33 min and centred on dec $+46.2^{\circ}$. The mean float altitude during the on-source periods was equivalent to an atmospheric pressure of about 4.2 mbar and the telescope was set at zenith angles between 16° and 33°.

To minimize systematic variations in the background due to changes in the zenith and azimuth of the telescope, the data for each scan were analysed separately and the results statistically combined. The only significant source of systematic variation in the background was found to be linearly related to the changes of the residual atmospheric pressure between 3.7 and 4.5 mbar. For each separate scan the background, corrected for the atmospheric pressure variation, was subtracted from the

measured on-source counting-rate. A mean excess of 3.3 ± 0.85 c.p.s. above 90 keV (2.9 ± 0.8 c.p.s. above 260 keV from the γ -ray telescope) was found when the data from the five drift-scans were combined.

In Fig. 1a, the correlation between the counting-rate excess above 260 keV and the angular distance between the estimated direction of MCG8 $-11 - \overline{1}1$ and the axis of the MISO telescope is shown. The error-box for the observed y-ray excess is plotted in right ascension and declination (for the 1950 equinox) in Fig. 1b together with the 4U and HEAO 1 (A2)⁵ error-boxes. The Ariel 5 error box1 has about the same dimension of the filled circle showing the position of MCG8-11-11. The limits of our error-box are represented by the 1σ points along the scanning direction, and by the total aperture of the telescope (6°) in the orthogonal direction, which represents the 100% probability of containing the emitting object. The uncertainty due to the pointing system has been included. The only X-ray source within our error-box, even if extended up to the 4σ level, is MCG8-11-11, apart from the OSO 7 source 1M0600+46 (ref. 10) for which the projection on the sky of the 1.14 square degrees error-box is not available.

The same method of analysis was used to evaluate the contribution of the source in individual energy-loss channels of both the main telescope and the hard X-ray detector, between 20 keV



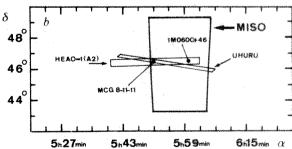


Fig. 1 a, γ -ray excess count rate above 260 keV versus the angle between the axis of the MISO telescope and the estimated direction of MCG8-11-11. b, The MISO error-box in right ascension and declination (for the 1950 equinox) is shown together with other astronomical data. The error-box of the OSO 7 X-ray source 1M 0600+46 (ref. 10) is not available. The Ariel 5 error-box of the X-ray source 2A 0551+466 (ref. 1) has about the same dimension of the filled circle showing the position of MCG8-11-11. The MISO error-box has 68% probability across the scan direction and 100% across the other. The uncertainty due to the pointing system has been included.

and 19 MeV. A photon spectrum at the top of the atmosphere was obtained by means of a matrix inversion technique which makes no a priori assumptions about the final shape of the emission spectrum. The absorption of photons in the residual atmosphere, the redistribution of photon energies through Compton interactions and pair-production, and the energy resolution of the detectors were taken into account. The error in this evaluation was estimated to be less than 10%. This photon spectrum is shown in Fig. 2 together with other measurements above 1 keV from the same region of the sky.

Our 2σ upper limits between 20 and 90 keV impose a severe constraint on the data fit at greater energies. In fact, an attempt to fit our data between 90 keV and 19 MeV with a single power-law photon spectrum leads to an estimated flux below 90 keV, a factor of two greater than the corresponding 2σ upper limits and to a reduced $\chi^2 = 2.77$ with 4 degrees of freedom ($\sim 2.5\%$ probability). Therefore, in a power-law representation of our spectral data a break, followed by a steeper spectrum, is needed between 2 and 4 MeV. At lower energies, the best fit is given by

$$I(E) = 7.9 \times 10^{-3} E^{-(1.0 \pm 0.7)}$$
 photons cm⁻² s⁻¹ keV⁻¹

corresponding to a reduced $\chi^2 = 1.45$ with 2 degrees of freedom. At higher energies the slope is about 3.8 if compatibility with the SAS 2 upper limit¹¹ is required.

In principle, we cannot exclude the possibility that the OSO 7 X-ray source 1M0600+46 (ref. 10) might be associated with the Seyfert galaxy MCG8-11-11. On the other hand, the OSO 7 error-box for this source is not available and therefore it is impossible to estimate the probability that the two X-ray sources are associated.

Assuming that we have detected a γ -ray emission from MCG8-11-11 and taking into account that its X-ray flux varies by a factor of 2 to 3 without evident spectral index variation^{2,12}, the HEAO 1 (A2)⁵ and MISO spectra could be in reasonable agreement.

The observed spectral feature at MeV energies is similar to that of the Seyfert galaxy NGC4151, in which a cutoff close to 3 MeV has been indicated by the MISO telescope observation⁶. The existence of the high-energy cutoff in the emission spectrum of these two Seyfert galaxies supports the suggestion¹¹ that a steepening of the spectrum between X-ray and γ -ray energies may be a general characteristic of the active extragalactic objects. This suggestion was based on the comparison between some measured X-ray spectra of active galaxies (for example, MKN501, Cen A, 3C273) and the corresponding positive results or intensity upper limits above 35 MeV.

A model has been recently proposed in which the cutoff at MeV energies is expected to be a 'universal' feature of the emission by active nuclei of galaxies, provided that a rapidly spinning Kerr black hole $(M>10^8\,M_\odot)$ surrounded by a hot $(T\sim10^9\,\text{K})$ accretion disk exists within these nuclei. In these conditions, the Penrose-Compton scattering mechanism¹³ stochastically generates γ -ray bursts with energies up to 3 MeV. The spectral shape is expected to be flatter than the X-ray spectrum in the tens of keV region and the burst duration is about $\Delta T \sim (2.2 \text{ h}) \times 10^{-8} \text{ M/M}_{\odot}$. Since the luminosity of MCG8-11-11 in the energy range 0.09-3 MeV, as obtained from our data, is $L(123 \text{ Mpc}) = 7 \times 10^{46} \text{ erg s}^{-1}$, on the basis of this model a lower limit of $5 \times 10^9 M_{\odot}$ is expected for the mass of the black hole. Thus, the γ -ray burst duration should be greater than 4.6 days, consistent with no evident flux variation during the 6.5 h observation of MCG8-11-11 by the MISO telescope. If we have detected a γ -ray emission from both the Seyfert galaxies NGC4151 and MCG8-11-11 during a balloon flight, the bursts must be rather frequent, at least during some periods of higher activity in these galaxies.

The spectrum of the cosmic γ -ray background⁸ shows a feature at MeV energies similar to that observed from the regions of MCG8-11-11 (Fig. 2) and NGC4151 (ref. 6). Moreover, the γ -ray background has a photon spectral index of about 1.7, not inconsistent within the statistical errors with those

observed at γ -ray energies from these two galaxies. Assuming that the present data pertain to MCG8-11-11, the contribution of Seyfert galaxies to the diffuse γ -ray background in the energy range 0.09-3 MeV can be estimated. The X-ray luminosity function for Seyfert galaxies obtained by the HEAO 1 (A2) experiment leads to a local volume emissivity above L_{Xmin} of 14

$$B_{\rm X}(2-10~{\rm keV}) = 2 \times 10^{38} \left(\frac{L_{\rm Xmin}}{10^{43}}\right)^{-0.75}~{\rm erg~s^{-1}~Mpc^{-3}}$$

Taking a γ -ray background emissivity up to 3 MeV of $B_{\gamma} = 1.8 \times 10^{39} \text{ erg s}^{-1} \text{ Mpc}^{-3}$ (ref. 15), the ratio of the two emissivities is

$$B_{\gamma}/B_{\rm X} = 9(L_{\rm Xmin}/10^{43})^{0.75}$$

Since $L_{\rm Xmin}$ is in the range of (2-5)×10⁴² erg s⁻¹ (refs 14, 16), then

$$B_{\rm y}/B_{\rm X} = 2.7 - 5.4$$

For MCG8-11-11 the ratio between the 0.09-3 MeV γ -ray luminosity and the 2-10 keV X-ray luminosity is:

$$L_{\gamma}/L_{x}(MCG8-11-11) = \frac{7 \times 10^{46} \text{ erg s}^{-1}}{(0.5-1.2) \times 10^{44} \text{ erg s}^{-1}}$$

= $(6-14) \times 10^{2}$

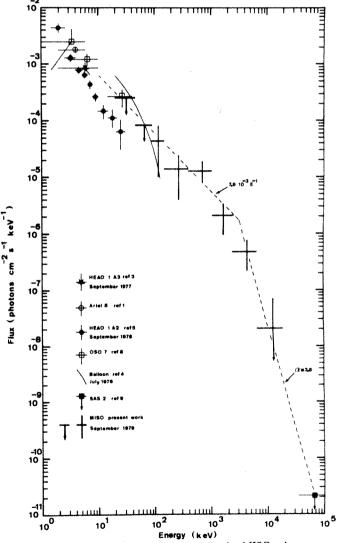


Fig. 2 The photon spectrum observed by the MISO telescope from the region of MCG8-11-11 is shown together with other measurements above 1 keV from the same region of the sky. When available, the date of observation is given. The dashed line represents the best fit power-law of the MISO data with a cutoff at about 3 MeV. The solid curve (ref. 4) represents the fit obtained by the authors assuming a bremstrahlung spectrum.

For NGC4151 the observed γ -ray luminosity is $(0.5-2)\times$ $10^{45} \, erg \, s^{-1}$ (refs 6, 17) and the 2-10 keV X-ray luminosity is in the range $(3.5-7) \times 10^{42}$ erg s⁻¹ (refs 16, 18), giving:

$$L_{\gamma}/L_{\mathbf{X}}(\mathbf{NGC4151}) = (1-5) \times 10^{2}$$

Consequently, we could assume $L_{\gamma}/L_{x} = 10^{2}$ as a characteristic ratio for this sample. The comparison between $L_{\gamma}/L_{\rm X} = 10^2$ and $B_y/B_x = 2.7-5.4$ implies that few per cent of all the Seyfert galaxies with $L_{\rm X}$ (2-10 keV) > $L_{\rm Xmin}$ = (2-5) × 10⁴² erg s⁻¹ could produce the observed diffuse background in the 0.09-3 MeV energy band. And since the y-ray background produced by Seyfert galaxies cannot exceed the total cosmic background, only a few per cent of Seyferts can have y-ray luminosities as high as those observed from NGC4151 and MCG8-11-11.

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- Cooke, B. A. et al. Mon. Not. R. astr. Soc. 182, 489 (1978). Ward, J., Wilson, A. S., Disney, M. J., Elvis, M. & Maccacaro, T. Astr. Astrophys. 59, L19

- Frontera, F., Fuligni, F., Morelli, E. & Ventura, G. Astrophys. J. 234, 477 (1979).

 Mushotzky, R. F., Marshall, F. E., Bolott, E. A., Holt, S. S. & Serlemitsos, P. J. Astrophys. J. 235, 377 (1980).

- Perotti, F. et al. Nature 282, 484-486 (1979). Leiter, D. Astr. Astrophys. 89, 370 (1980). Trombka, J. et al. Astrophys. J. 212, 925 (1978).
- Baker, R. E. et al. Nucl. Instrum. Meth. 158, 595 (1979). Markert, T. H. et al. Astrophys. J. Suppl. 39, 573 (1979).
- 11. Bignami, G. F., Fichtel, C. E., Hartman, R. C. & Thompson, D. J. Astrophys. J. 232, 649
- (1979). Mushotzky, R. F. & Marshall, F. E. Astrophys. J. Lett. 239, L.5 (1980). Piran, T. & Shaham, J. Astrophys. J. 214, 268 (1977).

- Firan, T. & Shahali, S. Sakoyini, 24, 244
 Ficcinotti, G. et al. Astrophys. J. (submitted).
 Schonfelder, V. Nature 274, 344-347 (1978).
 Elvis, M. et al. Mon. Not. Astr. Soc. 183, 129 (1978).
- 17. Perotti, F. et al. Astrophys. J. Lett. (submitted). 18. Tananbaum, H. et al. Astrophys. J. 223, 74 (1978).

Evidence for secondary origin of chondrules

Hiroko Nagahara

Geological Institute, University of Tokyo, Hongo, Tokyo 113, Japan

Although chondrules have been extensively studied chemically^{1,2}, petrologically³⁻⁵ and experimentally⁶⁻⁸, their origin is still not certain. I discuss here whether chondrules were condensed directly from the primitive solar nebula9-11 or were formed from precursory materials by various mechanisms, such as high velocity impact of small bodies 12,13, impact on the surface of a parental body^{14,15} or dust fusion¹⁶⁻¹⁸. Investigations of an Antarctic meteorite suggest that chondrules could have formed through melting of pre-existing materials.

The Antarctic meteorite ALH-77015(L3) has closely packed chondrules which show wide variations in texture, bulk chemical composition and mineralogy (work in preparation). They are classified into four textural groups; barred, radial, glassy and porphyritic chondrules. The porphyritic chondrules are most common and show wide variations in their crystal/liquid (groundmass) ratios and crystal shapes. Several chondrules contain two types of olivine. One, which has numerous dusty inclusions and a fuzzy 'dirty' appearance, is always found near the centre of the chondrules as a large anhedral grain, or as disaggregated small anhedral grains (Fig. 1a, b). The other is euhedral small grain which exists around the former olivine and has clear feature. This clear olivine also overgrows the 'dirty' large olivine crystal (Fig. 1 c, d).

These two types of olivine have different chemical compositions. Figure 2a shows the zonal pattern of MgO across the dirty and clear olivines in chondrule 2-4 (Fig. 1d). The dirty olivine shows 'reversal' zoning where the core is more enriched in iron than the rim. In contrast, the clear olivine shows 'normal' zoning

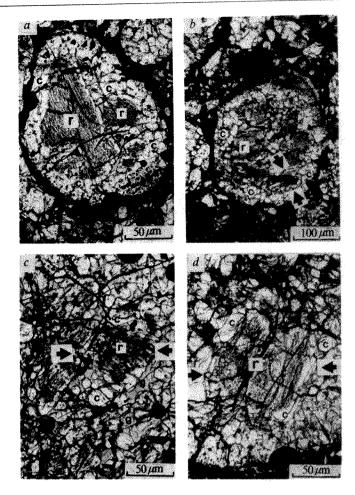


Fig. 1 a, Chondrule 2-1 containing a relic olivine. r, Relic olivine showing large, dirty and anhedral appearance; c, crystallized olivine showing small, clear and euhedral appearance; g, glass. b. Chondrule 2-2. Scanning profile of MgO across the grain between the arrows is shown in Fig. 2b. c, Closed view of relic olivine in chondrule 2-3. Clear part around the relic olivine is overgrown olivine. Scanning profile between the arrows is shown in Fig. 2c. d, Closed view of chondrule 2-4. Scanning profile between the arrows is shown in Fig. 2a.

where the core has more magnesium than the rim. It is impossible to form both types of olivine from a liquid during a single crystallization process. The dirty and relatively iron-rich olivine is probably a relic mineral which was originally rich in iron and was not completely melted when it was heated. On the other hand, the clear olivine seems to have crystallized directly from the liquid, which was probably formed by melting of the preexisting olivine, pyroxene and plagioclase. The fact that the overgrown rim of the relic olivine has the same composition as the clear one (Fig. 2a) supports this hypothesis. Figure 2b, c show the zonal patterns of MgO in the relic olivine with overgrown rim in chondrules 2-2 (Fig. 1b) and 2-3 (Fig. 1c). The overgrown olivine has more magnesium than the relic part, and there is compositional discontinuity between them.

The two types of olivine are also distinguished by their minor element content. Figure 3 shows the CaO content compared with $X_{Mg}(Mg/Mg+Fe)$ of the two olivines in four chondrules. In all cases, crystallized olivine contains more CaO than the relic olivine. In general, the olivine that crystallized at high temperature or that crystallized rapidly contains more CaO (ref. 19). This fact supports the suggestion that the clear olivine is crystallized from the liquid when the chondrules were heated and that the dirty olivine is a relic mineral which had been equilibrated at lower temperatures before chondrule formation.

Relic olivine is the first direct evidence for the presence of precursory material in the formation of chondrules; that is at

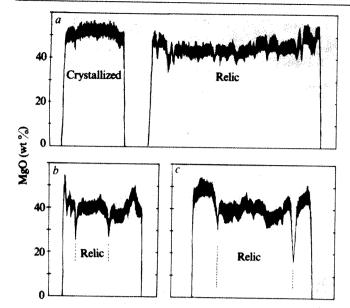


Fig. 2 a, Scanning profile of MgO in crystallized olivine and olivine in chondrule 2-4. Scanning position of relic olivine is shown in Fig. 1d. Chemical composition of rim of the two olivines is equal. b, Chondrule 2-2. Scanning position is shown in Fig. 1b. Overgrown part contains more MgO than the relic part. c, Chondrule 2-3. Scanning position is shown in Fig. 1c.

least some chondrules were not condensed directly from the gas of primitive solar nebula. In the present case, the relic olivine is so large that it can easily be distinguished from the crystallized olivine. However, if the relic olivine were small and/or were nearly completely melted, it would be difficult to distinguish relic minerals from the crystallized ones.

Some chondrules were, therefore, not necessarily crystallized from the completely melted liquid. In this case, the maximum temperature of chondrule formation would be lower than the liquidus temperature of chondrules, which is about 1,300-1,700 °C (ref. 8), and be consistent with recent experimental results (refs 8, 20, and H. N. and A. Tsuchiyama, in preparation). Dodd⁴ has discussed whether the microporphyritic chondrules were formed by fragmentation of rocks which crystallized from liquids and whether they are different in origin from the remelted chondrules such as barred or radial ones. Although his model seems to explain the difference in chondrule

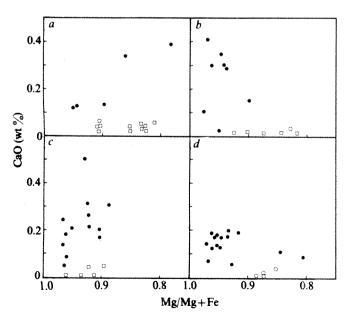


Fig. 3 CaO content in relic (□) and crystallized olivine (●) in four chondrules. a, Chondrule 2-1; b, chondrule 2-2; c, chondrule 2-4; d, chondrule 2-3.

texture, it does not account for microporphyritic texture which is difficult to form from wholly melted materials. The existence of pre-existing materials in the nucleus is an effective explanation for both the formation of porphyritic or microporphyritic minerals (H. N. and A. Tsuchiyama, in preparation). The fact that the bulk composition of the chondrules, which were clearly crystallized from the liquid, are mixture of olivine, pyroxene and plagioclase²¹ also strongly suggests that such chondrules at least were formed by melting of pre-existing materials.

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- 1. Osborn, T. W., Smith, R. H. & Schmitt, R. A. Geochim. cosmochim. Acta 37, 1909-1942
- Grossman, J. N., Kracher, A. & Wasson, J. T. Geophys. Res. Lett. 6, 597-600 (1979)
- 3. Dodd, R. T., Van Schmus, W. R. & Koffman, D. M. Geochim. cosmochim. Acta 31, 921-951 (1967).
- Dodd, R. T. Earth planet. Sci. Lett. 40, 71-82 (1978)
- Kimura, M. & Yagi, K. Geochim. cosmochim. Acta 44, 589-602 (1980).
- Blander, M., Planner, H. N., Keil, K., Nelson, L. S. & Richardson, N. L. Geochim. cosmochim. Acta 40, 889-896 (1976).
- Klein, L. C., Fasano, B. & Hewins, R. H. Proc. 11th Lunar planet. Sci. Conf. (in the press).
- Tsuchiyama, A., Nagahara, H. & Kushiro, I. Earth planet. Sci. Lett. 48, 155-165 (1980). Herndon, J. M. & Suess, H. E. Geochim. cosmochim. Acta 40, 233-236 (1977). McSween, H. Y. Geochim. cosmochim. Acta 41, 1853-1860 (1977).
- Wood, J. A. & McSween, H. Y. in Comets, Asteroids, Meteorites: Interrelations, Evolution and Origins (ed. Delsemme, A. H.) 365–373 (University of Toledo Press, 1977).

- and Origins (ed. Deisemme, A. H.; 363-5/3 (University of Toledo Press, 1977). Wasson, J. T. Rev. Geophys. Space Phys. 10, 711-759 (1972). Kerridge, J. F. & Keiffer, S. W. Earth planet. Sci. Lett. 35, 35-42 (1977). Kurat, G., Fredrikkson, K. & Nelen, J. Geochim. cosmochim. Acta 33, 765-773 (1969). King, E. A., Carman, M. F. & Butler, J. C. Science 175, 59-60 (1972). Martin, P. M. & Mills, A. A. Earth planet. Sci. Lett. 33, 239-248 (1976). Martin, P. M. & Mills, A. A. Earth planet. Sci. Lett. 38, 385-390 (1978). Sonatt C. P. & Revnolds, R. T. in Asteroids Lade Gabriels, T. & Matthews, M. S.) 823-844.

- Sonett, C. P. & Reynolds, R. T. in Asteroids (eds Gehrels, T. & Matthews, M. S.) 822-848 (University of Arizona Press, 1979)
- Donaldson, C. H., Usselman, T. M., Williams, R. J. & Lofgren, G. E. Proc. 6th Lunar Sci. Conf. 843-870 (1975).
- Tsuchiyama, A., Nagahara, H. & Kushiro, I. Geochim. cosmochim. Acta (in the press).
 Ikeda, Y. Mem. Natn. Inst. Polar Res. Jap. 17, 50-82 (1980).

Prebiotic atmospheric oxygen levels

J. H. Carver

Research School of Physical Sciences, Australian National University, Canberra ACT 2600, Australia

It has been argued 1-2 that the early Precambrian atmosphere contained a negligible amount of oxygen and that the transition to the modern oxygen-rich atmosphere began some 2,000 Myr BP when photosynthetic oxygen produced by blue-green algae oxidized ferrous iron in solution and escaped from the ocean to the atmosphere. Others3 have argued for an even earlier occurrence of an oxygen-rich atmosphere. Although photosynthesis has provided the oxygen of the present atmosphere, the photosynthetic model for Precambrian oxygen production has difficulties. There is the problem of identifying the large deposits of reduced carbon needed to account for the red beds4 and the predominant role of photosynthesis as an oxygen source in the early Precambrian has been questioned on both biochemical and geochemical grounds⁵. Before photosynthesis the only possible abiotic source of atmospheric oxygen would have been the direct photodissociation of water vapour by solar UV photons. What atmospheric oxygen level could have been produced by this purely abiotic process? It has been argued⁶ that the abiotic oxygen level was entirely negligible, ≤10⁻¹² PAL (present atmospheric level), but this assumed that the water vapour content of the palaeoatmosphere was the same as at present with a constant H2O mixing ratio of 3.8 p.p.m. above an altitude of 10 km. I argue here that this is too restrictive an assumption and calculate possible atmospheric oxygen levels for larger water vapour mixing ratios which cannot be ruled out by our limited knowledge of Precambrian conditions. Even a moderate oxygen content for the early Precambrian atmosphere could have had significant evolutionary implications because a biologically effective UV ozone screen would be established once the oxygen content exceeded 10⁻² PAL (refs 7-11).

In abiotic conditions the production of free oxygen in excess of atmospheric and surface oxidation would have depended on the amount of water vapour in the lower palaeoatmosphere and on transport processes that governed the escape rate of hydrogen through the exosphere. Abiotic production of atmospheric oxygen is limited by transport rather than by the water vapour photodissociation rate and, provided the exospheric temperature exceeds ~500 K, the hydrogen escape rate is given approximately by the limiting flux condition 12-14

$$\phi = \frac{b}{H} f_{\text{tot}} \tag{1}$$

where b is the binary diffusion parameter, H is the atmospheric scale height and f_{tot} is the total hydrogen mixing ratio at the homopause. Kasting et al. 15 have noted that the limiting flux condition may not apply exactly for palaeoatmospheres having high hydrogen escape rates $(f_{\text{tot}} \ge 10^{-3})$ and low mesospheric oxygen contents $(\le 10^{-5} \text{ PAL})$ owing to the partial ineffectiveness of ion-molecule reactions in converting H_2 into H in the thermosphere. Throughout most of the range of present interest, however, the limiting flux condition should provide a sufficiently good approximation.

For the Earth's atmosphere, $b/H \approx 2 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$ and the present stratospheric water vapour content of ~4 p.p.m. imply a hydrogen escape rate of ~1.6×108 H atoms cm⁻² s⁻¹. This is less than the current hydrogen emission rate from volcanoes estimated⁶ as $V \approx 2.5 \times 10^8 \,\text{H}$ atoms cm⁻² s⁻¹. Thus abiotic processes are now a negligible source of atmospheric oxygen and a net production of oxygen by photodissociation could have occurred in the past only for palaeoatmospheres containing significantly more water vapour than does the present atmosphere. It is unlikely that a highly reducing atmosphere could have survived for long under the photolytic influence of the solar UV radiation and it will be assumed that after an initial period of rapid outgassing the palaeoatmosphere 16 consisted mainly of carbon dioxide, water vapour and nitrogen with a volcanic emission rate comparable to the present value. With these assumptions the net hydrogen escape rate is

 $\psi = \phi - V \approx (2 \times 10^{13} f_{\text{tot}} - 2.5 \times 10^8)$ H atoms cm⁻² s⁻¹ (2) Larger values of f_{tot} imply increased rates of hydrogen escape (and hence oxygen production) but the oxygen level achieved depends on the loss rate. For the assumed palaeoatmosphere that does not contain large quantities of reduced gases the major loss process would be surface oxidation once the hydrogen escape rate exceeded the volcanic emission rate. Oxidation rates in present conditions probably vary as some fractional power of the atmospheric oxygen content¹⁷ because they depend on the rate of exposure of surface materials as well as on the intrinsic properties of those materials. At low oxygen levels in a palaeoatmosphere the supply of oxygen rather than the exposure of fresh material would have been the limiting factor and, following Brinkmann¹⁸, the oxidation rate L is assumed to have been proportional to the oxygen content $n(O_2)$

$$L = \frac{n(O_2)}{\tau} \tag{3}$$

where τ is a characteristic time for surface oxidation. At equilibrium the loss rate L is equal to the oxygen production rate P that corresponds to the net hydrogen escape rate given by equation (2).

The equilibrium atmospheric oxygen levels calculated with P = L are shown in Fig. 1 for $\tau = 0.1-100$ Myr and $f_{\text{tot}} = 10^{-6}-10^{-2}$. Brinkmann¹⁸ investigated Precambrian oxygen production and surface oxidation for a range of τ between 1 and 100 Myr. Hart¹⁹ assumed that τ was ~ 20 Myr. The solid lines in Fig. 1 at τ values of 1 and 10 Myr bracket the present oxidation time estimated by Holland as 3 Myr.

The very dry stratosphere of the present atmosphere^{20,21} is considered to result from circulation and condensation through the cold tropical troposphere with a typical temperature of ~195 K corresponding to a saturated humidity of ~4.5 p.p.m. Because of the approximately exponential dependence of humidity on temperature the water vapour content of the

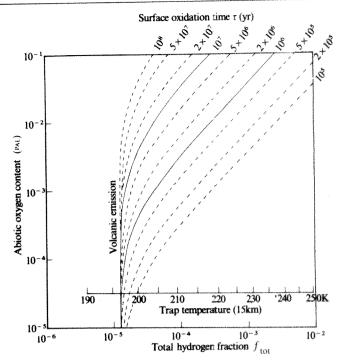


Fig. 1 Equilibrium abiotic oxygen content (PAL) as a function of surface oxidation time $(\tau \, \text{yr})$ and total hydrogen content (f_{tot}) in the lower atmosphere. Temperatures that would produce a water vapour content equal to f_{tot} for a notional cold trap at 15 km are indicated. The solid lines bracket estimates of the present surface oxidation time constant. Note that the atmospheric oxygen content is negligible unless f_{tot} exceeds its present value so that exospheric hydrogen escape overwhelms volcanic hydrogen input.

stratosphere would vary rapidly with changes in the temperature of the atmospheric cold trap. If, for example, the polar tropopause at ~ 225 K were the controlling temperature the stratospheric water vapour content would increase by nearly two orders of magnitude. Figure 1 also shows notional 'trap temperatures' corresponding to particular values of $f_{\rm tot}$ even though the palaeoatmosphere may not have had a true tropopause.

It is unlikely that the palaeoatmosphere would have possessed the particular combination of temperature profile and circulation pattern that produces the very dry stratosphere of the present atmosphere. That substantial changes in the radiation balance of the atmosphere have occurred during geological time is implied by the fact that the Earth's surface temperature has remained within the range 0-100 °C (as indicated by geological evidence for the continued presence of large amounts of liquid water) even though theories of stellar evolution imply that the Sun has brightened²² by 30-40% since it first joined the main sequence some 4,500 Myr ago. Sagan and Mullen²³ suggested that an ammonia greenhouse might have kept the Earth's mean surface temperature above the freezing point of seawater but it is unlikely that an ammonia-rich atmosphere could have persisted throughout the early Precambrian and a more probable explanation is that the greenhouse effect was provided by a CO₂ rich palaeoatmosphere^{24,25}. Other possible explanations for the non-freezing of the oceans under the weak early Sun are changes in the albedo or the rotation rate of the Earth²⁶. Any such explanation would profoundly affect the temperature profile and water vapour content of the palaeoatmosphere.

The range of total hydrogen contents, $f_{\rm tot}$, shown in Fig. 1 corresponds to notional cold trap temperatures at 15 km between 190 and 250 K. Figure 1 only indicates a range of possibilities for the equilibrium abiotic oxygen content of a palaeoatmosphere and any conclusions based on our meagre understanding of the Precambrian environment must be very tentative. The available evidence does, however, suggest that the Precambrian lower atmosphere may have been warmer and moister than at present so that significant quantities of atmos-

pheric oxygen could have been produced by abiotic means. Surface temperatures as high as 52 °C (325 K) at 1,300 Myr BP and 70 °C (343 K) at 3,000 Myr BP are suggested by the chert measurements of Knauth and Epstein²⁷. Schopf²⁸ has summarized evidence indicating Precambrian ocean surface temperatures of 50-70 °C. Model atmosphere calculations^{24,25} also suggest that early Precambrian surface temperatures may have been warmer than at present owing to the atmospheric greenhouse effect provided by a dense carbon dioxide blanket. For oxygen deficient atmospheres the photochemical region extends well down into the lower atmosphere with the water vapour photolysis rate being a maximum at an altitude of \sim 5 km (ref. 6). The hydrogen escape rate in the limiting flux condition is set by the water vapour content at the 10-15 km level and above ~20 km hydrogen molecules replace water vapour as the dominant hydrogen species. Even if the atmosphere is very cold above 20 km it will not affect the water vapour level that fixes the limiting flux.

Model atmosphere calculations suggest significant warming of the lower atmosphere in Precambrian conditions. The Precambrian models of Morss and Kuhn²⁹ indicate temperatures ~15 °C warmer than at present at 15 km which would imply $f_{\text{tot}} \sim 10^{-4}$ and $n(O_2) \sim 10^{-2}$ for present day surface oxidation rates and $n(O_2) \sim 10^{-3}$ for 10 times the present oxidation rate. These models, however, neglect variations in solar luminosity and more detailed calculations of palaeotemperature profiles are required before any very firm conclusions can be drawn from the models. Larger values of $n(O_2)$ are implied by the chert measurements of Knauth and Epstein²⁷ which indicate a possible surface warming of (40-50 °C). If this warming had extended throughout the lower palaeoatmosphere (if the lapse rate had been similar to the present one) the chert temperatures would imply total hydrogen fractions f_{tot} at 15 km in the range 10^{-3} 10⁻². In these conditions equilibrium abiotic oxygen levels $n(O_2)$ could have reached 0.1 PAL unless surface oxidation rates were substantially greater than at present. Thus if the temperatures were as high as suggested by the model atmosphere calculations and by the chert measurements abiotic atmosphere oxygen levels could have approached 10⁻³ PAL (even for a surface oxidation rate 10 times the present) and may have reached 10^{-2} -0.1 PAL during any particularly warm and moist Precambrian conditions. Atmospheric oxygen in such amounts would have been sufficient to have produced a biologically effective Precambrian ozone shield⁷⁻¹¹. The present calculations are consistent with the geological results of Grandstaff³⁰ who has shown that the occurrence of detrital uraninite does not require an essentially anoxic early Precambrian atmosphere.

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Cloud, P. Am. J. Sci. 272, 537-548 (1972).
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Surface viscosity of water

J. C. Earnshaw

Department of Pure and Applied Physics, The Queen's University of Belfast, Belfast BT7 1NN, UK

The recent suggestion that surface viscosity effects can arise from the molecularly diffuse nature of a fluid surface or interface has prompted this re-examination of data concerning capillary waves on the surface of water. Previous work2 has shown that the propagation of such waves is not compatible with the predictions of accepted theory for a surface having no abnormal rheological properties. These apparent discrepancies can be resolved in terms of one of the surface shear viscosities predicted by Goodrich. The existence of such surface viscosities for pure water suggests that caution must be exercised in interpreting surface viscosities observed for surface films in terms of molecular structure of, or interactions within the films.

Capillary waves on a fluid interface propagate in a manner governed by the viscoelastic properties of the system. The evolution of the waves in time can be described (in two dimensions; x in the surface) by a vertical displacement

$$\zeta = \zeta_0 \cos(qx) \exp(\alpha t) \tag{1}$$

where q is the surface wave-vector $(2\pi/\lambda)$ and α is a complex frequency:

$$\alpha = i\omega - \Gamma \tag{2}$$

In the case of clean liquid-air surface there is a well-established dispersion equation³ (based on linearized hydrodynamic theory) relating α to q and to the physical properties of the fluid. In first-order approximation this predicts a frequency

$$\omega^2 = \sigma_0 q^3 / \rho \tag{3}$$

and the damping constant

$$\Gamma = 2\eta_0 q^2 p \tag{4}$$

where σ_0 and η_0 are respectively the surface tension and the dynamic viscosity of the liquid. Non-propagating surface modes exist for sufficiently large q; they are not of present concern. Higher-order approximations for ω and Γ can be derived; they essentially agree with exact numerical solutions of the dispersion equation^{2,4}. Careful experiments² on thermally excited capillary waves of small amplitude on a water-air interface do not agree precisely with this theory. The observations imply a viscosity η_0 rather above the accepted value for water. Modification of the dispersion equation to allow for the viscosity of air5 only partially removes these systematic discrepancies. It has not been clear to date whether the discrepancies arise from the linearization of the hydrodynamic equations or from some modification of the properties of water close to the surface. Persuasive arguments can be presented against the former possibility.

Recently Goodrich has shown that the molecularly diffuse interfacial region between two fluids displays anisotropic momentum transport parallel to and normal to the interface; this anisotropy leads to surface viscosity effects. Goodrich identifies four surface viscosity effects, two (κ and κ_N) describing dilational viscosity within and normal to the interface and two $(\eta \text{ and } \eta_N)$ describing shear viscosity. These surface viscosity coefficients are usually associated with the viscoelastic behaviour of a fluid interface supporting a surfactant monolayer.

Margulis, L., Walker, J. C. G. & Rambler, M. Nature 264, 620-624 (1976). Dimroth, E. & Kimberley, M. M. Can. J. Earth Sci. 13, 1161-1185 (1976).

Van Valen, L. Science 171, 439-443 (1971). Towe, K. M. Nature 274, 657-661 (1978).

<sup>Howe, K. M. Nature 274, 657-661 (1978).
Kasting, J. F., Liu, S. C. & Donahue, T. M. J. geophys. Res. 84, 3097-3107 (1979).
Ratner, M. I. & Walker, J. C. G. J. atmos. Sci. 29, 803-808 (1972).
Blake, A. J. & Carver, J. H. J. atmos. Sci. 34, 720-728 (1977).
Levine, J. S., Hays, P. B. & Walker, J. C. G. Icarus 39, 295-309 (1979).
Katsumori, M. J. met. Soc. Jap. 57, 243-253 (1979).
Kasting, J. F. & Donahue, T. M. J. geophys. Res. 85, 3255-3263 (1980).
Hunten, D. M. J. atmos. Sci. 30, 726-732, 1481-1494 (1973).</sup>

Hunten, D. M. & Strobel, D. F. J. atmos. Sci. 31, 305-317 (1974) Liu, S. C. & Donahue, T. M. J. atmos. Sci. 31, 1118-1136 (1974).

Liu, S. C. & Donanue, F. M. J. atmos. Sct. 31, 1118-1136 (1974).
 Kasting, J. F. & Walker, J. C. G. J. geophys. Res. 86, 1147-1158 (1981).
 Walker, J. C. G. Evolution of the Atmosphere (Macmillan, New York, 1977).
 Holland, H. D. Proc. Symp. Hydrogeochemistry and Biogeochemistry, 68-81 (Clarke, Washington, 1973); The Chemistry of the Atmosphere and Oceans (Wiley, New York, 1978).

^{18.} Brinkmann, R. T. J. geophys. Res. 74, 5355-5368 (1969)

Hart, M. H. Icarus 33, 23-39 (1978).
Dobson, G. M. B. Exploring the Atmosphere (Clarendon, Oxford, 1963).

Mastenbrook, H. J. J. atmos. Sci. 25, 299-311 (1968).
 Newman, M. J. & Rood, R. T. Science 198, 1035-1037 (1977).
 Sagan, C. & Mullen, G. Science, 177, 52-56 (1972).
 Owen, T., Cess, R. D. & Ramanathan, V. Nature 277, 640-642 (1979).

Carver, J. H. 4th int. Symp. on Environmental Biogeochemistry, 55-64 (Australian Academy of Science, 1980).

Henderson-Sellers, A. & Meadows, A. J. Nature 270, 589-591 (1977)

Knauth, L. P. & Epstein, S. Geochim, cosmochim, Acta 40, 1095-1108 (1976)

Schopf, T. J. M. Paleoceanography (Harvard University Press, Cambridge, 1980)

Morss, D. A. & Kuhn, W. R. Icarus 33, 40-49 (1978).

^{30.} Grandstaff, D. E. Precambr. Res. 13, 1-26 (1980).

There seems, however, to be no a priori reason why such effects should not arise for clean fluid surfaces or interfaces. Goodrich's theory thus justifies an examination of the second possible explanation mentioned above.

Surface viscosity effects due to surfactant monolayers exert well-understood effects on capillary waves3. The predominant effect of the additional viscous dissipation is to increase the effective damping of the waves. Goodrich 1.6 shows that κ_N has no part in the linearized theory of capillary waves; further the shear modes decouple from the other modes and are not observed in the experiments mentioned above (these involve the scattering of light, which does not couple to surface shear modes). The hydrodynamic theory is generalized by introducing frequency dependence to the surface tension:

$$\sigma(\omega) = \sigma_0 + i\omega \eta_N \tag{5}$$

and a frequency dependent surface compressibility:

$$\varepsilon(\omega) = \varepsilon_0 + i\omega\kappa \tag{6}$$

Classically ε_0 , κ and η_N are all zero for clean surfaces but may assume non-zero values in the presence of a surface film. The spectrum of light scattered by surface waves reflects their temporal evolution (experiments directly yield values for ω and Γ); the theoretical form including surface viscosity effects has been calculated7 and can be used to estimate the viscoelastic properties of interfaces.

The effects of κ and η_N on ω and Γ are shown in Fig. 1; the two coefficients are seen to have very different effects. The increase in Γ caused by κ seems to saturate, whereas that due to η_N increases until eventually the capillary waves become overdamped. These effects of η_N are similar to those of an increased value of bulk viscosity, η_0 . The various surface viscosities have quite different effects on the dispersion properties of surface waves. Figure 2 shows that the relative effect of κ on Γ is approximately independent of q whilst that due to η_N increases with q. The effects of κ and η_N are comparatively small and rather large values were chosen for Fig. 2 to accentuate the general trends. The differences in behaviour of ω with q, if plotted on Fig. 2, would not be distinguishable (except for the higher η_N value at $q > 1,500 \text{ cm}^{-1}$).

Also shown in Fig. 2 are data² on Γ for waves on clean water-air surfaces; the data deviate increasingly from the classical assumptions ($\varepsilon_0 = 0$, $\kappa = \eta_N = 0$) as q increases. The wave frequencies are uniformly close to those for the classical

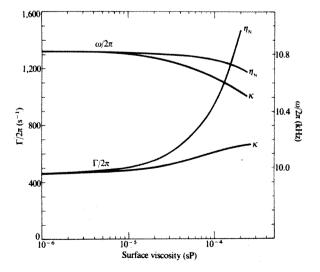


Fig. 1 The effects of surface viscosity $(\eta_N \text{ and } \kappa)$ on the frequency and damping constant of capillary waves $(q = 400 \text{ cm}^{-1})$ Note the different scales for ω and for Γ .

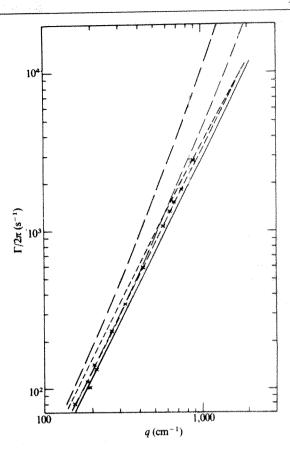


Fig. 2 The variation of Γ with q for water under various assumptions about the surface: —, classical surface, $\kappa = \eta_N = 0$; ———, $\eta_N = 0$ and $\kappa = 2 \times 10^{-5}$ sP (lower line) and 10^{-4} sP; ———, $\kappa = 0$ and $\eta_N = 2 \times 10^{-5}$ sP (lower line) and 10^{-4} sP. Also shown are the data of ref. 2 for water at 20 °C.

assumptions2. Taking as minimal assumptions the accepted values $\sigma_0 = 72.75$ dyne cm⁻¹ and $\eta_0 = 1.002$ cP (appropriate to T = 20 °C) and $\varepsilon_0 = 0$ dyne cm⁻¹, values for κ and η_N can be estimated using these data. The overall maximum likelihood estimates are $\kappa = 0$ sP (surface Poise—mN m⁻¹ s) and $\eta_N = 1.2$ $(\pm 0.4) \times 10^{-5}$ sP. The upper limit on κ is 1.5×10^{-5} sP. The limits quoted in both cases are 95% confidence limits.

Unfortunately quantitative data for comparison with that of ref. 2 is scarce. Several studies of capillary waves on water have used similar experimental techniques to those of ref. 2. The tabulated data of Langevin⁸ are all compatible with the present conclusions apart from the lowest q value investigated, for which the instrumental correction to Γ is largest. Bird and Hills derive a bulk viscosity slightly above the accepted value. The data of Hård et al. 10 yield estimates of η_0 below the accepted value; in this study, however, instrumental corrections were only made approximately and may have been overestimated. The damping of mechanically generated capillary waves on water seems to be consistent with the present conclusions.

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Goodrich, F. C. Proc. R. Soc. A374, 341–370 (1981).
 Byrne, D. & Earnshaw, J. C. J. Phys. D 12, 1133–1144 (1979); J. Colloid Interface Sci. 74,

3. Lucassen-Reynders, E. H. & Lucassen, J. Adv. Colloid Interface Sci. 2, 347-395 (1969).

Stone, J. A. & Rice, W. J. J. Colloid Interface Sci. 61, 160-169 (1977).
 Bellman, R. E. & Pennington, R. H. Q. appl. Maths. 12, 151-162 (1954).
 Goodrich, F. C. Proc. R. Soc. A260, 490-502 (1961).
 Langevin, D. & Bouchiat, M. A. Cr. hebd Séanc. Acad Sci., Paris B272, 1422-1425 (1971).

Langevin, D. JCS Faraday Trans. I 70, 95-104 (1974)

9. Bird, M. & Hills, G. Physicochemical Hydrodynamics (ed. Spaiding, D. B.) 609-625

10. Hård, S. Hamnerius, Y. & Nilsson, O. J. appl. Phys. 47, 2433-2442 (1976).

Raman investigation of ring configurations in vitreous silica

Shiv K. Sharma

Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA

Joseph F. Mammone* & Malcolm F. Nicol

Department of Chemistry, University of California, Los Angeles, California 90024, USA

Random network models of glass structures1 provide satisfactory qualitative descriptions of many properties of glasses (see reviews in refs 2-4). However, to obtain good quantitative agreement between theoretical analyses and experimental observations⁵⁻¹¹ it is often necessary to assume that specific ring structures in the random networks have special importance. In the case of vitreous silica (v-SiO₂), distributions of loops of SiO₄ tetrahedra in the random network have been invoked⁵ match calculated and experimental X-ray radial distribution functions (RDF). High resolution X-ray photoelectron spectra¹² of v-SiO₂ and quartz also provide evidence for the occurrence of four-, six- and higher-membered rings of SiO4 tetrahedra in v-SiO2. Raman spectra are also sensitive to local microstructures in vitreous solids. We have therefore examined the question of rings in the structure of v-SiO2 by comparing its Raman spectrum with spectra of crystalline silica polymorphs whose shortest loops contain four (coesite) and six (for example, α -quartz) tetrahedra. This comparison indicates that the sharp shoulder at 490 cm⁻¹ in the spectrum of v-SiO₂, previously attributed to a defect structure3 or a longitudinal optic mode14 can be assigned to four-membered ring structure. We discuss here possible basis for the stability of four-membered rings of SiO₄ tetrahedra in v-SiO₂ at ambient pressure. Possible reasons for the absence of the 490 cm⁻¹ band in the vibrational densityof-states derived for a random network model by Bell and co-workers 12,15 are discussed.

Figure 1 shows first-order room-temperature Raman spectra of polycrystalline samples of α -quartz and coesite and a bulk sample of high purity v-SiO₂. These spectra were measured in the 90° scattering configuration with a Jobin-Yvon Raman spectrometer and 488-nm line of an argon ion laser¹⁶. The positions and spectral characteristics of the bands in these spectra are given in Table 1. Examination of these spectra suggests that the very strong band at 437 cm⁻¹ and the sharp shoulder at 490 cm⁻¹ in the spectrum of v-SiO₂ are related to the strong bands at 465 and 521 cm⁻¹ in the spectra of α -quartz and coesite, respectively (Table 1).

Theoretical analyses 17-20 of the vibrational spectrum of v- SiO_2 have indicated that the 437 cm⁻¹ band belongs to $\nu_s(Si-O-$ Si), the symmetric stretch. In this mode, the bridging oxygen moves along the line bisecting the Si-O-Si bond angle. The frequency of this mode is sensitive to both the Si-O-Si bond angle and the Si-O band length 18,19. A third parameter which could affect $\nu_s(Si-O-Si)$ is the short-range connectivity of the network structure. Raman data for framework aluminosilicates with four-, five- and six-membered rings of TO₄ tetrahedra (T = Si or Al) indicate that, among crystals of isochemical compositions, the frequency of $\nu_s(T-O-T)$ increases with reduction in the ring size (Table 2). This correlation implies that the band at 437 and the shoulder at 490 cm⁻¹ in v-SiO₂ arise from tetrahedral network structures, composed of six- and four-membered rings, respectively. That these bands occur at lower frequencies in the spectrum of v-SiO2 than in the spectra of the crystalline polymorphs is probably due to differences in Si-O-Si bond angles in the rings in v-SiO2 and the crystalline

Table 1 Frequencies of the bands observed from Raman spectra

a. O		
α-Quartz	Coesite	Silica glass†
(cm^{-1})	(cm^{-1})	(cm^{-1})
	77 s	68 s, bd, wp
128 s*	116 s	1
-	151 m	
**************************************	176 s	
207 s, bd	204 m	
100 MARINE	220 w	e e e e e e e e e e e e e e e e e e e
264 m	269 s	
**********	314 m	and the state of t
*****	326 m	en e
356 m	355 m	
395 m(sh)		
	427 m	1
465 vs	466 m	437 vs, bd, p
Nationalism	521 vs	$490 \mathrm{s(sh)}, \mathrm{p}$
Meaning	NATIONAL PROPERTY AND ADDRESS OF THE PROPERTY ADDRESS OF THE PROPERTY AND ADDRESS OF THE PROPERTY ADDRESS OF THE PROPERTY AND ADDRESS OF THE PROPERTY	606 w, p
696 w		
795 w(sh)	785 w	netrosperio.
807 w	815 w	797 w, bd, dp
-100.000	837 w	830 w(sh)
***************************************	1,036 w	
1,066 w(sh)	1,065 w	1,060 w, bd, dp
1,083 w	1,144 w	
1,161 w	1,164 w	1,190 w, bd, dp
1,231 w	*****	

Measurement accuracy in the spectra of crystalline polymorphs, $\pm 2~{\rm cm}^{-1}$ for weak band and $\pm 1~{\rm cm}^{-1}$ for strong bands; in the spectrum of SiO₂ glass, $\pm 4~{\rm cm}^{-1}$ for sharp and strong bands and $\pm 10~{\rm cm}^{-1}$ for broad and weak bands.

polymorphs. Raman measurements of α -quartz under high pressures have shown that the frequency of the $464~\text{cm}^{-1}$ band is sensitive to compression²⁶. Studies of neutron irradiated v-SiO₂ also show that the frequencies of these bands increase on densification of the vitreous silica²⁷.

The large halfwidth of the 437 cm⁻¹ band of v-SiO₂ (Fig. 1) is usually attributed to the dispersion of Si–O-Si angles. However, the position of ν_s (Si–O-Si) also depends strongly on ring size (see Table 2). Thus, the ν_s (Si–O-Si) vibrational modes of five-, seven- and higher-membered rings of SiO₄ tetrahedra, which may also be present in the random network, may be responsible for the broadening of the 437 cm⁻¹ band.

The v-SiO₂ band at 606 cm⁻¹, which has been attributed to defects ^{13,27}, has no counterpart in the spectra of silica polymorphs (Fig. 1). Our data, therefore, support its assignment to defect structures involving partially broken Si-O bonds in the network²⁸.

The 400–600 cm⁻¹ region of the Raman spectrum of v-SiO₂ thus suggests that at least three different types of structural configurations occur in v-SiO₂. Each of these configurations, of course, will be associated with a range of Si–O–Si bond angles such that the material lacks long range order. The broad and complex bands in the $700-1,200 \, \mathrm{cm}^{-1}$ region of the v-SiO₂ spectrum (Fig. 1) also may be attributed to the presence of at least three different types of structural groups in metastable equilibrium in v-SiO₂.

The presence of three different types of structural groups in $v\text{-SiO}_2$ can be further supported by the density and Raman data²⁷ on neutron irradiated $v\text{-SiO}_2$. Both the density and intensity of the 490 cm⁻¹ band of $v\text{-SiO}_2$ increase on exposure to neutrons, and both properties show maxima at 5×10^{19} neutrons cm⁻². Neutron irradiation increases the intensity of the 606 cm^{-1} band at an even faster rate than the intensity of the 490 cm^{-1} band, but the 606 cm^{-1} intensity does not show a maximum. In contrast, the intensity of the 437 cm^{-1} band of unirradiated SiO_2 decreases with increasing exposure to neutrons²⁷. The different responses of the 437, 490 and 606 cm^{-1}

^{*} Present address: Textile Fibers Department, Experimental Station, E. I. Du Pont de Nemours and Co., Wilmington, Delaware 19898, USA.

^{*}w, Weak; s, strong; vs, very strong; bd, broad; sh, shoulder; p, polarized; dp, depolarized.

[†] The arrow indicates the presence of a strong continuum between the band maxima.

bands of v-SiO₂ to neutron irradiation clearly demonstrate that these bands belong to different species. The observed maxima in the intensity of the 490 cm⁻¹ band and in the density at 5×10^{19} neutrons cm⁻² can be attributed to the conversion of the five-, six- and higher-membered rings of SiO4 tetrahedra into fourmembered rings.

Recently, Mikkelsen and Galeener²⁹ reported that both the density and the intensity of the 606 cm⁻¹ band of v-SiO₂ (Suprasil W1) increase with the fictive temperature. The intensity of the 490 cm⁻¹ band was also enhanced as the fictive temperature of v-SiO2 increased (see Fig. 1 in ref. 29), although the magnitude of the increase could not be precisely determined.

The increase of the intensity of the 606 cm⁻¹ band with increasing fictive density suggests either that a higher density structure is responsible for the 606 cm⁻¹ mode, or that, while most of the network relaxes to a denser than average structure, this is accompanied by an increase in the concentration of a less dense structure responsible for the 606 cm⁻¹ band²⁹. The Raman data²⁷ on neutron-irradiated v-SiO₂ clearly show that the 606 cm⁻¹ band belongs to a less dense structure. At exposures of 2×10²² neutrons cm⁻², the intensity of the 606 cm⁻¹ band is 1.4 times that for v-SiO₂ exposed to only 5×10^{19} neutrons cm⁻²; but the additional irradiation decreases the density to 2.518 g cm^{-3} from 2.560 g cm^{-3} .

The intensities of the 490 and 606 cm⁻¹ bands increase with increasing fictive density as well as with increasing density of v-SiO₂ at low neutron irradiation (≤5×10¹⁹ neutrons cm⁻²); this suggests that there is a relationship between the fourmembered ring structure responsible for the 490 cm⁻¹ band and the defect centres responsible for the 606 cm⁻¹ mode. The silicon atoms at these defects carry a formal positive charge. We suggest that the four-membered rings in v-SiO2 at ambient pressure are stabilized by the excess positive charge on silicon atoms at the defect sites just as the four-membered rings of TO4 tetrahedra in crystalline feldspar are stabilized by cations³⁰. This interpretation suggests that the intensities of both the 490 and 606 cm⁻¹ bands will initially increase with the density of v-SiO₂ at ambient pressure. However, the extent to which defect centres can stabilize four-membered rings at ambient pressure is limited as indicated by the maxima in both the intensity of the 490 cm⁻¹ band and the density of unirradiated v-SiO₂ at an

exposure of 5×10^{19} neutrons cm⁻². Bell et al. 15 and Bell and Dean 17,31 have computed vibrational densities-of-states and other properties of SiO₂ for large (~200 SiO₄ molecular unit) continuous random-network clusters of almost perfectly tetrahedral units. Within each cluster, molecular units interact through short range (nearest-neighbour) bond forces while the cluster interacts with non-bridging oxygens at its surface. Raman spectra of v-SiO₂ have recently been computed¹¹ in terms of the behaviour of much smaller sections (typically 10-20 molecular units) of Bell and Dean's clusters. Computations with these models have given fairly good

Table 2 Relationship between $\nu_s(T-O-T)$ frequency and ring structures in some tectosilicates

Minerals	ν_s (T-O-T) in crystalline polymorphs	Repeat units and shortest rings of tetrahedra ²¹	Ref.
α-quartz	464 vs	6	22
β-quartz (700°C)	464 vs	6	22
α-crystabolite	416 vs	6	23
Coesite	521 vs	4	Present work
LiAlSi ₂ O ₆ -III	480 vs	6	24
LiAlSi ₂ O ₆ -II	492 vs	5	24
Low albite (NaAlSi ₃ O ₈)	506 vs	4	25
Orthoclase (KAlSi ₃ O ₈)	513 vs	4	25
Anorthite (CaAl ₂ Si ₂ O ₈)	503 vs	4	25

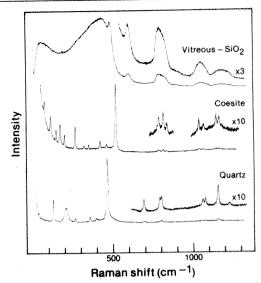


Fig. 1 Raman spectra of crystalline α -quartz, coesite and vitreous silica (v-SiO₂) at room temperature (laser 488.0 nm; slit and laser power at the sample used for crystalline polymorphs were 3 cm and 300 mW and for SiO₂ glass these were 5 cm⁻¹ and 500 mW, respectively).

agreement with observed X-ray and neutron diffraction data. Calculated spectra also resemble, to a first approximation, observed Raman spectra of v-SiO2. However, the sharp bands at 490 and 606 cm⁻¹ and the broad band at 1,200 cm⁻¹ do not have counterparts in the computed spectra ^{14,32}. This discrepancy may result from two factors that the v-SiO2 models of Bell and co-workers do not take into account: (1) the presence of defect centres involving partially broken Si-O bonds²⁸ in the interior of v-SiO₂ and (2) the ring statistics, especially the presence of four-membered ring structures which seem to be stabilized by the defect centres. It is now possible to calculate vibrational spectra of vitreous solids on the basis of random network models containing defect centres 10,33 and ring structures 10,11. The present results suggest that such calculations for v-SiO2 should be attempted to extend our understanding of the Raman spectrum as well as the structure of vitreous silica.

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- Zachariasen, W. H. J. Am. chem. Soc. 54, 3841–3851 (1932). Bockris, J. O'M. & Reddy, A. K. N. Modern Electrochemistry, 594–618 (Plenum, New York,
- 3. Cooper, A. R. in Borate Glasses, Structure, Properties, Application (eds Pye, L. D., Frechette, D. & Kreidl, N. J.), 167-181 (Plenum, New York, 1978).
- Gaskell, P. H. J. Phys. C: Solid St. Phys. 12, 4337-4368 (1979).
- Dean, P. Nature 210, 257-259 (1966).
- Dean, P. Nature 210, 257-259 (1966). Evans, D. L. & King, S. V. Nature 212, 1353-1354 (1966) Bell, R. J. & Dean, P. Nature 212, 1354-1356 (1966).
- King, S. V. Nature 213, 1112-1113 (1967).
- Bell, R. J. & Dean, P. Phil. Mag. 25, 1381-1398 (1972)
- Alben, R., Weaire, D., Smith, J. E. Jr & Brodsky, M. H. Phys. Rev. B11, 2271-2296 (1975). 11. Bell, R. J., Carnevale, A., Kurkjian, C. R. & Peterson, G. E. J. non-cryst. Solids 35/36,
- 1185-1190 (1980). Nucho, R. N. & Madhukar, A. Phys. Rev. B21, 1576-1588 (1980)
- 13. Stolen, R. H. & Walrafen, G. E. J. chem. Phys. 64, 2623-2631 (1976).
- Galeener, F. L. & Lucovsky, G. Phys. Rev. Lett. 37, 1474-1478 (1976)
- Bell, R. J., Bird, N. F. & Dean, P. J. Phys. C: Solid St. Phys. 1, 299-303 (1968).
 Sharma, S. K. Yb. Camegie Instn. Wash. 77, 902-904 (1978).
- 17. Bell, R. J. & Dean, P. in Amorphous Materials (eds Douglas, R. W. & Ellis, B.) 443-451 (Wiley, New York, 1972).18. Sen, P. N. & Thorpe, M. F. Phys. Rev. B15, 4030-4038 (1977).
- Laughlin, R. B. & Joannopoulos, J. D. Phys. Rev. B6, 2942-2952 (1977). Galeener, F. L. Phys. Rev. B19, 4292-4297 (1979).
- Zoltai, T. Am. Miner. 45, 960-973 (1960). Bates, J. B. & Quist, A. S. J. chem. Phys. 56, 1528-1533 (1972).
- Bates, J. B. J. chem. Phys. 57, 4042-4047 (1972). Sharma, S. K. & Simons, B. Am. Miner. 66, 118-126 (1981).
- White, W. B. in Infrared and Raman Spectroscopy of Lunar and Terrestrial Minerals (ed. Karr, C. Jr.) 325-358 (Academic, New York, 1975).
- Asell, J. F. & Nicol, M. J. chem. Phys. 49, 5395-5399 (1968).
 Bates, J. B., Hendricks, R. W. & Shaffer, L. B. J. chem. Phys. 61, 4163-4176 (1974).
- Greaves, G. N. J. non-cryst. Solids 32, 295-311 (1979). Mikkelsen, J. C. Jr & Galeener, F. L. J. non-cryst. Solids 37, 71-84 (1980).
- Megaw, H. D., Kempster, C. J. E. & Radoslovich, E. W. Acta crystallogr. 15, 1017-1035 (1962).

- Bell, R. J. & Dean, P. Discuss. Faraday Soc. 50, 55-61 (1970).
 Hass, M. Phys. Chem. Solids 31, 415-422 (1970).
 Dean, P. in Localized Excitations in Solids (ed. Wallis, R. F.) 109-116 (Plenum, New York,

Heat flow on the Hawaiian Swell and lithospheric reheating

R. S. Detrick*, R. P. von Herzen†, S. T. Crough‡, D. Epp§ & U. Fehn||

- * Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02281, USA
- † Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
- ‡ Department of Geosciences, Purdue University, West Lafayette, Indiana 47907, USA
- § Hawaii Institute of Geophysics, University of Hawaii, Honolulu, Hawaii 96822, USA
- University of Rochester, Rochester, New York 14627, USA

Most oceanic volcanic centres caused by hotspot activity are surrounded by sea floor that is unusually shallow for its age^{1,2}. There is increasing evidence that swells found on older sea floor, like the Hawaiian Swell, have formed from a broad-scale reheating and thinning of the lithosphere as it passes over a mantle hotspot^{3,4}. If the formation and subsequent disappearance of these swells are controlled by thermal processes, they should have an above-normal heat flux. We present here the results from 95 new heat flow measurements on the Hawaiian Swell. Along the older part of the swell the heat flow is 20–25% higher than the normal heat flow for crust of this age. This anomaly is consistent with the observed swell uplift. The shape and amplitude of the heat flow anomaly require that the reheating be largely confined to the lower half of the lithosphere.

The Hawaiian Swell is a broad, elongate region of anomalously shallow depths surrounding the youngest portion of the Hawaiian–Emperor seamount chain (Fig. 1). The swell has a maximum width of $\sim 1,200\,\mathrm{km}$ and extends more than 2,700 km WNW along the length of the Hawaiian chain. Southeast of Oahu the swell rises $\sim 1.5\,\mathrm{km}$ above the surrounding sea floor. The height of the swell gradually decreases to the northwest along the older portion of the island chain. While various models have been proposed for the origin of the Hawaiian Swell⁵⁻⁷ it has also been argued^{3,4} that the swell forms as a result of a broad-scale reheating of the lithosphere by the Hawaiian hotspot. The main evidence for this model is that the disappearance of the swell seems to follow a predictable thermal

subsidence curve³. If the lithosphere has been reheated, the swell should have an above-normal heat flux with the size and location of the heat flow anomaly depending on the manner in which the lithosphere is reheated⁴.

To test this hypothesis we recently obtained 95 reliable heat flow measurements at eight well-sedimented sites along the Hawaiian Swell (Fig. 1)⁸. The measurements were made with the Woods Hole Oceanographic Institution's digital heat flow instrument using thermistors externally mounted on either a piston core or a multipenetration pogo probe. Thermal conductivity measurements using the needle probe method⁹ were made on sediment samples recovered in piston cores at each site and a single mean conductivity determined for each area. The mean heat flow at each site, weighted according to the errors in individual heat flow measurements, is given in Table 1 and compared with the normal heat flow expected for crust of this age in Fig. 2.

The heat flow measurements at each site are remarkably uniform. The 95% confidence limits on the weighted site means are $<\pm4$ mW m⁻² (0.1 h.f.u.) at all but one site. The nearnormal heat flow on the youngest part of the swell precludes any significant reheating of the upper part of the lithosphere. However, the gradual increase in heat flow over the older part of the swell is consistent with reheating of the lower part of the lithosphere as it will take several million years for these relatively deep temperature changes to diffuse upwards. While part of the increase in heat flow between sites C and E is attributable to the \sim 10 Myr age offset across the Molokai fracture zone (Fig. 1), the heat flow at sites F, G and H is still 7-12 mW m⁻² higher than the expected heat flow for crust of this age. This anomaly, which is \sim 20-25% of the normal background heat flux, is clearly resolvable with the present data.

To determine if this heat flow anomaly is consistent with the amount of reheating required to uplift the swell, the expected heat flow for a simple reheating model has been calculated. The reheating process is assumed to begin at the lithosphere's base and proceed upwards, raising all lithospheric temperatures below a depth L to $T_{\rm m}$, the assumed temperature of the asthenosphere. The initial temperature above depth L remains unchanged. While other forms of reheating are possible, this model puts the added heat as deep as possible and satisfies the constraint that no large heat flow anomaly is associated with the youngest part of the swell. In this model, the sea floor depth and heat flow at any point along the swell are completely determined by the geotherm (age) of the lithosphere when reheated, the initial height of the swell and the time since reheating8. If we assume an age of 90 Myr for the lithosphere around Hawaii and a plate model geotherm for the lithosphere 10, then lithospheric thicknesses after reheating of 37-45 km are required to explain the observed swell uplift (corrected for the isostatic loading of

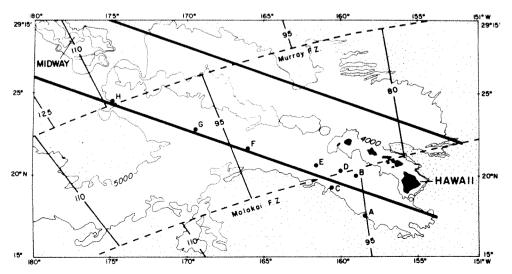


Fig. 1 Bathymetric map of the central Pacific with contours in metres. Isochrons (in Myr) and fracture zones (dashed lines) adapted from ref. 12. The extent of Hawaiian Swell is approximately indicated by the 5,000-m contour.

• Heat flow site locations. Average depths along thick, black lines are plotted in Fig. 3.

Table 1 Summary of heat flow data on the Hawaiian Swell

Site	Location	N	$(\mathbf{W}\mathbf{m}^{-1}\mathbf{K}^{-1})$	Ō (m₩	s.d.	95% confidence limits (mW m ⁻²)	t _c (Myr)	t _H (Myr)
Α	17°40′ N 158°20′ W	11	0.73	52.9	3.3	±2.3	95	2.1
В	20°00' N 159°00' W	8	0.77	51.5	2.0	±1.8	96	3.8
č	19°20′ N 160°25′ W	18	0.77	54.5	3.5	±1.8	98	5.0
Ď	20°15′ N 160°02′ W	4	0.74	58.7	3.4	±6.2	86?	5.0
É	20°30' N 161°30' W	8	0.81	58.0	2.9	±2.6	88	6.8
F	21°45′ N 166°00′ W	10	0.86	57.4	4.5	±3.4	94	12.1
Ğ	22°56′ N 169°36′ W	18	0.95	58.3	4.0	±2.1	97	16.3
Н	24°41′ N 174°38′ W	18	0.96	58.8	4.5	±2.3	109?	22.5

N, number of gradient measurements. \vec{K} , mean site thermal conductivity (corrected to in situ conditions). \vec{Q} , weighted mean heat flow with sample standard deviation s.d. t_c, Estimated crustal age. t_H, Inferred time since reheating. Crustal ages were estimated using the isochron map in ref. 12. Time since reheating was determined by projecting the site locations onto a line joining Hawaii and Midway and assuming an age of 27 Myr for Midway

100-200 m of sediment). These lithospheric thicknesses are about half the normal thickness of lithosphere of this age.

The predicted subsidence and anomalous heat flow for this range of values are shown in Fig. 3. The upper subsidence curve (L = 37 km) matches the data around Hawaii reasonably well, but seems too shallow over most of the rest of the swell. Inspection of the detailed bathymetry around the Hawaiian Islands¹¹ shows that there are two high spots on the swell, at 3 and 17 Myr, where smaller island chains (fracture zones?) crosscut the swell. Thus, the depths in these two areas may be anomalously shallow. The lower subsidence curve (L = 45 km) is a better fit over most of the swell. The heat flow anomaly observed on the swell also seems to be closer to the anomaly predicted by this lower subsidence curve, although the heat flow at the three oldest sites is less than predicted. This part of the swell actually formed when the hotspot was reheating slightly younger lithosphere (~80-Myr old) before it crossed the Molokai fracture zone (Fig. 1). When this is considered (dashed line in Fig. 3), the heat flow anomaly on this part of the Hawaiian Swell is probably large enough to be consistent with the observed swell uplift.

The main uncertainty in the present study is that reliable measurements of heat flow were not obtained on crust of the same age located off the swell, so the observed heat flow anomaly cannot easily be separated from any regional anomaly unrelated to the swell. The size of the observed anomaly is also small and close to the resolution of the data. Thus, although the

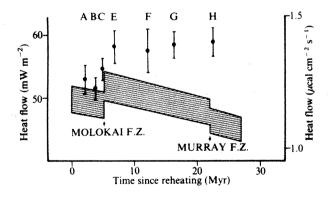


Fig. 2 Observed heat flow on the Hawaiian Swell with 95% confidence limits plotted against the time since each site passed over the Hawaiian hotspot. The shaded band is the normal heat flow expected for crust of this age estimated using a simple $(age)^{-1/2}$ relation with a scale factor of 11-12 h.f.u. (ref. 10). Assumed crustal ages and reheating times are given in Table 1. Site D was omitted from this plot because the heat flow is poorly determined (only four measurements) and the site is probably located on the Molokai fracture zone.

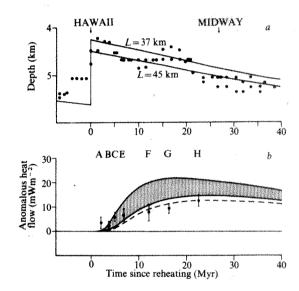


Fig. 3 a, Observed and predicted swell subsidence. Dots are 1° × 1° averages of the depth of the crest of the Hawaiian Swell along the lines shown in Fig. 1. Solid curves are predicted subsidence for reheating model where L is the lithospheric thickness after reheating. b, Anomalous heat flow observed on the Hawaiian Swell compared with predicted heat flow for L = 37 km (upper solid curve) and L = 45 km (lower solid curve) assuming an age of 90 Myr before reheating. These curves represent the upper and lower bounds on the heat flow anomaly required to explain the observed swell uplift. The dashed line shows the effect on the lower bound of reheating 80-Myr old rather than 90-Myr lithosphere (see text for discussion).

present measurements are consistent with lithospheric reheating, the strongest evidence for this model remains the manner in which the swell subsides.

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- Morgan, W. J. Nature 230, 42-43 (1971).
- Crough, S. T. & Jarrard, R. D., J. geophys. Res. (in the press).

 Detrick, R. S. & Crough, S. T. J. geophys. Res. 83, 1236-1244 (1978).

 Crough, S. T. Geophys. J.R. astr. Soc. 55, 451-569 (1978).

 Dietz, R. S. & Menard, H. W. J. Geol. 61, 99-113 (1953).

- Walcott, R. I. Tectonophysics 9, 435-446 (1970). McKenzie, D., Watts, A., Parsons, B. & Roufossi, M. Nature 288, 442-446 (1980).

- von Herzen, R. P., Detrick, R. S., Crough, S. T., Epp. D. & Fehn, U. (in preparation).
 von Herzen, R. P. & Maxwell, A. E. J. geophys. Res. 64, 1557–1563 (1959).
 Parsons, B. & Sclater, J. G. J. geophys. Res. 82, 803–827 (1977).
 Chase, T. E., Menard, H. W. & Mammerickx, J. Topography of the North Pacific (Institute of Marine Resources, University of California, San Diego, 1971).
 Sclater, J. G., Jaupart, C. & Galson, D. Rev. Geophys. Space Phys. 18, 269-311 (1980)
- 13. Dairymple, B. G., Clague, D. A. & Lanphere, M. A. Earth planet. Sci. Lett. 37, 107-116

Charnockite geobarometers based on coexisting garnet-pyroxene-plagioclase-quartz

D. Perkins III & R. C. Newton

Department of the Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

Numerous geothermometers based on compositions of coexisting minerals have been calibrated experimentally or theoretically during the past 10 yr. In contrast, few continuous-reaction geobarometers exist which have had wide application. One, based on the reaction of cordierite to garnet, sillimanite and quartz, is limited by a relatively narrow pressure range of application and also by conflicting calibrations^{1,2} because the mixing relations of Mg and Fe and the role of H2O in cordierite3 are not understood. Another successful barometer uses the assemblage garnet, plagiociase, Al₂SiO₅ and quartz^{4,5} Continuous geobarometers based on the reaction of orthoferrosilite to fayalite plus quartz⁶ and on the aluminium content of enstatite in equilibrium with garnet have been used to a lesser extent. The former barometer is limited by rarity of the requisite assemblage, and the latter by uncertain experimental calibration for crustal rocks. We calibrate here two mineralogical geobarometers from measured thermodynamic data. The participating minerals, pyroxenes, garnet, plagioclase and quartz, are frequently associated in high-grade metamorphic rocks. Calculation of pressures for several terrains show that the geobarometers yield reasonable and consistent results for the entire range of crustal pressures.

Most high-grade metamorphic terrains are in Precambrian shield areas and are dominated by orthopyroxene-bearing (charnockitic) acid to intermediate gneisses which invariably contain abundant basic segregations and lenses (basic granulites). The assemblage pyroxene-plagioclase-quartz is essential to charnockitic rocks, and garnet is present in many, including the type Madras charnockite occurrence. Garnet-pyroxene-plagioclase, frequently with quartz, is also characteristic of the basic units in many terrains. The four phase assemblages are represented by the reactions:

$$\begin{aligned} CaAl_2Si_2O_8 + Mg_2Si_2O_6 &= 1/3Ca_3Al_2Si_3O_{12}\\ &\text{anorthite} \end{aligned} \\ &+ 2/3Mg_3Al_2Si_3O_{12} + SiO_2\\ &\text{pyrope} \end{aligned} \tag{1}$$

$$\begin{array}{l} CaAl_2Si_2O_8 + CaMgSi_2O_6 = 2/3Ca_3Al_2Si_3O_{12} \\ \text{anorthite} & \text{diopside} \end{array}$$

$$+1/3Mg_3Al_2Si_3O_{12}+SiO_2$$
 (2)

Because these reactions have large volume changes they are suitable for geobarometry. The reactions are of high variance in rocks: plagioclase contains large amounts of NaAlSi₃O₈ (albite) in solid solution; the garnets and pyroxenes of granulites are complex, containing major amounts of Fe and smaller amounts of other elements. Previous attempts to calibrate these barometers^{9,10} have used experimental syntheses of the fourphase assemblages in simple systems^{11,12}, but long extrapolations are necessary from experimental temperatures and compositions of the phases were not reversed. An earlier thermodynamic calibration of reaction (1) was hampered by incomplete data for the phases¹³. Data are now complete enough for relatively rigorous formulations of the geobarometers from thermodynamic measurements.

The equilibrium condition that the Gibbs energy change is zero for reaction (1) at given temperature, T, and pressure, P, of metamorphism leads to the following expression:

$$-\Delta H^{\circ} + T\Delta S^{\circ} \cong RT \ln \frac{(\alpha_{Mg}^{Gt})^{2} \cdot (\alpha_{Ca}^{Gt})}{\alpha_{En}^{Opx} \cdot \alpha_{An}^{Pl}} + P\Delta V^{\circ}$$
(3)

where ΔH° and ΔS° are, respectively, the enthalpy and entropy changes of the reaction, ΔV° the molar volume change at T, and the α s denote the activities of MgAl_{2/3}SiO₄ and CaAl_{2/3}SiO₄ in garnet, Mg₂Si₂O₆ in orthopyroxene and CaAl₂Si₂O₈ in plagioclase. The approximation sign is used because of neglect of differential compressibilities and expansivities. A similar equilibrium condition is obtained for reaction (2).

Several recent thermochemical measurements allow evaluation of the left-hand side of equation (3), the standard Gibbs energy change, with moderate accuracy, thus avoiding the uncertain derivation from phase equilibrium studies. Enthalpy of solution measurements at 970 K for enstatite and pyrope diopside and grossular and anorthite give ΔH° as the difference in enthalpies of formation from the oxides. Low temperature adiabatic heat capacity measurements and high temperature heat content measurements, give ΔS° (refs 17–19). An entropy increment of 1.0 cal K⁻¹ is added to anorthite to account for Al, Si disorder 0.0 Over a temperature range of a few hundred degrees centred around 970 K, ΔH° and ΔS° are virtually constant.

The activities of Ca-Mg-Fe garnet components can be referred to constant W parameters for the bounding binary joins, such that the activity coefficient of MgAl_{2/3}SiO₄, $\gamma_{py} = \alpha_{py}/X_{py}$ is given by:

$$Rt \ln \gamma_{\text{py}} \cong W_{\text{MgFe}} X_{\text{Fe}}^2 + W_{\text{MgCa}} X_{\text{Ca}}^2 + (W_{\text{MgFe}} + W_{\text{MgCa}} - W_{\text{CaFe}}) X_{\text{Ca}} X_{\text{Fe}}$$
(4)

with a similar expression for $\gamma_{\rm gr}$ (ref. 21). $W_{\rm MgCa}$ has been measured calorimetrically and, for the ranges of compositions considered here, is, to a good approximation, $W_{\rm MgCa}$ (cal) = $3,300-1.5\,T$ (K)⁴. The simplest values of $W_{\rm CaFe}$ and $W_{\rm MgFe}$ compatible with high temperature and high pressure phase equilibrium measurements are zero^{22,23}. The unknown mixing properties of Mn in garnets restricts consideration to garnets with Mn \leq Mg/3. Enthalpy of solution studies on plagioclase¹⁶ lead to the 'Al-avoidance' activity model, expressed by

$$\alpha_{\rm an} = \gamma_{\rm an} \frac{X_{\rm an} (1 + X_{\rm an})^2}{4}$$

$$RT \ln \gamma_{\rm an} = (1 - X_{\rm an})^2 (2,075 + 9,318 X_{\rm an})$$
 (5)

Finally, the Mg₂Si₂O₆ and CaMgSi₂O₆ activities in pyroxene are taken as 'ideal two-site'

$$\alpha_{\text{en}}^{\text{Opx}} = X_{\text{Mg}}^{\text{M2}} \cdot X_{\text{Mg}}^{\text{M1}}$$

$$\alpha_{\text{di}}^{\text{Cpx}} = X_{\text{Ca}}^{\text{C2}} \cdot X_{\text{Mg}}^{\text{M1}}$$
(6)

where M1 and M2 are the pyroxene structural sites for nontetrahedral cations. Following Wood and Banno²⁴, Al^{VI}, Cr, Ti and Fe³⁺ are ordered in M1, Ca, Mn and Na are ordered in M2, and Mg and Fe²⁺ are equipartitioned in M1 and M2.

The above considerations lead to the following geobarometric expressions for reactions (1) and (2), respectively, with P in bar and T in K

$$P = 3,944 + 13.070T + 3.5038T \ln \frac{(\alpha_{\text{Ca}}^{\text{Gt}}) \cdot (\alpha_{\text{Mg}}^{\text{Gt}})^2}{\alpha_{\text{en}}^{\text{Opx}} \cdot \alpha_{\text{an}}^{\text{Pl}}}$$
(7)

$$P = 675 + 17.179T + 3.5962T \ln \frac{(\alpha_{Ca}^{Gt})^2 \cdot (\alpha_{Mg}^{Gt})}{\alpha_{Cpx}^{Cpx} \cdot \alpha_{an}^{Pl}}$$
(8)

The calculated pressures depend on mineralogical determination of T with its inherent uncertainties, but, fortunately, expressions (7) and (8) are not sensitively dependent on T. The major source of uncertainty is the thermochemically measured standard Gibbs energies, which create pressure uncertainties of 1,400 bar for the orthopyroxene barometer and 1,700 for the clinopyroxene barometer at a given temperature. The precision of the barometers is better than this, as the ΔG° errors are nearly

Table 1 Sample data and average calculated pressures

Locality	Description	Sample (No. of samples)	Ref.	Estimated temperature (°C)	P (opx) (bar)	P (cpx) (bar)
Huntley-Portsoy, Scotland Lochnagar, Scotland	Migmatite restite, aureoles of Newer Intrusives	10,557 (1) 82,891 (1)	25	690 780	3,849 2,845	
Nain, Labrador New York	Granulites, anorthosite aureole	NAK2893, 3909 (4)	39	750-800	3,299	
Adirondack Highlands,	Charnockites and metagabbros	ADAS-3 to ADTL-4 (12)	40	725–775	8,476	6,574
Nilgiri Hills, India Biligirirangan Hills, India Sittampundi Complex, India Madras, India	Charnockites and mafic granulites from South Indian shield	11-1, 11-3 (2) 7-5 (1) SITM-92 (1) MP44, 72 (2)	Unpublished Chicago data 7	825 800 830 790	8,723 9,493 7,406	6,167 6,530 7,993
Finnish Lappland Buksefjorden, Greenland Furua Complex, Tanzania Labwor, Uganda	Late Archaean-early Proterozoic granulites	47-III (1) 174087, 102 (2) TANZ 2-91 (14) AR51 (1)	41 10 29 42	740 800 800 950	7,384 7,537 10,689 8,087	5,166 5,026 8,350
Doubtful Sound, New Zealand Ronda Complex, Spain	Deep crustal mafic granulites	36453-68 (7) R-208-A (1)	28 27	750 800	12,602	10,971 11,100
Lashaine Volcano, Tanzania	Exotic granulite nodules	BD-727, 728 (2)	Unpublished Chicago data	950		15,550

constant. Assessment of accuracy of the barometers is more difficult. Information on this problem may be obtained by calculation of the pressures from a wide variety of granulite localities for which published mineral analyses exist.

Table 1 lists the localities, original sample numbers and descriptive data for several well documented occurrences of assemblages (1) and/or (2), representative temperatures, and calculated pressures. Immediately evident is that the clinopyroxene barometer reads 1-3 kbar lower than the orthopyroxene barometer. This discrepancy is within the combined uncertainties in ΔG° for reactions (1) and (2), and the ΔG° s could be adjusted to bring the barometers into agreement, on average. However, the pressure discrepancies may be real, and result from a greater tendency of clinopyroxene than orthopyroxene to re-equilibrate with plagioclase during uplift to lower pressures. Some information on the closure problem is given by the following pressure calculations from various terrains.

The Huntley-Portsoy and Lochnagar occurrences are hypersthene-bearing migmatites in the aureoles of the Newer Intrusions, southern Scottish Highlands²⁵. Both are sillimanitegrade, superposed on regional andalusite-bearing terrains, which suggests that the pressures should be lower than the experimental Al₂SiO₅ triple point. The Nain, Labrador granulites are in a high temperature aureole around a large anorthosite body. Pressure calculations based on the orthoferrosilite-fayalite-quartz reaction²⁶ give an average 3.2 kbar, in very good agreement with the present calculation. Buksefjorden, Greenland, Sittampundi Complex, Biligirirangan Hills and Nilgiri Hills, South India and Adirondack Highlands, New York, localities are all in large granulite tracts where sillimanite is the regional Al₂SiO₅ polymorph of intercalated metapelites. The orthopyroxene pressures agree with the Al₂SiO₅ diagram (Fig. 1) and seem more appropriate than previous pressure estimates, which would place the Adirondack⁶, Madras⁷ and Buksefjorden¹⁰ localities within the kyanite field. The Adirondack orthopyroxene pressure of $8,476 \pm 1,076$ bar based on 12 samples agrees with the current assessment²⁷ of 8±1 kbar based on revised orthoferrosilitefayalite-quartz barometry. Kyanite has been reported from the south-central Adirondacks²⁸, suggesting pressures close to the kyanite-sillimanite boundary. The Furua, Tanzania, granulite complex is a kyanite-bearing terrain²⁹, and the high calculated

orthopyroxene pressure is consistent with the experimental Al₂SiO₅ diagram (Fig. 1).

The Ronda aureole, southern Spain, and Doubtful Sound, New Zealand, granulites are tectonically uplifted portions of the deep crust^{30,31}. The Ronda granulites show a primary recrystallization at 11,100 bar followed by a secondary (corona) recrystallization at 6,480 bar. These estimates correspond closely to Obata's³² pressure estimates of two discrete recrystallizations of the Ronda peridotite mass. The Doubtful Sound mafic granulites are known, from gravity surveys, to be underlain by the upper mantle at a depth <10 km. At a distance of only 20 km inland, the New Zealand crust has a normal 40 km thickness. An average 2.8 g cm⁻³ crustal density and a 30-40 km uplift of the Doubtful Sound area implies pressures of 8.4-11.2 kbar of crystallization of the rocks now exposed at the surface.

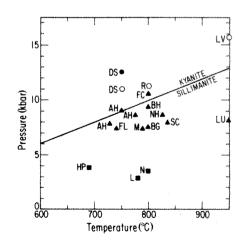


Fig. 1 Pressure calculations for various granulites. , Thermal aureoles (HP, Huntley-Portsoy; L, Lochnagar; N, Nain). , Precambrian granulite terrains (AH, Adirondack Highlands; FC, Furua Complex; FL, Finnish Lappland; M, Madras; BH, Biligirirangan Hills; NH, Nilgiri Hills; BG, Buksefjorden; SC, Sittampundi Complex; LU, Labwor, Uganda). , Deep crustal granulites (DS, Doubtful Sound; R, Ronda; LV, Lashaine Volcano). Filled symbols, orthopyroxene (reaction (1)) barometer. Open symbols, clinopyroxene (reaction (2)) barometer. Temperatures mostly from literature estimates (see Table 1).

The Lashaine Volcano, Tanzania exotic nodule suite contains clinopyroxene-plagioclase-garnet-scapolite granulites with occasional quartz, which may or may not be in textural equilibrium with the other minerals. The indicated clinopyroxene pressure of 15.5 kbar (Table 1) is an overestimate if quartz is not actually part of the primary equilibrium assemblage, but is in agreement with Irving's 33 experimental conclusion that very similar nodules from Delegate, Australia, equilibrated with their source at 14-17 kbar. Such scapolite granulites may be prominent components of the lower crust³⁴.

Everything that is known about the crystallization pressures of the occurrences tabulated here seems to agree with the orthopyroxene (reaction (1)) geobarometer. The strongest indications of its validity are comparison with the experimental Al₂SiO₅ diagram and the orthoferrosilite-fayalite-quartz geobarometer. This evidence suggests that reequilibration to lower pressures after the peak of metamorphism has had minimal effect on the pressure indication of reaction (1). The two barometers can be made to agree on the average within the uncertainties of the thermochemical measurements by a balanced readjustment of the ΔH° s and ΔS° s leading to the following expressions:

$$P(\text{opx}) = 3,694 + 12.820T + 3.5038T \ln K_1$$

$$P(\text{cpx}) = 1,425 + 17.929T + 3.5962T \ln K_2$$
(9)

These readjustments give the maximum allowable increase in the clinopyroxene pressure with only a small decrease in the orthopyroxene pressure. A test of the modified clinopyroxene barometer and some information on the delayed closure problem may be possible using lower-crustal two-pyroxene garnet granulite samples from explosive pipes³⁵, which are effectively quenched from deep-seated conditions. We have not yet found sufficient published data of this kind for a definitive test.

A firm result of this study is that the continental crust was, locally, at least, of thickness approaching or exceeding that of the present normal thickness as long ago as the late Archaean. A proposed high-temperature, low-pressure charnockite geotherm³⁶ is not supported. The remarkable cluster of Precambrian granulite pressures (Fig. 1) at $8.5 \pm 2 \text{ kbar}$ (opx barometer) for nine different terrains suggests that a certain repeated mechanism of high grade metamorphism has been operative. For the western Grenville this mechanism may have been continental collision, as the abortive rift of the North American continent which began ~1.400 Myr ago, closed ~1,100 Myr ago, generating the Grenville orogeny. The Grenville supracrustals were buried at 30 km depth in a doubled continent³⁷. Continental collision with temporary doubling of the crust has been proposed to explain the apparently high pressures of late Archaean metamorphism also³⁸. Great uplift in post-Precambrian times exposing deep crust and even upper mantle would seem to be required by the high pressures of the Doubtful Sound and Ronda occurrences.

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1. Hensen, B. J. & Green, D. H. Contr. Maner. Petrol. 38, 151-166 (1973)
```

15. Charlu, T. V., Newton, R. C. & Kleppa, O. J. Geochim. cosmochim. Acta 42, 367-375 (1978)

16. Newton, R. C., Charlu, T. V. & Kleppa, O. J. Geochim. cosmochim. Acta 44, 933-941 (1980).

Haselton, H. T. thesis, Univ. Chicago (1979).

Krupka, K. M., Robie, R. A. & Hemingway, B. S. EOS 58, 523 (1977).
Krupka, K. M., Kerrick, D. M. & Robie, R. A. EOS 60, 405 (1979).
Perkins, D., Westrum, E. F. & Essene, E. J. Geochim. cosmochim. Acta 44, 61–84 (1980).
Ganguly, J. & Kennedy, G. C. Contr. Miner. Petrol. 48, 137–148 (1974).

Cressey, G., Schmid, R. & Wood, B. J. Contr. Miner. Petrol. 67, 397-404 (1978). O'Neill, H. S. & Wood, B. J. Contr. Miner. Petrol. 70, 59-70 (1979).

Wood, B. J. & Banno, S. Contr. Miner. Petrol. 42, 109-124 (1973)

Ashworth, J. R. & Chinner, G. M. Contr. Miner. Petrol. 65, 379-394 (1978).

Bohlen, S. R. & Boettcher, A. L. Am. Miner. (in the press)

Bohlen, S. R., Essene, E. J. & Hoffman, K. S. Bull, geol. Soc. Am. 91, Pt I, 110–113 (1980). Boone, G. M. Geol. Soc. Am. Prog. 10, 34 (1978). Coolen, J. J. M. M. GUA (Amsterdam) Pap. 13 (Ser. 1) 1 (1980).

Loomis, T. P. Contr. Miner Petrol. 62, 1-22 (1977

Oliver, G. J. H. Contr. Miner. Petrol. 65, 111-121 (1977). Obata, M. J. Petrol. 21, 533-572 (1980).

Irving, A. J. J. Petrol. 15, 1-40 (1974).

Rogers, N. & Nixon, P. H. A. Rep. Res. Inst. Afr. Geol. 19, 38-41 (Dept. Earth Sci., Leeds University, 1975).

Griffin, W. L., Carswell, D. A. & Nixon, P. H. in The Mantle Sample (eds Boyd, F. R. & Meyer, H. O. A. 59-86 (American Geophysical Union, Washington, 1979). Saxena, S. K. Science 198, 614-617 (1977).

Sacina, S. R. Science 136, 614–617 (1977).

Seyfert, C. K. Bull, geol. Soc. Am. 91, Pt 1, 118–120 (1980).

Burke, K., Dewey, J. F. & Kidd, W. S. F. Tectonophysics 40, 69–100 (1977).

Berg, J. J. Petrol. 18, 399 (1977).

Bohlen, S. R. thesis, Univ. Michigan (1979). Hormann et al. Bull. geol. Surv. Finland 308 (1980).

42. Nixon, P. H. et al. Miner. Mag. 39, 420 (1973)

Preservation of polyunsaturated fatty acid in ancient Anasazi maize seed

David A. Priestley*, Walton C. Galinat† & A. Carl Leopold*‡

* Boyce Thompson Institute, Ithaca, New York 14853, USA † University of Massachusetts, Suburban Experiment Station, Waltham, Massachusetts 02154, USA

Oxidation of polyunsaturated fatty acids (PUFAs) is widespread in animal tissues and has been implicated in senescence¹ and as a cause of deterioration of stored seeds². Although signs of lipid oxidation have been detected in seeds during long-term storage3, little is known of the magnitude of the change induced in the PUFA fraction. In conditions of 'accelerated ageing' (saturating humidity and 45 °C) in soybean seeds, the PUFA content has been reported to decline within a few days4, but in somewhat milder conditions PUFAs are not oxidized during the time when germinability is lost⁵. An extreme case in which to estimate the extent to which PUFAs may be oxidized comes from the examination of ancient maize seeds from caves in the southwestern United States, where the arid climate is conducive to the preservation of biological materials⁶. We have now compared new seeds with seeds which were 1-17 centuries old, and report that most of the linoleic acid (by far the predominant PUFA in this species) is oxidized in the first 100-200 yr, but some is still detectable in seeds more than 1,500 yr old. We believe this represents the oldest sample of PUFA so far reported.

Modern maize seeds (Zea mays L.) contain ~5% lipid, most of which is neutral storage lipid. Four principal fatty acids are found (C18:2>C18:1>C16:0>C18:0). In a representative modern North American variety about half of the fatty acid is linoleic, C18:2 (Table 1; cv. Early Cornell Synthetic). The polar lipids of these seeds display a fatty acid profile rather similar to that of the storage lipid fraction. Increasing evidence indicates that maize has evolved through several thousand years of artificial selection from the annual teosinte (Z. mexicana (Schräder) Kuntze)^{7,8}. In agreement with an earlier study⁹, we

<sup>Hensen, B. J. & Green, D. H. Contr. Maner. Petrol. 38, 151-166 (1973).
Thompson, A. B. Am. J. Sci. 276, 425-454 (1976).
Newton, R. C. & Wood, B. J. Contr. Miner. Petrol. 68, 391-405 (1979).
Newton, R. C. & Haselton, H. T. in Thermodynamics of Minerals and Melts (eds Newton, R. C., Navrotsky, A. & Wood, B. J.) (Springer, New York, in the press).
Ghent, E. D., Robbins, D. B. & Stout, M. Z. Am. Miner. 64, 874-885 (1979).
Jaffe, H. W., Robinson, P. & Tracy, R. J. Am. Miner. 63, 1116-1136 (1978).
Weaver, B. L., Tarney, J., Windley, B. F., Sugavanam, E. B. & Venkata Rao, V. in Archaean Geochemistry (eds Windley, B. F. & Naqvi, S. M.) 177-204 (Elsevier, Amsterdam 1978).</sup> dam, 1978).

^{8.} Pichamuthu, C. S. J. Geol. Soc. Ind. 11, 273-275 (1970).

Wood, B. J. Earth planet. Sci. Lett. 26, 299-311 (1975).
 Wells, P. R. A. J. Petrol. 20, 187-226 (1979).
 Kushiro, I. & Yoder, H. S. J. Petrol. 7, 337-362 (1966).

Hensen, B. J. Contr. Miner. Petrol. 55, 279-292 (1976). Newton, R. C. in Archaean Geochemistry (eds Windley, B. F. & Naqvi, S. M.) 221-240 (Elsevier, Amsterdam, 1978).

^{14.} Charlu, T. V., Newton, R. C. & Kleppa, O. J. Geochim. cosmochim. Acta 39, 1487-1497

[‡] To whom correspondence should be addressed

Table 1 Total fatty acid content of maize and teosinte seeds

a, Recently grown seeds		% Of 1	total		mg Fatty acids per g
Sample	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	seed material
Z. mays (cv. Early Cornell Synthetic) Z. mexicana (race Nobogamé)	12.7 (0.2) 15.5	2.2 (0.1) 2.2	30.6 ((.1) 38.2	54.5 (0.2) 44.1 (0.1)	38.2 (0.2) 6.3 (0.1)
Z. diploperennis	(0.2) 14.6 (0.2)	(0) 1.9 (0)	(0.1) 37.0 (0)	46.5 (0.2)	13.5 (0.2)
b, Old seeds Mean of Sample estimated		% Of		(12.4)	mg Fatty acids per g
no. age (yr)	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	seed material
1,700	35.3 (0.5)	8.7 (0.2)	51.3 (0.1)	4.8 (0.3)	(0.3)
2 1,700	35.1 (0.3)	7.6 (0.1)	50.0 (0.5) 50.9	7.4 (0.3) 4.0	16.1 (0.9) 14.5
3 1,500	36.0 (0.5) 35.0	9.2 (0.8) 7.4	(2.7) 50.5	(1.5) 7.1	(0.9) 5.6
4 1,000 5 900	(0.8) 56.0	(0.8) 9.2	(0.2) 34.9	(0.4)	(0.5) 7.3
6	(1.2) 35.3	(0.3) 6.9	(0.8) 46.4	11.5	(0.7) 13.4
7 78	(0.2) 18.2 (0.2)	(1.9) 3.9 (0.5)	(0.1) 44.2 (0.2)	(1.9) 33.8 (0.5)	(1.7) 21.9 (0.9)

The lipids of ground seed particles (0.8-1.6 g) were extracted with chloroform-methanol (2:1, v/v) and the fatty acids prepared for gas chromatography as previously described. Separations were performed either on a 1.8×4 mm internal diameter glass column packed with 3% Silar-5CP on 100/120-mesh Gas Chrom Q or a 1.8×2 mm glass column packed with 10% DEGS-PS on 80/100-mesh Supelcoport. Additional details are given in ref. 5. Values given are the mean (and range) of two injections. Traces of linolenic acid (C18:3) in recent samples have been ignored.

Table 2 Provenance of old maize seeds

Sample no.	Race or variety	Date AD (and cultural affiliation)	Location	Ref.
1	Evolved Chapalote	100-500 (Basketmaker)	Cave II, March Pass, Kayenta, Arizona (PM: A 2481)	12
2	Early Chapalote	100-500 (Basketmaker)	As above (PM: A 2481)	12
3	Near Chapalote	200-700 (Basketmaker)	Site SR 16-6, Dirty Devil-Waterhole Flat, Utah (PM: 10/264)	24
4	Near Maíz de Ocho	700-1300 (Kayenta Anasazi)	Cave 8, Sagiotsosi Canyon, Kayenta, Arizona (PM: A 3520)	. 12
5	Dented Chapalote	950-1200 (Fremont)	Site PR4-31, Nine Mile Canyon, Utah (PM: A 7769)	24
6	Florida White Dent	1883	Central Kentucky (WCG)	
7	Leaming Dent	1902	Agricultural Expt. Station, Urbana, Illinois (WCG)	

The age ranges given for the ancient seeds are derived from the stratigraphy of the sites and are consistent with the overall pattern of maize evolution. Further details are given in ref. 12. PM, Catalogue number of the Peabody Museum, Harvard University; WCG, private collection of Dr Walton C. Galinat.

found that contemporary seeds of annual teosinte contain less lipid than maize. The proportions of the fatty acids are similar for maize, annual teosinte and a recently discovered perennial teosinte (Z. diploperennis Iltis, Doebley and Guzmán)¹⁰ (Table 1). In each of these contemporary seeds, linoleic acid constitutes >40% of the total fatty acids present¹¹. It is likely that the original content of linoleic acid in the ancient maize seed would have matched or exceeded this value.

Maize has been associated with the Anasazi culture of the American South-West from the first appearance of its earliest representatives (the Basketmaker people) around AD 100. This association continued throughout the Classic Pueblo period and into the recent era of European influence. The changing morphology of the maize races during this long period has been studied in some detail¹². We examined four samples of maize from various periods of the Anasazi culture (Tables 1 and 2, samples 1-4) and one sample from the closely related Fremont

culture (sample 5). We also investigated maize seed grown during the past 100 yr (samples 6 and 7). The provenance of the seeds is detailed in Table 2. The ages assigned to the seeds are consistent with the stratigraphy of the sites from which they were obtained and with the overall pattern of maize evolution 12. All these seeds were non-viable. When received for analysis their water content was ≤5-6%. All the ancient seeds showed excellent external preservation, and gave a light or dark brown meal when ground. The single Fremont sample (no. 5) was unusual in having adhering soil particles (presumably indicative of a moist environment) and in being extremely friable, readily crumbling into a fine dust when ground.

With the exception of the Fremont seed, all the samples contained linoleic acid (Table 1). The relative proportion of the three other fatty acids followed the customary maize-teosinte pattern (C18:1>C16:0>C18:0). Similar results were obtained whether we used a cyanosilicone phase or a polyester

phase for the gas chromatographic analysis. The presence of the C18:2 in the oldest seeds has been further confirmed by combined gas chromatography-mass spectrometry (data not shown). Results were comparable with those of Table 1 when the fatty acids of the polar (presumably primarily membrane) lipid subfraction were examined (data not shown). The PUFAs of the storage lipid fraction and the membrane lipid fraction were therefore about equally susceptible to oxidation. The data of Table 1 suggest a relatively rapid decline in linoleic acid during the first 100-200 yr of storage, leaving a small proportion of linoleic acid which is comparatively stable for much longer.

The proportion of palmitic acid (C16:0) in the Fremont sample exceeded that of oleic acid (C18:1). In these badly preserved seeds, even the monoenoic fatty acid was probably subject to enhanced degradation compared with the fully saturated acids. The well known deleterious effects of damp soil on ancient seeds13 probably led to loss of oleic acid in the Fremont seeds. On the other hand, prolonged dry storage of the well preserved Anasazi seeds (samples 1-4) was sufficient to remove only ~80% of the linoleic acid and scarcely any of the oleic.

Although preservation of monoenoic fatty acids has been noted in well stored Roman oil samples from the second century AD^{14,15} and brain lipids of a 40,000-yr-old frozen mammoth¹⁶, a badly deteriorated Egyptian oil sample originating from the sixth century BC lacked all but the fully saturated fatty acids¹⁷. PUFAs were absent in each case.

It is unlikely that the linoleic acid in the Anasazi seeds represents contamination, microbial or otherwise, because both the total (primarily neutral) lipid fraction and the quantitatively minor polar (membrane) lipid subfraction yielded similar results and it is most unlikely that these two different fractions would be contaminated to approximately the same extent in every sample.

The long-term preservation of linoleic acid is consistent with reports that some membrane structure can be preserved in ancient seeds, which suggests the presence of sufficient membrane lipid to permit a bilayer structure. Both membrane profiles in Egyptian wheat seeds from the third and fifth millenia BC18 and ample membrane preservation in 'fossil' Indian lotus seeds¹⁹ (exact age uncertain)²⁰ have been reported. The widespread occurrence of tocopherols in seeds²¹ doubtless serves to minimize the prevalence of PUFA oxidation during storage.

Our data refer to changes which occur in maize seeds post mortem, and do not relate directly to the debate about the relevance of lipid peroxidation to loss of seed viability^{4,5,22,23} However, they do indicate that the extensive PUFA oxidation which may occur in seeds during a few days or weeks of 'accelerated ageing' in the laboratory may take many decades or centuries in drier and cooler conditions. Furthermore, the retention of significant amounts of linoleic acid after 1.500 yr or more of storage emphasizes the unusual capacity of dry seeds to protect at least part of their susceptible components from atmospheric oxidation.

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- Barber, A. A. & Bernheim, F. Adv. gerontol. Res. 2, 355-403 (1967).
- Villiers, T. A. in Seed Ecology (ed. Heydecker, W.) 265-288 (Pennsylvania State University, University Park, 1973).
- 3. Spencer, G. F., Earle, F. R., Wolff, I. A. & Tallent, W. H. Chem. Phys. Lipids 10, 191-202
- 4. Stewart, R. R. C. & Bewley, J. D. Pl. Physiol. 65, 245-248 (1980)
- Priestley, D. A. & Leopold, A. C. Pl. Physiol. 63, 726-729 (1979). Guernsey, S. J. & Kidder, A. V. Pap. Peabody Mus. 8(2), 1-121 (1921). Galinat, W. C. Bull. Torrey bot. Club 102, 313-324 (1975).

- Doebley, J. F. & Iltis, H. H. Am. J. Bot. 67, 892-993 (1980).

 Melhus, I. E., Aguirre, F. & Scrimshaw, N. S. Science 117, 34-35 (1953).

 Iltis, H. H., Doebley, J. F., Guzmán-M., R. & Pazy, B. Science 203, 186-188 (1979).

 Reiners, R. A. & Gooding, C. M. in Corn: Culture, Processing, Products (ed. Inglett, G. E.)
- 241-261 (AVI, Westport, Connecticut, 1970).
 Galinat, W. C. & Gunnerson, J. H. Bot. Mus. Leafl. Harv. Univ. 20, 117-160 (1963)
- Keepax, C. J. archaeol. Sci. 4, 221-229 (1977).
- Jáky, M., Perèdi, J. & Pálos, L. Fette Seifen Anstr-Mittel 66, 1012-1017 (1964).
 Seher, A. Jb. Römisch-Germanischen Zentralmuseums Mainz 12, 199-203 (1965).
 Kreps, E. M. et al. Zhur. Evol. Biokhim. Fiziol 15, 227-238 (1979).

- Seher, A., Schiller, H., Krohn, M. & Werner, G. Fette Seifen Anstr-Mittel 82 (in the press).

- Hallam, N. D. in Seed Ecology (ed. Heydecker, W.) 115-143 (Pennsylvania State University, University Park, 1973).
- Zhukova, G.Ya. & Yakovlev, M. S. Bot. Zh. 61, 869-872 (1976).
 Wester, H. V. Hortscience 8, 371-377 (1973).
- Grams, G. W., Blessin, C. W. & Inglett, G. E. J. Am. Oil Chem. Soc. 47, 337-339 (1970). Harman, G. E. & Mattick, L. R. Nature 260, 323-324 (1976).
- Priestley, D. A., McBride, M. B. & Leopold, A. C. Pl. Physiol. 66, 715-719 (1980).
- Kidder, A. V. & Guernsey, S. J. Bull. Bur. Am. Ethnol. 65, 1-228 (1919).

Lack of correlation between extracellular polysaccharide and nodulation ability in Rhizobium

Richard Sanders*†, Elisabeth Raleigh† & Ethan Signer†

- * Department of Chemistry, University of Colorado, Boulder, Colorado 80309, USA
- † Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Rhizobia are Gram-negative bacteria normally capable of nodulating the roots of leguminous plants, and the failure of five EPS (extracellular polysaccharide)-deficient mutants to nodulate suggested that EPS is required for this nodulation. However, we report here that among a larger sample of mutants with altered EPS production, isolated from two species of Rhizobium (including one of the original set plus 34 new ones), production of EPS is not correlated with ability to nodulate appropriate hosts. Therefore, although involvement of a minor EPS component cannot be ruled out, there is no evidence that gross EPS is required for nodulation.

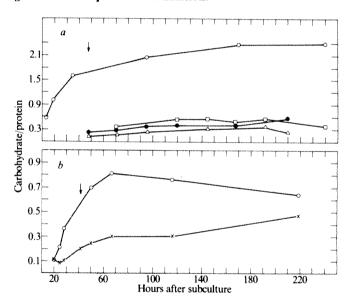


Fig. 1 a, Bacteria were grown in MBM⁴; 1 ml of a fresh stationary-phase culture was inoculated into 100 ml of fresh medium and shaken at 30 °C, and a 10-ml sample was taken at each time point. Arrows indicate the onset of stationary phase. Aliquots (5 ml) of each sample were passed through a 0.45-µm Millipore filter and the filtrate dialysed against three changes of distilled water before being assayed for carbohydrate using the anthrone method3. The remaining 5 ml were dialysed as above and assayed for protein by the Lowry method8. Numbers are glucose-anthrone units per bovine serum albumin Lowry unit. Each point represents the average of a least three independent experiments. We have examined the residual anthrone-positive material made by R. leguminosarum 128c53 str' rif' RS1 and found that ~50% of it is excluded from Sepharose 4B, where essentially all wild-type material enters the column. This peak of aggregated or highmolecular-weight material is rich in 2-keto-3-deoxyoctanoic acid9, suggesting that it consists of cell wall fragments rather than extracellular material 10. Therefore, low EPS values are likely to be overestimates, and we do not consider that the assay used here is accurate below 10% of wild-type production. O, 128c53 str' rif';

 \bullet , RS4; \triangle , RS5; \square , RS9. b, Bacteria were grown and assayed as described in a. Each point is the average of two independent determinations. \bigcirc , 127K26; \times , ER12.

Table 1 Extracellular polysaccharide production and nodulation ability of mutants isolated by a, the membrane-filter technique and b, phage selection

Strain a 128c53 str rif RS1 RS2 RS3 RS4 RS5 RS6 RS7 RS8 RS9 RS10 RS11 RS12 RS13 RS14 RS15 RS16	% Wild-type EPS (100) 6.1 8.9 15.5 9.6 9.1 6.1 6.5 13.0 20.0 7.4 6.4 5.5 12.6 6.5 11.6 13.8	% Wild-type nodules (100) ≤2 10±5 25±5 110±5 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2 ≤2	Strain b 127K26 FB30 ER2 ER19 ER3 ER13 ER17 ER24 ER26 ER21 ER23 ER204 ER15 ER6 ER8 ER12 ER14 ER16 ER203 FB308 ER1	% Wild-type EPS (100) 108 32 28 6.9 8.3 8.1 6.0 4.3 22 23 11 10 4.7 3.4 9.2 6.4 12 5.8 8.9 36	% Wild-type nodules (100) 100 ≥60 ≥60 ≥60 ≥60 ≥60 ≥60 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.
					<0.2 <0.2

a, Strains were assayed for EPS at 96 h after a 1:100 dilution of a stationary-phase culture into fresh medium as described in Fig. 1 legend. All values represent the average of at least three independent experiments. For nodulation assays pea seeds were surface sterilized by stirring in 30% H₂O₂ for 60 min, planted in a sterile 1:1 mixture of perlite and vermiculite (Grace), and watered daily with sterile distilled water. R. leguminosarum 128c53 str' rif' and its derivatives (5 ml) were grown with shaking at 30 °C to stationary phase in modified Bergersen's medium (MBM)⁴, resuspended in 5 ml of sterile water and inoculated onto 18-day-old Early Market (Agway) pea seedlings. The seedlings were grown at 26 °C with a 14-h light/10-h dark cycle, illumination by 3,000 ft-candles, and collected at day 21. All values represent the average of at least three independent experiments, each of which contained at least three plants. In each experiment at least three seedlings were inoculated with R. leguminosarum 128c53 str rif as positive control. Negative controls for each experiment were several uninoculated plants equal to the number of experimentally inoculated seedlings. Experiments in which the negative controls contained any nodules were discarded. Nodules were surface sterilized by stirring in 70% ethanol for 5 min, crushed, resuspended in sterile water, and spread on plates containing MBM for inspection of colony morphology. From RS2, 3 and 4 we recovered only non-slime-forming colonies identical to the inoculum. From rare nodules formed on plants inoculated with RS1 and RS5-16 we occasionally recovered colonies, usually a mixture of wild (slime-forming) and mutant types but sometimes mutant only; the slime-formers nodulated with wild-type efficiency and are presumed to be revertants. We found rare nodules with RS1, in contrast to the initial study, possibly due to differences in cultivar or conditions of temperature and light. b, Strains which prefixed ER were isolated from 127K26; FB308 was isolated from FB30, a streptomycin-resistant derivative of 127K26 (E.R., in preparation). For convenience, mutants are grouped by classes, to be described elsewhere. Cultures were inoculated 1:500 into fresh medium and collected 48 h after stationary phase was attained. Values are the average of three independent experiments for all except FB30, ER1 and ER8, which are the average of two independent experiments. ER1, which cannot use glutamate, was grown in MBM+0.2%(NH₄)₂SO₄; the value is in comparison with 127K26 grown in the same medium (which gave a value of 98% of that for MBM alone). For the nodulation assays, five independent colonies of each mutant strain, six of FB30 and seven of 127K26 were grown in YAP (0.1% yeast extract, 0.1% L-arabinose, 0.5×10⁻⁴ M Na₂HPO₄, 6.65 × 10⁻³ M KH₂PO₄, pH 6.6)⁵ with 5 mM MgSO₄ added, for 3 days. Plants were inoculated with ~5 × 10⁸ bacteria for each culture. Each culture was tested at the time of inoculation by cross-streaking against five typing phages. Plants were prepared for the nodulation assay according to Beringer⁶ except that Fahraeus medium⁷ containing 1% agar was used. Seedlings were grown as described in a. Nodulation was monitored every 2-3 days by inspection for 15-20 days, at which time the plants were collected. Nodules began to appear on day 6; all strains recorded as >60% for nodulation had produced red nodules by day 12, and gave positive acetylene reduction assays for excised nodules at the time of collection. Plants inoculated with wild-type 127K26 had 50-150 nodules (average ~100) in various stages of development at the time of collection. Nodules were counted individually, up to 70, and the number remaining was estimated by eye. No nodules were found for any strain listed as <0.2, ER204 yielded one white nodule, among the five plants inoculated, which contained bacteria like the inoculum; two plants inoculated with ER6 yielded a total of 28 nodules which contained only revertant bacteria, as judged by colony morphology and phage type. A plant inoculated with YAP only had no nodules

RS1 of Rhizobium leguminosarum 128c53 str' rif' is EXO-1 of ref. 1; the remaining mutants of this strain were isolated by the membrane-filter technique previously described1. EPSdeficient derivatives of Rhizobium phaseoli 127K26 were found among survivors of exposure to bacteriophage F1, isolated from soil².

Figure 1 shows the kinetics of EPS production after subculture (see Fig. 1 legend) for R. leguminosarum 128c53 str' rif', R. phaseoli 127K26 and several of their EPS-deficient mutants. Table 1 compares EPS production, as measured by the anthrone method3, at 96 h after subculture (compare with Fig. 1), with nodulation ability for all mutants.

The main finding is that one mutant of R. leguminosarum, RS4, and five mutants of R. phaseoli, ER3, 13, 17, 24 and 26, make <10% of wild-type anthrone-positive material, yet nodulate normally. Other mutants have intermediate EPS and normal nodulation (ER2, 19), intermediate EPS and intermediate nodulation (RS3), intermediate EPS and little or no nodulation (RS8, 9, 13, 15, 16; ER1, 15, 16, 21, 23, 204), little EPS and poor nodulation (RS2), and even excess EPS and no nodulation (ER20).

Obviously, the crude methods used here do not distinguish between different carbohydrate components of EPS and our data would not reveal whether a minor component of EPS was

essential for nodulation. Nevertheless, it is clear that gross EPS production cannot be used as an indicator of nodulation ability and, therefore, that there is no evidence of a requirement for EPS in nodulation.

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- Sanders, R. E., Carlson, R. W. & Albersheim, P. Nature 271, 240-242 (1978).
- Raleigh, E. & Signer, E. R. (in preparation). Dische, Z. Meth. Carbohydrate Chem. 1, 478-512 (1962). Bergersen, F. Aust. J. biol. Sci. 14, 349 (1961).
- Ludwig, R. A. & Signer, E. R. Nature 267, 245-248 (1977). Beringer, J. E. J. gen. Microbiol. 84, 188-198 (1974).
- Vincent, J. M. A Manual for the Practical Study of Root Nodule Bacteria (Blackwell, Oxford, 1970).
- owry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265-275
- A. & Hurwitz, J. J. biol. Chem. 234, 705-709 (1958).
- 10. Munford, R. S., Hall, C. L. & Rick, P. D. J. Bact. 144, 630-640 (1980).

Do carbonate skeletons limit the rate of body growth?

A. Richard Palmer

Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9
Bamfield Marine Station, Bamfield, British Columbia, Canada V0R 1B0

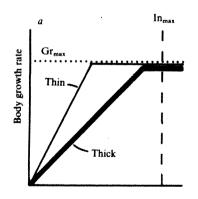
Many marine invertebrates produce mineralized skeletons whose form suggests an economical construction. Porous as opposed to solid skeletal components are produced by scleractinian corals1.2, balanoid and coronuloid barnacles3, ostreid and hippuritid bivalves4, and almost all modern echinoderm classes^{5,6}. They were also produced by rudist bivalves which were widely successful in Cretaceous tropical seas7. Marine prosobranch gastropods produce a stout external shell sculpture in place of a uniformly thick, ultimately stronger shell^{8,9}. The costs implied by these patterns of economical skeleton construction, however, are unknown¹⁰⁻¹². The term 'cost' refers to an evolutionary cost measured in terms of reduced fitness. Note that not all such costs are energetic; non-energetic constraints may also influence fitness. I present here evidence that both thick- and thin-shelled morphs of Thais (=Nucella) lamellosa (Gastropoda, Prosobranchia) produce shell material at a remarkably similar rate during maximal growth. Thick-shelled animals, however, exhibit a significantly slower rate of body growth. These results suggest that rates of skeletal growth can limit the rate of body growth and that this limitation represents a potentially important evolutionary cost.

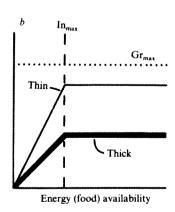
Costs associated with carbonate skeletons can be subdivided into at least three categories: (1) an energetic, depositional cost including organic matrix production and mineralization; (2) an energetic, post-depositional cost, limited primarily to organisms that must expend energy transporting a skeleton; and (3) a non-energetic, growth-rate-limitation cost where the rate of maximal body growth may be set not by the energy available for growth but rather by the maximum rate at which skeleton may be produced. This latter cost is important to recognize because different patterns of energy consumption and growth will be exhibited with increasing availability of energy than would be expected if skeleton costs were strictly energetic.

Figure 1 illustrates the relationship between body growth and increasing energy (food) availability for three alternative hypotheses about factors which potentially limit body growth rate. Predictions about the relative rates of shell growth and rates of energy consumption for each hypothesis are included in Fig. 1 legend. The first hypothesis (Fig. 1a) predicts that if a physiologically determined upper limit to the rate of tissue production (dotted line) is reached before some upper limit to the rate of ingestion or assimilation (dashed line), rates of body growth in organisms with thick and thin skeletons will be identical when energy availability is high. Given an energetic cost to shell material, however, organisms with a thick or dense skeleton will require more energy to achieve the same rate of body growth because of additional energy expended in producing more skeletal material. The second hypothesis (Fig. 1b) predicts that if an ingestion (or assimilation) limit (dashed line) is reached before a tissue production limit (dotted line), then organisms with thick or dense skeletons will exhibit a lower maximal rate of body growth than those with thin or porous skeletons. Here, organisms with thick and thin skeletons should ingest (assimilate) equal amounts of energy at high energy availability, but because of energy invested in shell, thick-shelled animals should have a slower rate of body growth.

The third hypothesis (Fig. 1c) predicts that if a shell production limit is reached before either an ingestion or a tissue production limit, organisms with thick skeletons will again show slower rates of body growth at high energy availability, as with hypothesis b. However, because body growth cannot proceed in advance of skeleton growth, organisms with thick or dense skeletons should consume less energy (food) than those with thin or porous skeletons.

To test these hypotheses, pre-reproductive individuals of similar body weight (Table 1) were selected from a thick- and a thin-shelled population of the morphologically variable, intertidal gastropod, Thais lamellosa. Although having similar body weights, thick-shelled individuals had almost 50% heavier shells (Table 1). Growth experiments (Tables 1, 2) demonstrated that both thick- and thin-shelled animals added significantly less shell (mean, 22.6% less) and less tissue (mean, 25.2% less) at the upper than the lower tidal level. At both tidal levels, however, thick-shelled animals consumed fewer barnacles (Table 3) and also gained less body weight (mean, 32.5% less). Of particular interest is the fact that thick- and thin-shelled animals of the same initial body size produced almost equal weights of shell material at both tidal heights. These patterns of growth and prey consumption are inconsistent with the first two hypotheses (Fig. (1a, b), and support the skeleton-limitation hypothesis (Fig. (1c))





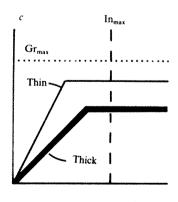


Fig. 1 Graphical illustration of hypothetical growth patterns with increasing rate of energy (food) supply for thick- and thin-shelled organisms subject to different growth constraints. a, Maximum rate of body growth limited by maximum rate of tissue production. b, Maximum rate of body growth determined by maximum rate of ingestion (or assimilation). c, Maximum rate of body growth determined by maximum rate of shell production. Predicted differences in rates of shell production at maximum body growth rate are: a, b, thick > thin; c, thick = thin. Expected energy (food) intakes at maximum body growth rate are: a, thick > thin; b, thick = thin; c, thick < thin. These differences will only be true when animals are growing at a maximum rate. In_{max} , potential maximum rate of ingestion (assimilation). Gr_{max} , potential maximum rate of body growth. Thin-lined curve, organism with low-density skeleton or thin shell; thick-lined curve, organism with high-density skeleton or thick shell.

Note for hypothesis c that the inflection points of the two curves need not coincide on either axis, in contrast to hypotheses a and b.

Table 1 Size and growth parameters for thick- and thin-shelled T. lamellosa

				Initial		Total we	ight change
Tidal height (ft)	Morph	n	Shell length (mm)	Shell weight (g)	Body weight (g)	Shell weight (g)	Body weight (g)
0.0	Thick Thin Thick Thin	10 9 14 15	21.9 (1.08) 22.2 (1.26) 21.6 (0.95) 21.5 (1.67)	1.68 (0.370) 1.06 (0.169) 1.59 (0.208) 0.95 (0.216)	0.23 (0.052) 0.24 (0.042) 0.22 (0.039) 0.23 (0.045)	1.43 (0.309) 1.49 (0.177) 1.06 (0.260) 1.19 (0.329)	0.37 (0.133) 0.50 (0.092) 0.25 (0.074) 0.41 (0.138)

Values are means and standard deviations of size and growth parameters for thick- and thin-shelled *T. lamellosa* pooled from replicates at two tidal heights. Thick-shelled animals were collected from False Bay and thin-shelled animals from Turn Rock, both located on San Juan Island, Washington, USA. Shell and body weights were measured non-destructively by methods described elsewhere Replicate groups of individually marked, measured and weighed animals of each shell type were placed in plastic screen cages mounted on pilings at two tidal heights (0.0 and +2.0 ft above MLLW⁴¹) at the Friday Harbor Laboratories, Friday Harbor, Washington, USA. Throughout the experiment the *Thais* were supplied freely with their preferred prey, *Balanus glandula*, on small stones 1. Prey were made available at all times in high densities so that searching times were negligible. Snails were supplied with barnacles from 13 August to 5 September and 14 September to 14 October, and final body weights (wet) were measured on 13 November, 1978. n, Total number of individuals in each treatment. Numbers in parentheses are pooled standard deviations. There were two replicate cages for each treatment at the lower, and three for each treatment at the upper tidal level.

Table 2 Analysis of variance on initial values and two growth parameters of thick- and thin-shelled T. lamellosa at two tidal heights

7			Init	tial			Growth p		
		Shell Body weight weight		-	Shell weight change		Body weight change		
Source of variation	d.f.	MS	P	MS	P	MS	P	MS	P
Main effects Shell morph Tidal height Interaction Residual Total	1 1 44 47	5.101 0.056 0.009 0.063 0.170	0.001* 0.339 0.701	0.0002 0.0013 0.0001 0.0020 0.0021	0.737 0.419 0.844	0.118 0.880 0.014 0.088 0.112	0.244 0.002* 0.686	0.283 0.103 0.001 0.014 0.022	0.001* 0.008* 0.857

d.f., Degrees of freedom; MS, mean square; P, exact probability of F values obtained by dividing the component mean squares by the residual mean square. Data are from Table 1.

* Significant F ratios.

Table 3 Consumption rates of barnacles, Balanus glandula, by thick- and thin-shelled T. lamellosa at two tidal heights

Consu	Consumption rates			Two-way analysis of variance on consumption rates				
	Shell morph							
Tidal height (ft)	Thick	Thin	Source of variation	d.f.	MS	P		
0.0	0.93 1.01	1.15 1.28	Main effects					
			Shell morph	1	0:120	0.029*		
+2.0	0.56 0.63	0.85 1.02	Tidal height	1	0:231	0.007*		
	0.85	0.78	Interaction	1	0:002	0.725		
			Error	6	0.015			
			Total	9	0.049			

Consumption rates were measured as barnacles eaten per snail per day from 14 September to 14 October, 1978. Each value represents an average for five snails (individual consumption rates are not known as five snails were contained in each cage). An analysis of variance was performed on these average values: d.f., degrees of freedom; MS, mean square; P, exact probability.

* Significant F ratios.

that rate of shell production limits the maximum rate of body growth. However, these data cannot rule out the possibility that animals from the thick-shelled population consumed barnacles more slowly for other reasons and that the production of equal weights of shell material was coincidental. That this occurred at both tidal heights due to chance alone seems unlikely.

Two otherwise enigmatic patterns of invertebrate growth may be explained by the skeleton-limitation hypothesis. First, shell production continues in the absence of feeding in species of barnacles¹³, meso-¹⁴ and neogastropods (A.R.P., unpublished results), and both pteriomorphian^{15,16} and heterodont¹⁷ bivalves. Although difficult to explain energetically, this may reflect either an increase in a shell's habitable volume in anticipation of future body growth or a reinforcement of thinner shell produced during periods of more rapid growth¹⁸. Second,

scleractinian corals frequently show seasonal changes in the density of skeletal material produced. Higher density bands are generally believed to form during periods of slower growth (refs 19-22). In both examples, energetic considerations would predict a decrease in skeleton production when tissue growth decreases or stops, yet calcification continues. Both examples would thus seem to be more readily explained as a response to skeleton-limited rates of body growth, rather than as a result of a high energetic cost to skeletal material.

A third growth pattern may also be explained by the skeleton-limitation hypothesis: rapidly growing organisms frequently produce less skeleton per unit body weight (for example, species of ramose scleractinian corals^{20,23}, pteriomorphian²⁴ and heterodont²⁵ bivalves, echinoids²⁶ and echinoderms in general²⁷). In addition, a number of organisms exhibit ontogenetic

changes in skeletal growth. Juveniles, for whom maximal growth may be critical^{9,28}, exhibit more porous or, in some cases, significantly less massive skeletons than adults (for example, species of balanoid and coronuloid barnacles^{29,30}, strombid and cypraeid gastropods^{31,32} and some extinct armoured heterostracan fishes³³). Although juveniles are more vulnerable than adults to most predators, a heavier skeleton could limit the rate of body growth, increasing the time spent at smaller, more vulnerable sizes⁸. When mature and slow growing, skeletal reinforcement would no longer restrict potential growth. Where there is a refuge in size from predation, trade-offs between rate of growth and extent of morphological defence may represent an important compromise.

Step(s) which limit the rate of skeleton production are unknown. Neither rates of ion transfer across epithelia³⁴ nor availability of dissolved calcium carbonate in surface seawater (<100 m; refs 35-37) are likely to limit the rate of skeletal growth. A probable limiting step is the rate at which crystals can grow from a biologically maintainable precipitating medium. If this is the case, during rapid growth crystal growth limitations would favour shell microstructures with many small crystals because of the increased surface for precipitation. Limited data reveal such a pattern in comparisons of seasonally rapid and slow growth increments in two bivalve species³⁸. Furthermore, both bivalved^{4,39} and shelled gastropod³⁹ molluscs exhibit an evolutionary loss of nacre, a shell microstructure composed of large crystal elements. Thus both ecological and evolutionary patterns in skeletal architecture may be explained by the skeleton-limitation hypothesis.

The above evidence indicates that a potentially important non-energetic 'cost' exists due to growth constraints imposed by carbonate skeletons, both for marine gastropods and possibly for other marine invertebrates. The prevailing attempt to explain morphological adaptations purely in terms of energetic costs and benefits may be too narrow a perspective.

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- 1. Chamberlain, J. A. Jr Palaeobiology 4, 419-435 (1978).
- Sorauf, J. E. Palaeontology 15, 88-107 (1972).
- Sorman, W. A. & Ross, A. San Diego Soc. nat. Hist. Mem. 9, 1–108 (1976).
 Carter, J. G. in Skeletal Growth of Aquatic Organisms (eds Rhoads, D. C. & Lutz, R. A.) 69-114 (Plenum, New York, 1980).
- 5. Raup, D. M. in The Physiology of Echinodermata (ed. Boolootian, R. A.) 379-395 (Wiley, New York, 1966)
- 6. Weber, J., Greer, R., Voight, B., White, E. & Roy, R. J. ultrastruct. Res. 26, 355-366
- Kauffman, E. G. & Sohl, N. F. Verh. naturf. Ges. Basel 84, 399-467 (1974).
- Palmer, A. R. Evolution 33, 697-713 (1979)
- Vermeij, G. J. Biogeography and Adaptation: Patterns of Marine Life, 416 (Harvard
- 10. Rachootin, S. in Encyclopedia of Paleontology (Dowden, Hutchinson & Ross, Stroudsburg,
- 11. Simkiss, K. in The Mechanisms of Mineralization in Invertebrates and Plants (eds Watabe, N.

- Simkiss, K. in The Mechanisms of Mineralization in Invertebrates and Plants (eds Watabe, N. & Wilbur, K. M.) 1-32 (University of South Carolina Press, 1976).
 Taylor, J. D. & Layman, M. Paleontology 15, 73-87 (1972).
 Bourget, E. & Crisp, D. J. J. mar. biol. Ass. U.K. 55, 231-249 (1975).
 Zischke, J. A., Watabe, N. & Wilbur, K. M. Malacologia 10, 423-429 (1970).
 Galtsoff, P. S. Ecol. Monogr. 4, 481-490 (1934).
 Worsnop, E. & Orton, J. H. Nature 111, 14-15 (1923).
 Rhoads, D. C. & Lutz, R. A. J. mar. Res. 28, 150-178 (1970).
 Zolotarev, V. N. & Ignat'ev, A. V. Sov. J. mar. Biol. 3, 352-358 (1977).
 Highsmith, R. C. J. exp. mar. Biol. Ecol. 37, 105-125 (1979).
 Buddemeier, R. W. & Kinzie, R. A. III Oceanogr. mar. Biol. A. Rev. 14, 183-225 (1976).
 Buddemeier, R. W., Maragos, J. E. & Knutson, D. W. J. exp. mar. Biol. Ecol. 14, 179-200 (1974).
- Polyakov, D. M. & Krasnov, E. V. Sov. J. mar. Biol. 2, 391-396 (1976).
- 23. Goreau, T. F. Biol. Bull. 116, 59-75 (1959).
- Hickman, R. W. Mar. Biol. 51, 311-327 (1979).
 Swan, E. F. Ecology 33, 365-374 (1952).
- Raup, D. M. J. Geol. 66, 668-677 (1958).
- 27. Raup, D. M. in Physiology of Echinodermata (ed. Boolootian, R. A.) 379-395 (Interscience, New York, 1966).
- 28. Seed, R. & Brown, R. A. J. Anim. Ecol. 47, 283-292 (1978)
- 29. Darwin, C. A Monograph of the Subclass Cirripedia. The Balanidae, the Verrucidae, etc. (Ray
- Society, London, 1854). 30. Pilsbry, H. R. U.S. nat. Mus. Bull. 93, 1-366 (1916).
- Abbott, R. T. American Seashells, 663 (Van Nostrand Reinhold, New York, 1974).
- 32. Keen, A. M. Sea Shells of Tropical West America, 1064 (Stanford University Press, 1971).

- 33. White, E. I. in Studies on Fossil Vertebrates (ed. Westoll, T. S.) 212-234 (Athlone, London, 1958).
- Crenshaw, M. A. in Skeletal Growth of Aquatic Organisms (eds Rhoads, D. C. & Lutz, R. A.) 115-132 (Plenum, New York, 1980).
 35. Ingle, S. E. *Mar. Chem.* 3, 301-319 (1975).
 36. Li, Y. H., Takahashi, T. & Broecker, W. S. *J. geophys. Res.* 74, 5507-5525 (1969).

- Lees, A. Mar. Geol. 19, 159-198 (1975).
- 38. Jones, D. S. Palaeobiology 6, 331-340 (1980). 39. Taylor, J. D. Palaeontology 16, 519-534 (1973).
- 40. Palmer, A. R. Malacologia (in the press)
- 41. Palmer, A. R. thesis, Univ. Washington (1980).

Segment-specific organization of leg motoneurones is transformed in bithorax mutants of Drosophila

Steven H. Green

Division of Biology 216-76, California Institute of Technology, Pasadena, California 91125, USA

In Drosophila, genes controlling segmentation in the thorax and abdomen are clustered in one region of the genome known as the bithorax complex. Studies of the genetics of this complex suggest that loss of activity of a gene causes transformation of a particular segment to a more anterior one, mesothorax representing the ultimate transformation¹. This transformation is well described for the epidermis, but it is not clear whether other segmentally arranged tissues are also transformed. The segmental ganglia are fused in Drosophila into a single compact mass termed the thoracic ganglion but the segmental organization of the nervous system is still apparent. There are discrete regions of neuropil, termed neuromeres, corresponding to the three thoracic segments: prothorax, mesothorax and metathorax. A small terminal neuromere corresponds to the abdominal segments. Evidence is presented here that the leg motoneurones of each of the three thoracic segments are arranged in a segment-specific pattern in the thoracic ganglion. In mutant flies which have the metathoracic cuticle transformed to mesothoracic, the arrangement of the metathoracic leg motoneurones can be altered to resemble that of the mesothoracic leg motoneurones.

Horseradish peroxidase (HRP) filling of cells was used to study the innervation of the leg. This enzyme is applied to a cut made in the tarsus and is transported through those sensory axons that have been cut. It is also taken up by motoneurone terminals and transported in a retrograde fashion to fill the entire cell. The HRP-filled cells can be visualized by any one of several methods^{2,3}. Figure 1 is a composite drawing showing leg motoneurones in each neuromere. The arrangement of the leg motoneurones is different in the three neuromeres: in the prothoracic (anterior) neuromere, the cell bodies are in a region of the cortex anterior to the neuropil. The axons project back into the neuropil, into which they branch, collect into a loose bundle and course laterally and ventrally to leave the ganglion in the prothoracic leg nerve. In the mesothoracic (middle) neuromere, the cell bodies are in the cortex anterior and ventral to the neuropil. The axons project posteriorly to exit through the mesothoracic leg nerve. Branches are directed dorsally into the lateral neuropil, segregated from sensory neurone arborizations which are in the medial region. In the metathoracic (posterior) neuromere, the motoneurone cell bodies lie laterally and somewhat posteriorly to the neuropil. Their axons project anteriorly from the cell bodies, then loop back and run posteriorly to the nerve. All the axons loop back at the same point although the cell bodies are spread through a region 30-60 µm posterior to the loop. As in the mesothoracic neuromere, the arborizations of the motoneurones are laterally placed in the neuromere, not overlapping the medially placed arborizations of the sensory neurones. In each neuromere a single motoneuron sends a branch contralaterally. These cross in commissures located posteriorly in the prothoracic and mesothoracic neuromeres but anteriorly in the metathoracic neuromere. In the mesothoracic neuromere this motoneurone has its cell body located anteriorly to the other motoneurone cell bodies. Thus, in terms of the positions of the leg motoneurone cell bodies and of the commissure, the metathoracic neuromere is the reverse of the anterior two neuromeres along the anteriorposterior axis.

Similar results were obtained by cobalt filling of axons at the cut end of the coxa, using a method described elsewhere⁴. Furthermore, observations of silver-stained ganglia by Power⁵ and repeated in this laboratory reveal large cells with an arrangement and nuclear morphology like that of the backfilled motoneurones. Therefore, the differences in the motoneurone pattern in the three neuromeres do not seem to result from uptake of HRP by different subpopulations of an identically arranged motoneurone population in each neuromere. The arrangement of the leg motoneurones as described above is the same in males and females of the *Drosophila melanogaster* wild-type strains Canton-S and Oregon-R, the mutant spineless-aristapedia and *Drosophila virilis*.

The bithorax complex mutations abx (ref. 6) and bx transform anterior metathorax to anterior mesothorax; pbx does the same to the posterior compartment. Flies of the genotypes abx bx^3 pbx/abx bx^3 pbx and abx bx^3 pbx/Df(3R)P2 (ref. 7) (derived from stocks provided by Dr E. Lewis) can have the cuticle of the metathoracic segment almost identical to that of the mesothoracic, although expression (degree of change) is variable and incomplete transformations are seen. In all cases studied, the

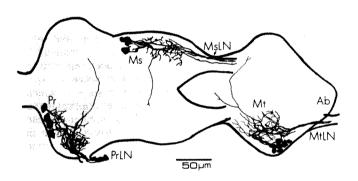


Fig. 1 A wild-type thoracic ganglion is shown in horizontal plane. This drawing is a composite of three separate backfills, traced from photomicrographs on to one thoracic ganglion outline. Only one side is shown for each neuromere. As many as nine motoneurones have been filled from a given leg but generally fewer are seen as in the pro- and mesothoracic examples shown here. In anterior to posterior order, the prothoracic (Pr), mesothoracic (Ms), metathoracic (Mt) and abdominal (Ab) neuromeres are labelled. Leg nerves: PrLN, prothoracic; MsLN, mesothoracic; MtLN, metathoracic. Sensory axons filled in these preparations were not included in the drawings. The motoneurones were filled by applying 20% HRP+3% α -lysolecithin to a cut at the distal end of a leg. Backfill time was 10-14 h. The ventral thorax as removed and fixed with 1.25% glutaraldehyde + 1% formaldehyde in 0.1M phosphate buffer (pH 7.3) for 30 min. The ganglion was removed from the cuticle, fixed for an additional 45 min and washed three times for 30 min in phosphate buffer + 10% sucrose. The HRP was visualized by one of the following two methods: (1) The ganglion was washed two times for 5 min in Yates reagent (1.5 mg ml⁻¹ in 0.05M Tris buffer, pH 7.6), and then incubated for 3-6 min in fresh reagent + 0.006% hydrogen peroxide. The reaction, which colours the HRP-filled neurones reddish-brown, was observed with a dissecting microscope to determine the appropriate end point. The ganglion was then washed three times for 5 min in Tris buffer, dehydrated in ethanol (1 min each 70% and 95%, three times for 1 min in 100%), cleared in methyl salicylate and mounted in immersion oil. (Method modified from ref. 2.) (2) The ganglion was kept for 15 min in a medium made up as follows: 0.25 ml of 3,3', 5,5'-tetramethylbenzidine solution (2 mg ml⁻¹ in 100% ethanol) was added to 10 ml of sodium ferricyanide solution (1 mg ml⁻¹ in 0.01M acetate buffer, pH 3.3), both solutions freshly made up. Hydrogen peroxide was then added to a final concentration of and the ganglion kept in this for 4-6 min. This reaction colours the HRP-filled neurones blue. As above, the actual reaction time was determined by observing the reaction with a dissecting microscope. The ganglion was washed three times for 5 min with acetate buffer and further processing was as described above. (Method modified from ref. 3.)

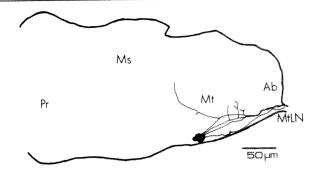


Fig. 2 A thoracic ganglion from an animal of genotype $abx bx^3 pbx/abx bx^3 pbx$ is shown in horizontal plane. A metathoracic leg was backfilled and the HRP visualized as for method (1) in Fig. 1 legend. Labelling as in Fig. 1.

metathoracic leg itself was completely transformed into a mesothoracic leg, as determined by the pattern of leg bristles. Metathoracic legs of these flies were cut and HRP applied as for the wild-type flies. Of 27 successful backfills, four showed altered patterns of motoneurone arrangement that resembled mesothoracic motoneurones and nine showed intermediate phenotypes; unusually positioned motoneurones, suggestive of a mesothoracic pattern but resembling neither pattern entirely. The remaining 14 backfilled legs showed the wild-type arrangement of motoneurones. Of 41 successful metathoracic leg backfills in flies wild-type for bitherax, no deviations from the metathoracic pattern were ever observed. This is consistent with the hypothesis that the central nervous system (CNS) can be segmentally transformed by the mutations. As with the epidermis, expression is variable although the expression and penetrance (fraction of flies affected) of the mutations are far less in the CNS than in the cuticle.

Figure 2 exemplifies a metathoracic neuromere with a mesothoracic pattern of motoneurone arrangement. The motoneurone cell bodies lie anteriorly and ventrally in the cortex and the axons project posteriorly through the neuropil. Figure 2 also shows that the cell which projects contralaterally is anterior to the other cells, just as in the mesothoracic neuromere. Thus, a unique metathoracic neurone seems to be converted into its mesothoracic homologue. Nevertheless, the transformation to the mesothoracic is not complete. The contralaterally projecting fibre takes an anterior route as in the wild-type metathoracic neuromere.

Two examples of intermediate phenotypes are shown in Fig. 3: a shows a metathoracic neuromere containing motoneurones in intermediate positions; b shows a ganglion with metathoracic leg motoneurones that are positioned properly but have axons that project posteriorly into the nerve instead of looping anteriorly. Possibly, cell body position and direction of axon growth are under separate control by anterior-posterior information.

Previous neuroanatomical studies^{8,9} of bithorax using these mutants to study sensory projections from the ectopic cuticle raised the question of whether the mutations affect the CNS itself. Palka et al.⁸ found sufficient differences between bithorax and wild-type ganglia and sensory pathways to suggest that the CNS was altered by the mutations. They found additional evidence for this by considering the pattern of projection of sensory neurones from clones of mutant cuticle into presumably wild-type ganglia. Ghysen⁹, stressing the overall anatomical similarity of wild-type and bithorax ganglia and sensory pathways concluded that the CNS was not altered in the specific pathways studied.

I have approached the question directly by studying neurones with cell bodies in the CNS, using the recently constructed combination abx bx^3 pbx to obtain more extreme transformations than were previously available. The observations reported here do not imply that the genes of the bithorax complex directly control the CNS segmental pattern. They may

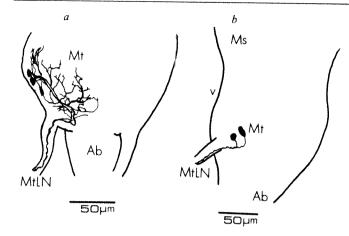


Fig. 3 Two 'intermediate' transformations. a, A thoracic ganglion from an animal of genotype abx bx3 pbx/abx bx3 pbx in horizontal plane. A metathoracic leg was backfilled and the HRP visualized using method (1) of Fig. 1 legend. b, A thoracic ganglion from an animal of the genotype $abx bx^3 pbx/$ Df(3)P2 in sagittal plane, V marking the ventral surface. A metathoracic leg was backfilled and the HRP visualized using method (2) of Fig. 1 legend. Labelling as in Fig. 1.

result from a direct effect of the genes in another tissue, which in turn affects the CNS by induction or by mechanical constraints on CNS growth. Experiments using mosaic flies should determine which tissue must be mutant for the pattern of organization of leg motoneurones to be transformed. This should help to elucidate the mechanisms underlying segmental differences in the CNS.

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- 1. Lewis, E. B. Nature 267, 565-570 (1978).
- Mesulam, M.-M. J. Histochem. Cytochem. 24, 1273–1280 (1978).
 Hanker, J. S., Yates, P. E., Metz, C. B. & Rustioni, A. Histochem. J. 9, 789–792 (1977).
 Bacon, J. P. & Altman, J. S. Brain Res. 138, 359–363 (1977).
 Power, M. E. J. comp. Neurol. 88, 347–409 (1948).

- Lewis, E. B. Abstr. 16th Int. Congr. Ent. Kyoto, 161 (1980). Lewis, E. B. Drosoph. Inf. Serv. 55, 208 (1980). Palka, J., Lawrence, P. A. & Hart, H. S. Devl Biol. 69, 549-575 (1979).
- Ghysen, A. Nature 274, 869-872 (1978)

Establishment in culture of pluripotential cells from mouse embryos

M. J. Evans* & M. H. Kaufman†

Departments of Genetics* and Anatomy†, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Pluripotential cells are present in a mouse embryo until at least an early post-implantation stage, as shown by their ability to take part in the formation of chimaeric animals1 and to form teratocarcinomas2. Until now it has not been possible to establish progressively growing cultures of these cells in vitro, and cell lines have only been obtained after teratocarcinoma formation in vivo. We report here the establishment in tissue culture of pluripotent cell lines which have been isolated directly from in vitro cultures of mouse blastocysts. These cells are able to differentiate either in vitro or after innoculation into a mouse as a tumour in vivo. They have a normal karyotype.

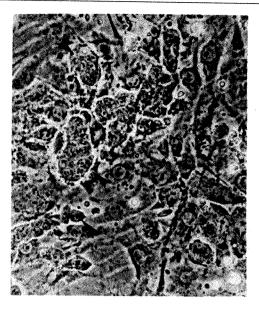


Fig. 1 Groups of pluripotential embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~3 weeks and the pluripotential cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference

Previous attempts to obtain cultures of pluripotential cells directly from a mouse embryo have been unsuccessful3. although cells with a similar appearance have been reported to be present transiently^{5,6}. We considered that success might depend on three critical factors: (1) the exact stage at which pluripotential cells capable of growth in tissue culture exist in the embryo; (2) explantation of a sufficiently large number of these precursor cells from each embryo; and (3) tissue culture in conditions most conducive to multiplication rather than differentiation of these embryonic cells. These considerations have been discussed at greater length elsewhere. An indication of the optimal stage of embryonic development might be gained by a comparison of the properties of embryonic cells at various stages with established cultures of embryonal carcinoma (EC) cells. Cell-surface antigen expression and the patterns of protein synthesis revealed by two-dimensional electrophoresis have suggested that neither the cells of the 62-day ectoderm nor those of the $3\frac{1}{2}$ -day inner cell mass show homology with EC cells, but that epiblast cells of the early post-implantation embryo at $5\frac{1}{2}$ days post coitum may do so⁸ (the day of finding coital plug is termed day $\frac{1}{2}$). Cells from embryos of an early post-implantation stage seem to be the best candidates for direct progenitors of pluripotential cells in culture. As these embryos are difficult to isolate, and as the cell number in the isolated epiblast is small, we chose an alternative route to obtain embryo cells at this stage of development.

Mouse blastocysts may be induced to enter a state of diapause just before implantation. This delay in implantation depends on the maternal hormonal conditions, and may be induced experimentally by ovariectomy at an appropriate stage⁹. Embryos in implantational delay hatch from the zona but remain free-floating in the uterine lumen. A gradual increase in cell number occurs 10, and the primary endoderm may be formed but no further development takes place until implantation occurs, under the control of hormonal stimuli.

129 SvE mice were caged in pairs and examined for mating plugs each morning. They were ovariectomized on the afternoon of day $2\frac{1}{2}$ of pregnancy, injected subcutaneously with 1 mg Depo-Provera (Upjohn), and delayed blastocysts were recovered 4-6 days later. The blastocysts were cultured intact in

groups of about six embryos in small drops of tissue culture medium under paraffin oil on tissue culture plastic Petri dishes for 4 days. The blastocysts attached within 48 h and the trophectoderm cells grew out and differentiated into giant trophoblast cells. The inner cell mass cells subsequently developed into large egg cylinder-like structures, with a group of small round cells surrounded by endodermal cells growing attached to the Petri dish. The egg cylinder-like structures were picked off the dish, dispersed by trypsin treatment and passaged on to gelatinpretreated Petri dishes containing mitomycin C-inactivated STO fibroblasts. All culture was carried out in Dulbecco's modified minimal essential medium supplemented with 10% fetal calf serum and 10% newborn calf serum. The cultures were examined daily and passaged by trypsinization every 2-3 days. Actively proliferating colonies of cells closely resembling EC cells were apparent from an early stage. These colonies were picked out, passaged and mass cultures grown. The cell cultures had the appearance and general growth characteristics of feeder-dependent EC cells (Fig. 1).

The embryos used to initiate these cultures are from normal 129 SvE strain mice, that is, from the same strain of mice as many EC cell lines, in particular those grown in this laboratory. Therefore it was important to exclude any possibility of contamination of these cultures with EC cells from established cell lines. Cell cultures were established from different embryos in three separate experimental series, but the best indication of their separate identity came from their karyotype. Cultures were initiated from 6-12 embryos, thus it might be expected that both male and female cells should be present. None of the 129 embryonal carcinoma cell lines in this laboratory have a normal karyotype, and, in particular-in common with most available embryonal carcinoma cell lines—they do not contain a Y chromosome. These embryo-derived cells have a completely normal karyotype. An XY karyotype is shown in Fig. 2. Three additional cell lines have been analysed; two of these are normal 40XX and one is normal 40XY. We have termed these directly embryo-derived cells EK to distinguish them from EC cells. EK cells grow rapidly in culture and have been maintained for over 30 passages in vitro.

Cultures of EK cells were collected by trypsinization, and $\sim 10^6$ cells injected subcutaneously into the flank of syngeneic male mice. Tumours grew in all cases, and histological examination of these revealed that they were teratocarcinomas. When the EK cells were passaged without feeder cells they formed embryoid bodies which, when kept in suspension, became cystic.

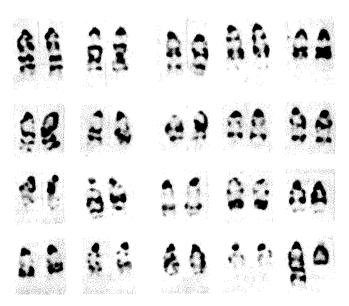


Fig. 2 Karyotype of an embryo-derived pluripotential cell line, 40XY. Over 80% of the spreads of this clonal line possessed 40 - chromosomes and had a clearly identifiable Y chromosome.

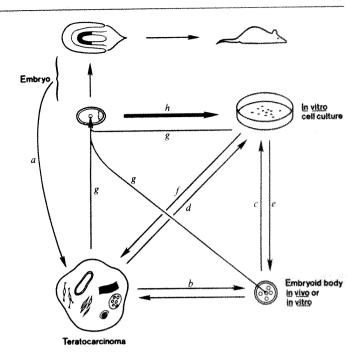


Fig. 3 Inter-relationships of cell lines, teratocarcinomas and embryoid bodies with normal mouse embryos. Arrows indicate routes of cell transfer: a, formation of teratocarcinoma by ectopic implantation of embryos; b, formation of embryoid bodies from teratocarcinoma and vice versa; c, derivation of cell culture from embryoid bodies; d, cell culture obtained directly from solid tumours; e, differentiation to embryoid bodies from culture; f, formation of solid tumours on reinjection of cells from culture; g, transfer of embryonal carcinoma cells either from cell culture or from the core of an embryoid body or from a solid tumour back to a blastocyst. All these procedures may result in chimaerism of the resulting mouse; h, the missing link supplied here.

Embryoid bodies allowed to attach to a Petri dish spread out and differentiated in the usual way into a complex of tissues. Preliminary observations indicate that, like early ectoderm cells of the mouse embryo and EC cells, EK cells carry the cell-surface antigens recognized by M1-22-25 (Forssman)^{8,11} and anti-I Ma (lacto-N-iso-octaosyl ceramide)^{12,13} and also that two dimensional gel electrophoretic separations of their proteins very closely resemble those of the EC cell line PSMB.

We have demonstrated here that it is possible to isolate pluripotential cells directly from early embryos and that they behave in a manner equivalent to EC cells isolated from teratocarcinomas. The network of inter-relationships between the mouse embryo and pluripotential cells derived from it has previously lacked only the direct link between the embryo and cells in culture for completion. We have now demonstrated this (Fig. 3).

Teratocarcinoma cells are now being widely used as a model for the study of developmental processes of early embryonic cell commitment and differentiation. Their use as a vehicle for the transfer into the mouse genome of mutant alleles, either selected in cell culture or inserted into the cells via transformation with specific DNA fragments, has been presented as an attractive proposition. In many of these studies the use of pluripotential cells directly isolated from the embryos under study should have great advantages. We have now shown that these EK cell lines are readily established from cultures of single blastocysts and so far have 15 lines of independent embryonic origin, some of which have been isolated from non-129, outbred mouse stocks. We are now studying the chimaeric mice formed from these cells.

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Gardner, R. L. & Papaioannou, V. E. in The Early Development of Mammals (eds Balls, M. & Wild, A. E.) 107-132 (Cambridge University Press, 1975).
 Stevens, L. C. Devl Biol. 21, 364-382 (1970).

Cole, R. J. & Paul, J. in Preimplantation Stages of Pregnancy (eds Wolstenholme, G. E. W. & O'Connor, M.) 82-122 (Churchill, London, 1965).

Sherman, M. I. Cell 5, 343-349 (1975).

Atienza-Samols, S. B. & Sherman, M. I. Devl Biol. 66, 220-231 (1978).
Solter, D. & Knowles, B. Proc. natn. Acad. Sci. U.S.A. 72, 5099-5102 (1975).
Evans, M. J. J. Reprod. Fert. 62, 625-631 (1981).
Evans, M. J. Lovell-Badge, R. H., Stern, P. L. & Stinnakre, M.-G. INSERM Symp. 10, 115-129 (1979). McLaren, A. J. Endocr. 50, 515-526 (1971).

10. Kaufman, M. H. in Progress in Anatomy Vol. 1 (eds Harrison, R. J. & Holmes, R. L.) 1-34 (Cambridge University Press, 1981).

11. Stern, P. L. et al. Cell 14, 775-783 (1978).

- Feizi, T. Blood Transfusion Immunohaemat 23, 563-577 (1980).
- 13. Kapadia, A., Feizi, T. & Evans, M. J. Expl Cell Res. 131, 185-195 (1980).

Stage-specific embryonic antigen involves $\alpha 1 \rightarrow 3$ fucosylated type 2 blood group chains

H. C. Gooi*, T. Feizi*, A. Kapadia*, B. B. Knowles†, D. Solter† & M. J. Evans‡

- * Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK
- † Wistar Institute of Anatomy, Philadelphia, Pennsylvania 19104, USA
- ‡ Department of Genetics, University of Cambridge, Cambridge CBQ 3EH, UK

There is much interest in developmentally regulated molecules which may have function in cell interactions and sorting during embryogenesis and differentiation. Numerous antisera have been raised which detect antigens that are expressed in early embryonic cells and become restricted during differentiation, being expressed in only a minority of adult cells (reviewed in refs 1-3). The precise antigenic determinants recognized by such antisera have not been defined. However, studies using a hybridoma antibody against mouse spleen cells⁴ and monoclonal autoantibodies of patients with cold agglutinin disease⁵ have shown that two defined carbohydrate antigen systems, the Forssman and the Ii antigens, have stage-specific expression in early mouse embryos. We now describe evidence that the stage-specific embryonic antigen SSEA-1 (ref. 6) involves the carbohydrate sequence

Gal
$$\beta$$
1 \rightarrow 4GlcNAc \uparrow 1.3 Fuc α

This determinant is formed by $\alpha 1 \rightarrow 3$ fucosylation of blood group I or i antigens which are branched or linear oligosac-charides, respectively⁷⁻⁹, built of Gal β 1 \rightarrow 4GlcNAc units and known as type 2 precursor chains 10 of the major blood group antigens. Thus, we introduce the concept of simple glycosylation changes as a basis for stage-specific expression of embryonic antigens.

A relationship between the stage-specific embryonic antigen, SSEA-1, and blood group I antigen was suggested by: (1) some similarities in the distribution of these two antigens in early post-implantation embryos and teratocarcinomas^{5,11}, and (2) the reactivities of anti-I and -SSEA-1 reagents with a glycosphingolipid fraction of erythrocytes¹². Subsequent immunocytochemical studies (to be described elsewhere) have clearly shown that the two antigens are not identical. Furthermore, in binding assays using radioiodinated blood group Iactive glycoproteins¹³ isolated from sheep gastric mucins and human meconium, the anti-SSEA-1 reagent gave a binding curve only with the latter glycoprotein (Fig. 1).

The specificity of anti-SSEA-1 reagent was further investigated by inhibition assays using blood group substances with known A,B,H,Lewis^a, Lewis^b, I and i activities, some of which are shown in Fig. 1. These substances were clearly divisible into two groups: those with potent inhibitory activities, giving 50% inhibition at 0.3-6 µg ml⁻¹, and those with negligible or weak activities requiring concentrations >100 µg ml⁻¹ for 50% inhibition. Almost all the substances with strong SSEA-1 activity lacked blood group ABH activities but expressed Le^a and/or Ii activities.

The two ovarian cyst glycoproteins with the strongest SSEA-1 activity, designated N-1 10% and F1, contained 8.5% and 1.6% fucose, respectively^{14,15}. In experiments to be described elsewhere, we showed that their reaction with anti-SSEA-1 was abolished by mild acid hydrolysis. Thus, it seemed likely that the SSEA-1 determinant involved fucose residue(s) and that the fucosyl linkage was other than those associated with A, B, H, Lea or Leb activities 10. This agrees with previous evidence for the presence of unusual fucosyl glycopeptides20 in undifferentiated cells of mouse embryos and teratocarcinomas.

Inhibition of binding assays were next performed with chemically synthesized²¹⁻²³ and natural oligosaccharides^{14,24} containing type 1 $(Gal\beta 1 \rightarrow 3GlcNAc)$ or type 2 $(Gal\beta 1 \rightarrow$ 4GlcNAc/Glc) precursor chain¹⁰ sequences (Fig. 2). Whereas Ii-active synthetic tri- and pentasaccharides consisting of type 2 precursor chains were inactive as inhibitors of anti-SSEA-1, several fucose-containing oligosaccharides showed inhibitory activities. The most active inhibitor (ID₅₀ 0.5 nmol) was the oligosaccharide designated N-1 R_L 0.71a, containing a fucosylated type 2 sequence:

$$Gal\beta 1 \rightarrow GlcNAc$$

$$\uparrow^{1.3}$$

$$Fuc\alpha$$

This oligosaccharide was one of several isolated from glycoprotein N-1 after partial alkaline degradation¹⁴. The very poor inhibitory activity of 3-fucosyl lactose indicated that subterminal N-acetyl glucosamine is an important component of

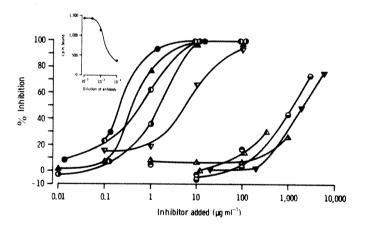


Fig. 1 Double antibody radioimmunoassay using anti-SSEA-1 and 125 I-labelled human meconium. The immunoassay procedure was a modification of that described previously 13. The anti-SSEA-1 reagent 6.11 was ascites fluid from a pristane-primed BALB/c was ascites fluid from a pristane-primed BALB/c mouse injected with antibody-producing hybrid cells. Normal mouse serum, 1:100 dilution, was used as carrier and undiluted rabbit anti-mouse immunoglobulin serum (Dako-immunoglobulins) as second antibody. The binding curve is shown in the inset. Inhibition assays were performed using the anti-SSEA-1 reagent at 1:3,000 dilution. As inhibitors blood group substances of human and animal origins were used. Symbols, blood group activities and designations (the latter in parentheses) of representative samples are given below. The following were human ovarian cyst gly-coproteins: \bullet , Le^aI (N-1 10%)¹⁴; \triangle , Ii (FI)¹⁵; \bullet , Ii (484 ¹³; \blacktriangledown , Le^a (445); \triangle , HLe^b (JS)¹⁶; \ominus , A (438)¹³. Also tested were gastric mucosal glycoproteins from sheep, \triangle , Ii (sheep 1 + 10)¹⁷ and from hog, \blacktriangledown , AH (hog A + H)¹⁸, a glycoprotein-rich extract from human meconium of non-secretor type, ①, Le*Ii (Mec)13 and polyglycosyl ceramides isolated from human erythrocytes, O, HIi (ref. 19).

the SSEA-1 antigenic determinant. The Le^a-active oligosaccharides tested having the type 1-based terminal sequence

were almost 10 times less active than their type 2 isomers. Lacto-N-difucohexaose I (Le^b active), lacto-N-fucopentaose I (H active) and 3-fucosyl lactose were 200-1,000 times less active as inhibitors; 2-fucosyl lactose, lacto-N-tetraose and lacto-N-neotetraose were inactive.

Further investigation is required to determine whether the weak inhibitory activity observed with the Lea-active oligosaccharide represents a cross-reaction or whether it is due to the presence of their type 2 isomers in the preparations tested. It is also of interest to determine whether the 3-fucosyl N-acetyl lactosamine structure is the entire antigenic determinant recognized by anti-SSEA-1 or whether, as with Ii antigens 7-9.2 additional internal structures are involved. A recent observation¹² that a mixture of erythrocyte glycosphingolipids (H4 fraction) with blood group H and I activities precipitates with anti-SSEA-1, indicated that the SSEA-1, H and I antigens might co-exist on the same macromolecules. Glycosphingolipids with 3-fucosyl-N-acetyl lactosamine sequence are known to accumulate in adenocarcinomas26 but it has not been demonstrated on erythrocyte glycosphingolipids of the H1-4 series, although its presence in small amounts has not been completely ruled out (S. Hakomori, personal communication).

Previous studies^{5,27} have shown that the earliest embryo cells of the mouse and similarly, undifferentiated teratocarcinoma cells, express I antigen which is a branched oligosaccharide built of *N*-acetyl lactosamine units^{7,8} as follows:

$$Gal\beta 1 \rightarrow 4GlcNAc\beta 1$$

$${}^{3}Gal\beta 1 \rightarrow 4GlcNAc...$$
 $Gal\beta 1 \rightarrow 4GlcNAc\beta 1$

Presumably, a proportion of these chains become glycosylated with fucose in $\alpha 1 \rightarrow 3$ linkage in the late eight-cell stage, when SSEA-1 activity is first found⁶. At the start of differentiation, i is expressed on visceral endoderm cells in addition to I (ref. 5) and SSEA-1 (refs 9, 11). The i antigen is a linear structure also built of N-acetyl lactosamine units⁹:

$$Gal\beta1 \rightarrow 4GlcNAc\beta1 \rightarrow 3Gal\beta1 \rightarrow 4GlcNAc...$$

Subsequently, during embryogenesis and embryonal carcinoma cell differentiation, several types of differentiated epithelia are found which lack I, i and SSEA-1 activities. A reciprocal increase in blood group H activity has been demonstrated in some of these⁵. The relationship between the I, i, SSEA-1 and H antigens is schematized in Fig. 3. It is now well established that the I and i antigens are masked in the presence of the blood group H-active fucose in $\alpha 1 \rightarrow 2$ linkage to galactose^{9,28} and a similar conclusion can be reached from the present studies, with respect to masking of SSEA-1 antigen by such fucosylation. Alternatively, sialylation may occur at the terminal galactose; in $\alpha 2 \rightarrow 3$ linkage the sialic acid causes partial or complete masking of the I and i antigens^{7,8} and possibly the SSEA-1 antigenic determinants. We can extrapolate further and envisage that, depending on the relative activities of the various glycosyl transferases (and perhaps glycosidases) in individual differentiated tissues, the proportions of these several antigenic determinants will vary. For example, variation in fucosylation might explain the presence of SSEA-1 in some blastomeres but not in others 1-3,6

Thus, simple glycosylation steps involving changes in proportions of linear and branched structures and their fucosylated or sialylated derivatives provide a mechanism for stage-specific appearance and disappearance of antigens. It will be of interest to investigate which of the other various embryonic antigens¹⁻³ that disappear on differentiation reflect subtle glycosylation changes.

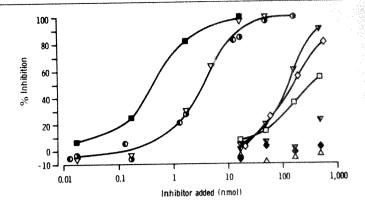


Fig. 2 Inhibition of binding of anti-SSEA-1 to ¹²⁵I-labelled meconium by oligosaccharides. Symbols and structures for the oligosaccharides in order of their inhibitory activies are as follows:

■ Gal
$$\beta$$
1 → 4GlcNAc β 1 → 6?—3 hexenetetrol(s)
↑ 1.3
Fuc α N-1 R_L 0.71a

$$\nabla$$
, Gal β 1 \rightarrow 3GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc \uparrow 1.4 Fuc α Lacto-N-fucopentaose II

$$\begin{array}{cccc} \Psi, \; Gal\beta 1 \; \rightarrow \; 3GlcNAc\beta 1 \; \rightarrow \; 3Gal\beta 1 \; \rightarrow \; 4Glc \\ & \uparrow_{1,2} & \uparrow_{1,4} \\ & Fuc\alpha & Fuc\alpha & Lacto-N-difucohexaose I \end{array}$$

$$\Diamond$$
, Gal β 1 \rightarrow 4Glc
 $\uparrow_{1,3}$
Fuc α 3-Fucosyl lactose

$$∇$$
, Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc
Lacto-N-tetraose

$$\triangle$$
, Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3al β 1 \rightarrow 4Glc Lacto-N-neotetraose

The following synthetic oligosaccharides²¹⁻²³ were inactive at the highest levels tested shown in parentheses:

$$Gal\beta1 \rightarrow 4GlcNAc\beta1 \rightarrow 3Gal (55 nmol),$$
 $Gal\beta1 \rightarrow 4GlcNAc\beta1 \rightarrow 6Gal (92 nmol),$
 $Gal\beta1 \rightarrow 4GlcNAc\beta1$
 $Gal\beta1 \rightarrow 4GlcNAc\beta1$
 $Gal\beta1 \rightarrow 4GlcNAc\beta1$

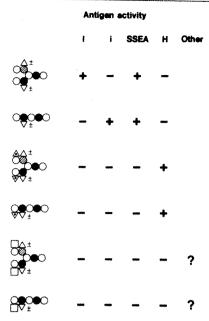


Fig. 3 Schematic presentation of the proposed structure for the SSEA-1 antigenic determinant and its relationship to blood group I, i or H active oligosaccharides and to sialylated structures of undetermined antigenic specificity. Symbols: \bigcirc , $\beta 1 \rightarrow 4$ linked galactose; \bullet , $\beta 1 \rightarrow 3$ linked N-acetyl glucosamine; \emptyset , $\beta 1 \rightarrow 6$ linked N-acetyl glucosamine; \triangle , $\alpha 1 \rightarrow 3$ linked fucose; \triangle , $\alpha 1 \rightarrow 2$ linked fucose; \Box , $\alpha 2 \rightarrow 3$ linked sialic acid; \pm implies optional substitution with $\alpha 1 \rightarrow 3$ linked fucose, such that in the absence of other peripheral monosaccharides, the I, i or the SSEA-1 antigenic determinants are expressed. It is not known whether the $\alpha 1 \rightarrow 2$ fucosylation or the sialylation steps precede or follow the $\alpha 1 \rightarrow 3$ fucosylation of these precursor chains.

The biological significance of the orderly changes of these and the major blood group antigens ABH (ref. 29) during development and differentiation is unknown. If these various carbohydrate sequences function as recognition structures involved in cell interactions and tissue organization, it can be anticipated that there will be a progressively increasing list of complementary molecules (endogenous lectins) specifically reactive with them. From the present studies it can be predicted that a 3-fucosyl N-acetyl lactosamine-mediated recognition system, analogous to the previously described lectin-like compound on hepatocytes³⁰, is operating at the embryonic cell surface, and it is tempting to speculate that such a lectin may be involved in the process of compaction. This occurs at the eight-cell stage of the mouse embryo when blastomeres become tightly associated with each other and undergo substantial changes in their organization during differentiation³¹. Thus, there is now a need to investigate the significance of different carbohydrate structures and lectins^{32,33} on mammalian embryonic cells. They may be involved in sugar-protein interactions which are crucial and specific for phenomena as varied as receptor-mediated uptake of circulating glycoproteins 30,34,35 and the homing of enzymes to appropriate intracellular compartments 34,36.

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Note added in proof: Further studies to be described elsewhere (E. F. Hounsell, H. C. Gooi and T. Feizi, in preparation) have shown that the inhibition of anti-SSEA-1 by the type 1 oligosaccharide, lacto-N-fucopentaose II, is due to the presence of the type 2 isomer, lacto-N-fucopentaose III, in this preparation. Received 19 January; accepted 30 March 1981.

- Jacob, F. Curr. Topics dev. Biol. 13, 117-137 (1979)
- Solter, D. & Knowles, B. B. Curr. Topics dev. Biol. 13, 139-165 (1979). Wiley, L. M. Curr. Topics dev. Biol. 13, 167-193 (1979).
- Willison, K. R. & Stern, P. L. Cell. 14, 785–793 (1978). Kapadia, A., Feizi, T. & Evans, M. J. Expl Cell Res. 131, 185–195 (1981).
- Solter, D. & Knowles, B. B. Proc. natn. Acad. Sci. U.S.A. 75, 5565-5569 (1978) Watanabe, K., Hakomori, S., Childs, R. A. & Feizi, T. J. biol. Chem. 254, 3221-3228
- (1979). Feizi, T., Childs, R. A., Watanabe, K. & Hakomori, S. J. exp. Med. 149, 975-980 (1979). Niemann, H. et al. Biochem. biophys. Res. Commun. 81, 1286-1293 (1978). Watkins, W. M. Adv. Hum. Genet. 10, 1-136, 379-385 (1980).

- Fox, N. et al. Deol Biol. (in the press). Nudelman, E. et al. Biochim. biophys. Res. Commun. 97, 443-451 (1980).

- Wood, E., Lecomte, J., Childs, R. A. & Feizi, T. Molec. Immun. 16, 813-819 (1979).
 Lloyd, K. O., Kabat, E. A. & Licerio, E. Biochemistry 7, 2976-2990 (1968).
 Watkins, W. M. & Morgan, W. T. J. Vox Sang. 4, 97-119 (1959).
 Schiffman, G., Kabat, E. A. & Thompson, W. Biochemistry 3, 113-120 (1964).
 Wood, E., Hounsell, E. F., Langhorne, J. & Feizi, T. Biochem. J. 187, 711-718 (1980).
 Feizi, T. et al. J. exp. Med. 133, 39-52 (1971).
 Koscielak L. in Humen Blood Gers.
- Koscielak, J. in Human Blood Groups (eds Mohn, J. F. et al.) 143-149 (Karger, Basle,
- 20. Muramatsu, T. et al. Cell 18, 183-191 (1979)
- Augé, C., David, S. & Veyrières, A. Nouv. J. Chim. 3, 491–497 (1979). Feizi, T., Wood, E., Augé, S. & Veyrières, A. Immunochemistry 15, 733–736 (1978). Wood, E. & Feizi, T. FEBS Lett. 104, 135–140 (1979).
- Kobata, A. Meth. Enzym. 28, 262-271 (1972).
 Feizi, T. Revue fr. Transfusion Immuno-hémat. 23, 563-577 (1980).
- 26. Yang, H.J. & Hakomari, S. J. biol. Chem. 246, 1192-1200 (1971).
 27. Feizi, T., Kapadia, A., Gooi, H. C. & Evans, M. J. in Teratocarcinoma and Cell Surface (eds Muramatsu, T. & Ikawa, Y.) (Japan Scientific Societies Press, Tokyo, in the press).

 28. Feizi, T. et al. J. Immun. 106, 1578-1592 (1971).
- 29. Szulman, A. E. in Human Blood Groups (eds Mohn, J. P. et al.) 426-436 (Karger, Basle, SZUIMAN, A. E. H. ALLENDAN, A. P. 1977).
 Pricels, J.-P. et al. Proc. natn. Acad. Sci. U.S.A. 75, 2215–2219 (1978).
 Ziomek, C. A. & Johnston, M. H. Cell 21, 935–942, 1980.
 Prenl Cell Res. 92, 122–126 (1975).

- Johnes, C. A. & Johnston, M. H. Cell 21, 933-942, 1980.
 Oppenheimer, S. B. Expl Cell Res. 92, 122-126 (1975).
 Grabel, L. B., Rosen, S. D. & Martin, G. R. Cell 17, 477-484 (1979).
 Neufeld, E. & Ashwell, G. in The Biochemistry of Glycoproteins and Proteoglycans (ed. Lennarz, W. J.) 241-266 (Plenum, New York, 1980).
- 35. Day, J. F., Thornburg, R. W., Thorpe, S. R. & Baynes, J. W. J. bial. Chem. 255, 2360-2365
- 36. Stahl, P. et al. Cell 19, 207-215 (1980).

Erythrocyte membrane sidedness in lectin control of the Ca²⁺-A23187-mediated diskocyte echinocyte conversion

Richard A. Anderson & Rex E. Lovrien

Biochemistry Department, Gortner Laboratory, University of Minnesota, St Paul, Minnesota 55108, USA

Increasing the cytoplasmic calcium concentration of human erythrocytes with ionophore A23187 drives the transformation from diskocyte to echinocyte morphology¹. This transformation is closely linked to the intracellular ATP level, which drops when calcium is introduced across the membrane^{2,3}. The echinocyte morphology reverts to the diskocyte after restoration of normal ATP and calcium levels4.5. ATP control of cell morphology is thought to depend on the action of a protein kinase and phosphatase which reversibly modify the membrane cytoskeleton 6-9. The cytoskeleton in turn is in contact with the outside membrane surface via transmembrane proteins 10-11. We show here that binding of the lectin, wheat-germ agglutinin (WGA), to the erythrocyte membrane surface blocks the morphological conversions diskocyte = echinocyte in both directions. WGA seems to work via a mechanism involving the transmembrane glycoprotein, glycophorin 13,14.

Wheat-germ agglutinin was used as a convenient tool to control in a reversible way the morphological conversion initiated by Ca²⁺-A23187 on the cytoplasmic side. The internal echinocytic reactions initiated by Ca²⁺-A23187 are not blocked by WGA binding to the membrane; instead they go to completion and are 'stored' until finally expressed, as a morphological change, when WGA is displaced from the membrane.

Fig. 1 Control of human erythrocyte morphology by wheat germ agglutinin (WGA) on the cell's outside surface in relationship to internal calcium loading and cell metabolism. Final erythrocyte concentration for sequence I is $1 \times$ 10⁷ cells ml⁻¹ . Buffer (mM): 10 KC1, 130 NaCl, 2 MgCl2, 15 Tris, pH 7.4, 25 °C. Erythrocytes were drawn and washed as described before 14. In sequence I the concentration of WGA is 2 µg ml⁻¹; this results in 18×10⁵ molecules bound per cell. In reaction (2), 0.2 mM Ca² and 5 µM A23187 were added, giving the final cytoplasmic calcium concentration at equilibrium of 0.4 mM. Addition of ionophore without calcium caused no added morphological change. Calcium influx into the cell was 50% complete after 30 s, 80% complete

Sequence I

Co NAG WGA

after 60 s and 100% complete after 100 s. The influx was followed using 45 Ca $^{2+}$ (ref. 15). The binding of WGA to the cell did not change the flux of calcium across the membrane or the final cytoplasmic calcium concentration. Calcium was then withdrawn from the cell using 2 mM EGTA, leaving $< 10^{\circ}$ µ, μ , 150 s after addition of EGTA. Lectin was displaced from the membrane by addition of 30 mM NAG. The unbinding was followed with 125 I-WGA 14 , and was complete ($< 2 \times 10^5$ molecules per cell) within 30 s of addition of NAG. In sequence II, diskocytes are converted to eckinocytes using the above conditions. In reaction (2) calcium was removed with 2 mM EGTA and ionophore A23187 with a 0.3% bovine serum albumin wash. The red cells were > 90% echinocytes at this point, but upon re-energizing with 5 mM glucose and 2 mM inosine [reaction (3)], 95% of the cells reverted to diskocytes within 2 h. In reactions 4–6, the cell concentration was lowered to 2×10^6 cells per ml to avoid lectin-induced agglutination during the 2-h glucose—inosine incubation. The WGA concentration was 1 μ g ml $^{-1}$, with 20×10^5 molecules bound per cell.

The sequences of morphological changes are illustrated in Fig. 1. Figure 2 shows scanning electron microscope photographs of red cells in the critical stages of the reactions.

For the experiments of sequence I (Fig. 1), WGA was added to diskocytes such that the amount bound, ν_{WGA} , was 1.8×10^6 molecules of WGA per cell. This amount did not produce any change in cell morphology, as was expected ¹⁴. When the cytoplasmic calcium concentration was increased, using 0.2 mM Ca²⁺ and 5 μ M A23187, the cells with this level of bound lectin completely retained their normal diskocyte morphology. However, erythrocytes treated identically, but without lectin, lapsed to the echinocyte morphology within a few minutes. Figure 2a and b contrasts lectin-protected and unprotected cells.

The lectin does not control morphological conversion by blocking transport of calcium into the cell by ionophore A23187 because when the Ca²⁺ flux into the cell was quantified using ⁴⁵Ca²⁺ (ref. 15), both the rate of Ca²⁺ influx and the final cytoplasmic concentration of calcium were found to be identical for cells with bound lectin, and control cells.

WGA is rapidly displaced from protected cells by N-acetyl-glucosamine (NAG). Reaction (3) of sequence I shows that within 30 s of addition of NAG to lectin-protected Ca^{2+} -loaded diskocytes, the lectin was displaced and the diskocyte \rightarrow echinocyte conversion completed. The velocity of this reaction was greater by an order of magnitude than that of the conventional Ca^{2+} -A23187-driven conversion (see Fig. 3), indicating that the erythrocytes were already calcium loaded when NAG was added.

Reaction (2) of sequence I shows that WGA completely blocks the echinocyte effects of Ca^{2+} -A23187. Reactions (4) and (5) show that the lectin does not block internal reactions initiated by Ca^{2+} . In reaction (4), EGTA was used to remove cytoplasmic Ca^{2+} . Using $^{45}Ca^{2+}$, removal of Ca^{2+} was shown to be complete (within experimental error of the measurement of cytoplasmic Ca^{2+} which is $\pm 10 \,\mu\text{M}$) within 150 s of addition of EGTA. The products of reaction (4) are diskocytes which have had Ca^{2+} -A23187 influxed, then completely removed, while fully protected by WGA. However, when WGA is displaced by NAG, as shown in reaction (5), the Ca^{2+} -free diskocytes

immediately change to echinocytes. Thus the internal reactions initiated by Ca²⁺-A23187 are not erased by removal of Ca²⁺, but their final expression, formation of echinocytes, is permitted only when the external lectin is removed.

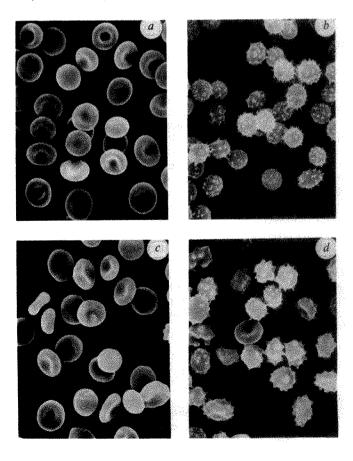


Fig. 2 Scanning electron micrographs of erythrocytes from stages indicated by the letters a, b, c and d in Fig. 1. Scale bar, $5 \mu m$.

The effects of calcium on the cell morphology are reversible if the initial calcium concentration is < 0.5 mM and the incubation time with calcium < 15 min. In these conditions cytoskeletal cross-linking 16,17 by endogenous transglutamidase or disulphide coupling 5 does not occur to a significant extent. This is supported by the fact that the echinocyte morphology is reversed on restoration of normal calcium and ATP levels. Calcium also activates an endogenous phospholipase C which results in the hydrolysis of phosphotidylinositol and the accumulation of 1,2-diacyglycerol 18,19 . In our conditions < 0.4% ($< 10^6$ molecules per cell) of the phospholipids would be hydrolysed 19 . It is unlikely that this small amount of 1,2-diacylglycerol would effect a morphological conversion 24 .

The forward and reverse rates of the diskocyte \rightleftharpoons echinocyte conversion in the absence of WGA and the profound decrease in these rates effected by the lectin are shown in Figs 3 and 4. The ordinate value in these figures, r, is the fraction of cells which are echinocytes; at r = 0, all cells are diskocytes.

Reaction (1) of sequence II represents a conventional diskocyte \rightarrow echinocyte conversion by Ca²⁺-A23187. When this conversion is 100% complete, the Ca²⁺ is removed by EGTA and the ionophore A23187 by serum albumin. If glucose and inosine are then added to allow regeneration of ATP, these calcium-unloaded echinocytes revert slowly to the diskocyte form. At least 95% diskocytes (r = 0.05) are obtained after 2 h.

In reaction (4) WGA is used to control the Ca²⁺-A23187-produced echinocytes. If lectin is added to calcium-unloaded echinocytes followed by incubation in glucose for 2 h (the time necessary for completion of the conventional echinocyte →diskocyte conversion), the cell morphology does not change from the echinocyte. If the lectin is then displaced with NAG, the echinocyte → diskocyte conversion proceeds to completion within seconds. It is clear that the reactions needed to erase the impact of Ca²⁺-A23187 on the membrane take place by the end of the 2-h incubation with metabolite. Thus displacement of WGA from echinocytes, which have been emptied of Ca²⁺-A23187 and re-energized, causes a return to the diskocyte that is much more rapid than the conventional reversal in reaction (3).

The diskocyte ≠echinocyte conversion is the result of a number of molecular events within the membrane. Increased cytoplasmic calcium activates internal reactions, which drive the change in cell shape. The data presented in Figs 3 and 4 indicate that the internal reactions for the diskocyte ≠echinocyte morphological conversions are not blocked by lectin binding to the outside of the membrane. Instead lectin binding to one membrane component, which is crucial in the sequence of molecular events, blocks this component's participation in the sequence or pathway leading to morphological conversion, thereby completely blocking the conversion.

The receptor for WGA on the human erythrocyte is the major sialoglycoprotein, glycophorin^{13,14}. WGA binds to the sialic acid groups of glycophorin; when these groups are removed, lectin binding and control of cell morphology are eliminated¹⁴. Glycophorin is a transmembrane protein and is thought to be associated with band III within the membrane¹¹. It can thus interact with the cytoskeleton via the linkage band III–ankyrinspectrin^{10,12}. Accordingly, WGA may influence the cytoskeleton and cell morphology, via the transmembrane proteins, in the following way:

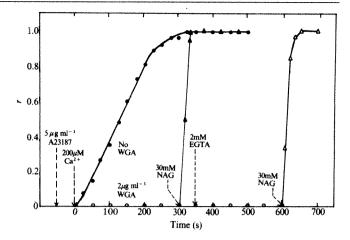


Fig. 3 Time course of the diskocyte → echinocyte conversions from sequence I, Fig. 1. r, Fraction echinocytes. Points lying on the abscissa (r = 0) represent WGA-protected diskocytes.

The existence of a cross-linking mechanism whereby the lectin randomly cross-links glycophorins to form an exocytoskeleton seems unlikely as: (1) freeze-fracture electron microscopy has shown that lectins do not cause clustering of receptors on intact cells^{20,21}, (2) the glycoprotein receptors are uniformly distributed in the membrane, ~200 Å apart² (3) the carbohydrate binding sites on WGA are 40 Å apart²⁵ and thus WGA could not span the distance separating the glvcophorins. It is more likely that each WGA binds specifically to one glycophorin molecule, or, perhaps, to a dimer. Support for this view comes from work with peanut lectin, which can control change of shape of the desialated erythrocyte. Peanut lectin binds to desialoglycophorin with high specificity²³ and, at a concentration of 3×10^5 molecules bound per cell, completely blocks the diskocyte → echinocyte conversion. As there are only about 6×10^5 monomers of glycophorin per cell¹⁴, it is very

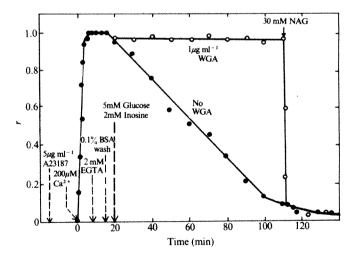


Fig. 4 Time course of the echinocyte → diskocyte conversions from sequence II, Fig. 1.

Metabolism Cytoskeletal Diskocyte External Transmembrane Internal glycophorin ↔ control of 1 glycophorin Lectin → glycophorin → cytoskeleton cell shape Echinocyte saccharide interaction Ca2+

unlikely that random cross-linking takes place with such a small amount of lectin bound. A more reasonable proposition is that the lectins exert their control of morphology by intramolecular binding to a glycophorin dimer. This is supported by the fact that the molecular ratio of peanut lectin: glycophorin is 1:2 when morphology is completely blocked (R.A.A. and R.E.L. unpublished results).

The transmembrane protein glycophorin is a crucial component in cytoplasmic Ca2+-induced morphological change, and lectins may exert their control of morphology by specifically binding to glycophorin. The 'sidedness' or asymmetric nature of glycophorin enables carefully aimed use of lectins, specific for glycophorin's external carbohydrate, to block the expression of internal biochemical reactions. The indirect action of Ca2+ on the cell cytoskeleton is paramount in this morphology conversion. Thus it is likely that glycophorin-cytoskeletal contact is also important in this morphological conversion.

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- Weed, R. L., LaCelle, P. L. & Merrill, E. W. J. clin. Invest. 48, 795-811 (1969)
- Taylor, D., Baker, R. & Hochstein, P. Biochem. biophys. Res. Commun. 76, 205-211 (1977).
- Edmondson, J. W. & Li, T. K. Biochem, biophys. Acta 443, 106-113 (1977)
- Feo, C. & Mohandas, N. Nature 265, 166-168 (1977). Palek, J., Liu, P. A. & Liu, S. C. Nature 274, 505-507 (1978).
- Birchmeir, W. & Singer, S. J. J. Cell Biol. 73, 647-659 (1976) Sheetz, M. P. & Singer, S. J. J. Cell Biol. 73, 638-646 (1976).
- Fischer, S., Tortolero, M., Piau, J. P., Delaunay, J. & Schapira, G. Biochim. biophys. Acta 598, 463-471 (1980).
- Pinder, J. C., Bray, D. & Gratzer, W. D. Nature 270, 152-154 (1977).
 Bennett, V. & Stenbuck, P. J. Nature 280, 468-473 (1979).
- 11. Tyler, J. M., Hargraves, W. R. & Branton, D. Proc. natn. Acad. Sci. U.S.A. 76, 5192-5196
- Nigg, E. A., Bron, C., Girardet, M. & Cherry, R. J. Biochemistry, 19, 1887–1893 (1980).
 Adair, W. L. & Kornfeld, S. J. biol. Chem. 249, 4696–4704 (1974).
- Lovrien, R. E. & Anderson, R. A. J. Cell Biol. 85, 534-548 (1980)
 Ferreira, H. G. & Lew, V. L. Nature 259, 47-49 (1976).
- Lorand, L., Siefring, G. E. & Lowe-Krentz, L. J. supramolec. Struc. 9, 427-440 (1978).
 Siefring, G. E., Apostel, A. B., Velasco, P. T. & Lorand, L. Biochemistry 17, 2598-2606
- 18. Allen, D., Billah, M. M., Finean, J. B. & Michell, R. H. Nature 261, 58-60 (1976).
- Allen, D. & Michell, R. H. Biochem. J. 166, 495-499 (1977). Schekman, S. & Singer, S. J. Proc. natn. Acad. Sci. U.S.A. 73, 4075-4079 (1976).
- Kuettner, C. A., Staehelin, L. A. & Gordon, J. A. Biochim. biophys. Acta 448, 114-120
- Elgsaeter, A. & Branton, D. J. Cell Biol. 63, 1018-1033 (1974).

 Carter, W. G. & Sharon, N. Archs Biochem. Biophys. 180, 570-582 (1977).

 Lovrien, R., Tisel, W. & Pesheck, P. J. biol. Chem. 250, 3136-3141 (1975).
- Wright, C. S. J. molec. Biol. 141, 267-272 (1980).

A new protein that gels F actin in the cell cortex of Dictyostelium discoideum

John Condeelis & Jeffrey Salisbury

Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461, USA

Keigi Fujiwara

Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02138, USA

It is thought that the dense actin filament networks found in the cortex of many cell types 1-4 might account for the gelled consistency of the cortex⁵ and the exclusion of other organelles from this region of the cytoplasm⁶⁻⁸. We now demonstrate that a new actin-binding protein from Dictyostelium that gels actin in vitro is concentrated in the cell cortex, suggesting that this protein, in association with actin, accounts for the gel-like properties of this region of the cell.

Cell extracts from the amoeboid stage of the cellular slime mould Dictyostelium discoideum contain protein factors that gel actin filaments in vitro9. We have recently purified to homogeneity one of these gelation factors that has a molecular weight of 120,000 (120K)¹⁰. It is a soluble, trypsin-sensitive and carbohydrate-free protein that increases the viscosity and sedimentation rate of F actin, inhibits the actin-stimulated Mg²⁺-ATPase activity of myosin and nucleates actin filament assembly in vitro 10. The interaction of this actin-binding protein with F actin is not inhibited by millimolar ATP or micromolar calcium. Electron microscopy demonstrates that this protein promotes both side-to-side and end-to-side interactions between actin filaments in vitro 8,10

To ascertain the function of this protein in vivo we have used the indirect immunofluorescence technique to determine its location in spreading and migrating cells. Antisera were raised against purified native 120K protein in rabbits and characterized as monospecific by double diffusion and immunoelectrophoresis in agar¹¹ (Fig. 1). Dictyostelium amoebae were cultured in suspension in HL5¹⁰, washed in 20 mM KCl, 1 mM CaCl₂, 2 mM

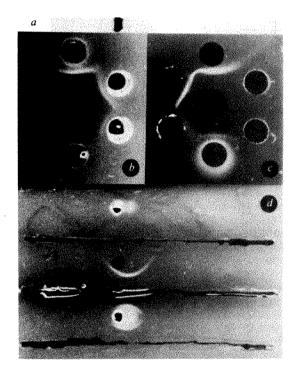


Fig. 1 The 120K protein was purified from *Dictyostelium* amoebae as described elsewhere ¹⁰. a Shows SDS-polyacrylamide gel electrophoresis of the purified antigen. Rabbits were immunized with native 120K protein by subcutaneous injection of antigen in complete Freund's adjuvant and boosted once with antigen in incomplete Freund's adjuvant 4 weeks after initial immunization. Antiserum was characterized using double immunodiffusion in 1% agarose 11. b Shows result with antiserum in the centre well with a clockwise progressive twofold dilution of native 120K protein in the outer wells. c Shows the result with antiserum in the centre with outer wells filled with the following, clockwise from the top: a complex of 95,000 MW protein-120K protein-actin 10; 95,000 MW protein alone; 95,000 MW protein plus actin; whole cell extract; 1/5th dilution native 120K protein; native 120K protein. In both cases single precipitin arcs result showing monospecificity and fuse to demonstrate immunological identity. No reaction was found in samples containing actin or 95,000 MW protein, the other major actin-binding protein found in Dictyostelium 9.10. d, Immunoelectrophoresis was performed as described previously in 1% agarose 11. Wells from top to bottom contain cell extract, native 120K protein and cell extract. Troughs from top to bottom contain pre-immune serum, antiserum to 120K protein and antiserum to 120K protein. A single precipitin line with the same electrophoretic mobility is formed against 120K protein and the cell extract.

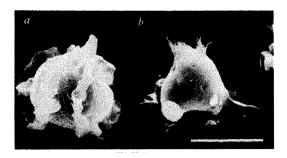


Fig. 2 Scanning electron micrographs of spreading (a) and migrating (b) amoebae on poly-L-lysine. Cells were prepared for microscopy by fixation in 2% glutaraldehyde in 40 mM sodium phosphate pH 7.0 for 10 min, dehydrated in ethanol and critical point dried against CO₂. Scale bar, 10 μM.

MgSO₄ and 40 mM sodium phosphate pH 6.4 and allowed to spread and migrate on poly-L-lysine-coated coverslips. In these conditions, when the cells first make contact with the adhesive surface, they become flattened against the substrate on the bottom and proceed to throw ruffles from the surface back over the cytoplast, which remains rounded (Fig. 2a). The cells then progressively flatten and begin to migrate by sending out large flat ruffles or pharopods (Fig. 2a). In randomly migrating cells such as those shown here the pharopods are usually extended from all sides of the cell with only one or two gaining dominance at the leading edge during net locomotion (Fig. 2b). Migrating cells usually remain slightly rounded in the cytoplast region which contains the nucleus and other organelles, even when exhibiting rapid migration. At no stage during spreading or locomotion are stress fibres or large microfilament bundles observed within these cells in either the light or electron microscope, respectively8.

Immunofluorescence for 120K protein in cells undergoing various stages of spreading and migration on poly-L-lysine is shown in Fig. 3. All the cells have bound antiserum and exhibit at least one of two types of fluorescence: weak diffuse fluorescence which seems to be distributed throughout the cytoplasm exclusive of the nucleus, and strong peripheral fluorescence which is concentrated in the cell cortex, ruffles and pharopods. In spreading cells (Fig. 3c), ruffles at both the cell margin and those thrown back over the cytoplast stain for the presence of 120K protein. In migrating cells (Fig. 3d), 120K protein is concentrated primarily in pharopods that extend randomly from the cell margin. In both cases a thin rim of fluorescence is usually seen around most of the periphery of the cell.

To determine whether 120K protein was present in mammalian cells, human (HeLa) and rat kangaroo (Ptk-2) tissue culture cells were fixed, permeabilized and stained with antiserum against 120K protein as described above. None of these cells demonstrated immunofluorescence for the protein.

The concentration of 120K actin-binding protein in structures associated with the cell periphery is analogous to the immunofluorescence localization in the cell cortex of macrophage actin-binding protein 12 and filamin 13 in macrophages and cultured fibroblasts, respectively. The localization of 120K protein in the cell cortex, ruffles and pharopods of *Dictyostelium* is quite similar to that of macrophage actin-binding protein in lung macrophages 12 but less similar to the location of filamin in cultured fibroblasts, where this protein is also found in stress fibres. This latter difference might be due more to the absence of actin-containing stress fibres from *Dictyostelium* amoebae and macrophages than to dissimilarities in function between 120K protein and filamin.

The properties of purified 120K protein in vitro and its concentration in the cell cortex in situ suggest possible functions for this protein in vivo. The location of 120K protein in the cell corresponds well with that of actin determined by immunofluorescence¹⁴ and electron microscopy⁸. This protein

cross-links and gels filamentous actin and hence may be required with actin for the formation of the cortical gel long known to be present in amoeboid cells⁵. The presence of weak fluorescence for 120K protein throughout the cytoplasm of *Dictyostelium* amoebae suggests that small amounts of the protein in association with actin may also account for the presence of the diffuse filamentous network seen throughout the cytoplast⁸. The concentration of 120K protein in ruffles and pharopods indicates that cytoplasm within these structures has an increased potential for the formation of a rigid actin gel but does not prove that the cytoplasm in this region is gelled continuously, because gels formed from 120K protein and actin *in vitro* are readily solated by micromolar Ca²⁺ in the presence of 95K protein^{6,9,10}.

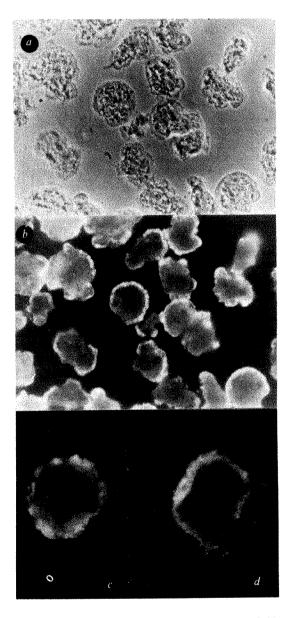


Fig. 3 Light microscopy of amoebae on poly-L-lysine. Cells were fixed in 3.7% formaldehyde in 0.85% NaCl, 10 mM sodium phosphate pH 7.2 for 10 min, permeabilized in -20° C acetone and stained with rabbit antiserum against 120K protein followed by goat anti-rabbit IgG conjugated to rhodamine (Cappel). Controls were as follows: pre-immune serum in place of antiserum against 120K protein, deletion of immune serum before staining with goat anti-rabbit IgG, cells fixed but not permeabilized with acetone, use of antiserum preabsorbed with native 120K protein. Staining was not detected in any of these controls. a, Phase and b, fluorescence of cells permitted to spread and migrate on poly-L-lysine; c, spreading cell, and d, migrating cell at higher magnification. a and b, $\times 1,200$; c and d, $\times 2,500$.

This protein also nucleates assembly of actin filaments, thereby promoting end-to-side filament attachments resulting in branched filaments which are frequently observed in the cortex of Dictyostelium in situ^{4,8}. Concentration in the cell cortex of actin nucleation activity associated with 120K actin-binding protein would have substantial significance in the generation of new actin filaments in this region of the cell during sol-gel transformations and pseudopod extension, and might result in the production of large numbers of free actin filament ends which could be used to advantage by the cell to establish membrane-filament attachments⁶.

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- 1. Mooseker, M. & Tilney, L. J. Cell Biol. 67, 725-743 (1975).
- Goldman, A., Yerna, M. & Schloss, J. J. supramolec. Struct. 5, 155-183 (1976). Begg, D., Roderwald, R. & Rebhun, L. J. Cell Biol. 79, 846-852 (1978).
- Condeelis, J. & Wolosewick, J. Proc. 38th Meet. Electron Miscrosc. Soc. Am. 420-423
- Taylor, D. & Condeelis, J. Int. Rev. Cytol. 56, 57-143 (1979). Condeelis, J. Int. Cell Biol. (ed. Schweiger, H.) 306-320 (Springer, Berlin, 1980/81).
- Wolosewick, J. & Porter, K. J. Cell Biol. 82, 114-139 (1979). Woloswick, J. & Condeelis, J. Cell (in the press).

- Woloswick, J. & Condeelis, J. Cell (in the press).
 Condeelis, J. Neurosci. Res. Prog. Bull. 19, 83-99 (1981).
 Condeelis, J. & Geosits, S. J. biol. Chem. (in the press).
 Fujiwara, K. & Pollard, T. J. Cell Biol. 71, 848-875 (1976).
 Stendahl, O., Hartwig, J., Brotschi, E. & Stossel, T. J. Cell Biol. 84, 215-224 (1980).
- 13. Heggeness, M., Wang, K. & Singer, S. J. Proc. natn. Acad. Sci. U.S.A. 74, 3883-3887
- 14. Eckert, B. & Lazarides, E. J. Cell Biol. 77, 714-721 (1978).

T cell-dependent IgA anti-polysaccharide response in vitro

Park E. Trefts, Denis A. Rivier & Martin F. Kagnoff

Department of Medicine, University of California at San Diego, La Jolla, California 92093, USA

The induction in mice of humoral antibody to purified polysaccharides, such as dextran B1355, dextran B512 and other bacterial capsular materials, is generally regarded as being thymus independent¹⁻⁵, that is, not requiring conventional carrier-specific T-cell help6. Such responses are largely immunoglobulin M (IgM), often transient although occasionally cyclic, and there is little evidence of immunological memory The selective formation of an IgG response to polysaccharides or haptenated polysaccharides (for example, dextran B512, dinitrophenol (DNP)-Ficoll) has also been characterized as thymus independent 12,13. Recent reports have indicated that the murine IgG response to such antigens is restricted largely to the IgG₃ subclass ^{14,15} although lesser amounts of other IgG subclasses have been reported after stimulation with thymusindependent antigens such as DNP-Ficoll and DNP-Levan 16,17 In the latter study, T cells were shown to influence certain IgG subclass responses to haptens on a thymus-independent carrier¹⁷. Here we report that BALB/c mice produce a substantial IgA anti- $\alpha(1,3)$ dextran B1355 response in vitro that is highly T-cell dependent and age-dependent. This finding represents a significant departure from previous observations and may have particular relevance for mucosal immunity. It also suggests the need for a re-evaluation of the induction and regulation of anti-polysaccharide antibody responses.

To study the induction and regulation of the Igh-1^a allotypelinked anti- $\alpha(1,3)$ antibody response to the purified polysaccharide dextran B1355 (43% $\alpha(1,3)$ and 57% $\alpha(1,6)$ glucan linkages) we used Mishell-Dutton in vitro culture methods¹⁸. As shown in Table 1, substantial IgA anti- $\alpha(1,3)$ dextran responses were seen only when spleen cells were obtained from old (10-17-month-old) BALB/c mice. In contrast, spleen cells from

young (4-month-old) or old (12-month-old) BALB/c (Igh-1") mice could be stimulated by dextran B1355 to produce IgM anti- $\alpha(1,3)$ dextran responses over a 3-5-day culture period. Similar results (not shown) were obtained in cultures using mesenteric lymph node (MLN) cells. The IgM and IgA antidextran B1355 antibody in spleen and MLN cultures shared idiotypic determinants with the two well-characterized anti- $\alpha(1,3)$ glucan myelomas, MOPC 104E (IgM λ_1) and J558 $(IgA \lambda_1)^{19}$ as determined in specific inhibition studies with anti-J558 or anti-MOPC 104E antiserum.

To determine whether the absence of the IgA anti- $\alpha(1,3)$ dextran response in spleen or MLN cells from younger mice was due to cell-mediated suppression, we compared the responses in cultures containing young, old or age-mixed spleen or MLN cells. Cells from young mice did not suppress the anti-dextran response of cells from old mice (see Fig. 1).

The IgA anti- $\alpha(1,3)$ dextran response was markedly T-cell dependent (see Fig. 2). Thus, anti-Thy 1.2 and complement treatment ablated the in vitro IgA anti- $\alpha(1,3)$ dextran response in spleen and MLN cells and such responses were totally reconstituted by the addition of T cells.

To clarify the nature of the T-cell regulation of the IgA response, we used dextran B1355 derivatized with trinitrophenol (TNP)-lysine (TNP-dex)20 as an in vitro antigen. Our experiments demonstrate that the IgA response after stimulation with TNP-dex was limited to dextran $\alpha(1,3)$ determinants. Both IgA and IgM anti- $\alpha(1,3)$ dextran responses were induced by this antigen (Fig. 3) whereas there was only an IgM response to the TNP determinant in either normal (Fig. 3a) or TNP-protein-primed (Fig. 3b) mice.

The results clearly demonstrate the age-dependence of the in vitro IgA anti- $\alpha(1,3)$ dextran B1355 response in BALB/c mice. Antigen-stimulated cultures from 2-8-month-old BALB/c mice did not produce significant IgA anti- $\alpha(1,3)$ glucan antibody although such cultures were competent in terms of anti-sheep red cell (SRBC) (not shown) and IgM anti- $\alpha(1,3)$ glucan responses. Note that although we did not prime the old mice used in our study, the mice may not be antigenically naive. Polyglucans are ubiquitous antigens in the mammalian digestive tract and the increase in the IgA anti- $\alpha(1,3)$ glucan response in ageing mice may be due to T- and/or B-cell priming with

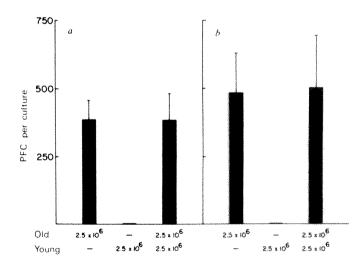


Fig. 1 Failure of lymphoid cells from young BALB/c mice to suppress IgA anti-α(1,3)dextran B1355 response in cultures containing lymphoid cells from old BALB/c mice. Spleen cells (a) or MLN cells (b) from 12-month-old (eld) or 6-8-week-old (young) mice were cultured alone or mixed at a 1:1 ratio, Cultures containing 2.5×10^6 old cells, 2.5×10^6 young cells or 2.5×10^6 old cells and 2.5×10^6 young cells in 0.5 ml were stimulated with optimal concentrations of dextran B1355. Cultures were assayed on day 5. Data represent the mean ± s.e.m. of seven experiments (a) and four experiments (b). Culture conditions were as described in Table 1 legend.

Table 1	Age-dependence and kinetics of the in vitro
	anti-\alpha(1,3)dextran B1355 response

Age of mice (months)	Dextran B1355 (ng per culture)		nse* l cells) IgA				
		Day 3	Day 4	Day 5	Day 3	Day 4	Day 5
	None	9	< 2	< 2	NT	NT	NT†
4	0.1	67	106	16	< 2	< 2	< 2
	1.0	78	111	32	< 2	< 2	< 2
	None	7	< 2	< 2	NT	NT	NT
12	0.1	102	303	99	195	509	391
	1.0	80	259	218	199	449	289

The table shows a representative experiment in which spleen cells from 4- or 12-month-old BALB/c mice were cultured at 2.5×10^6 cells per 0.5 ml in Falcon mutiwell tissue culture plates (no. 3008) and stimulated with 0.1 or 1.0 ng dextran B1355. Culture medium RPMI 1640 containing 10% fetal calf serum (FCS, lot 614 from Gibco), 2-mercaptoethanol (5×10^{-5} M), penicillin (40 U ml^{-1}) and streptomycin (40 µg ml^{-1}) was enriched with 12% cocktail 16. Results are means of duplicate cultures.

* Specific anti- $\alpha(1,3)$ responses were assayed using palmitoyl dextran B1355 coupled to HRBC²¹. IgM and IgA anti-dextran plaques were totally inhibited by dextran B1355 but not by dextran B512 (95% $\alpha(1,6)$ and 5% $\alpha(1,3)$ glucan linkages) added to the plaque-forming cell (PFC) assay²¹. IgG responses, including IgG₃, were not detected using polyvalent and subclass-specific enhancing reagents.

 \dagger NT, not tested. In other experiments, low background levels of IgA anti-dextran PFC (that is, from 10 to 40 PFC per 10^6 cultured cells) were noted regularly in lymphoid cell suspensions derived from mice > 10 months old but not in younger mice.

naturally occurring environmental antigens at mucosal surfaces $^{21-23}$.

We point out that the age at which IgA responses were observed in vitro was considerably delayed compared with our previously reported in vivo results in which responses were seen between 1 and 6 months of age²¹. Because over 95% of the anti-dextran B1355 antibody in vivo and in vitro showed idiotypic determinants that cross-reacted with the two well-characterized myeloma proteins J558 and MOPC104E, we conclude that both systems produce antibodies that are similar or identical. We have no convenient explanation for the differences observed in vivo and in vitro. Cell-mediated suppression does not seem to explain the lack of the in vitro IgA response in young mice (see Table 1), but a relative deficiency of specific T or B cell or the size (that is, molecular weight $\sim 4 \times 10^7$), structural or chemical characteristics of the molecule may explain the fact that immunization with dextran B1355 is less efficient in vitro than in vivo. In addition, differences between the in vitro and in vivo systems may depend on the microenvironment or adherent cell requirements^{24,25} for immunocompetence.

The T-dependence of the in vitro IgA anti- $\alpha(1,3)$ dextran response was striking and confirms our previous in vivo report²¹. Invariably, the removal of T cells from spleen or MLN populations eliminated the IgA anti- $\alpha(1,3)$ response and such responses were restored by the addition of T cells. Moreover, the T-dependent IgA anti- $\alpha(1,3)$ response did not seem to require conventional carrier-specific T-cell help⁶. Thus, with TNP-dextran as an immunogen, we routinely observed IgM and IgA anti- $\alpha(1,3)$ dextran responses and IgM anti-TNP responses in spleen and MLN cultures from unprimed or TNP-proteinprimed mice, whereas such cultures produced no significant IgA anti-TNP response. The specificity of the response for $\alpha(1,3)$ glucan determinants argues against polyclonal activation by dextran in this system²⁶ or a role for IgA isotype-specific T-helper cells²⁷. However, we cannot exclude the possibility that the IgA anti- $\alpha(1,3)$ glucan precursor B cell that responds to T-cell help has special cell-surface determinants or properties that are lacking on normal or TNP-primed precursor B cells. We prefer the interpretation that T-cell help for the IgA anti- $\alpha(1,3)$ dextran response is directed to idiotypic determinants on

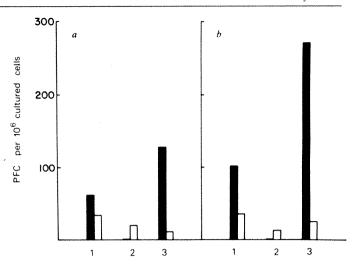


Fig. 2 Representative experiments showing the T-cell dependence of the IgA anti- $\alpha(1,3)$ dextran B1355 response in BALB/c mice. Cultures containing spleen cells (a) or MLN cells (b) were treated with complement alone (1), or anti-Thy 1.2 and complement (2). Additional cultures (3) were treated with anti-Thy 1.2 and complement and reconstituted with MLN which were depleted of B cells before mitomycin C treatment according to a two-step panning procedure $^{37}(a)$ or with mitomycin C-treated spleen cells (b). Cells from triplicate cultures were pooled and assayed on day 5 for specific IgA (solid bars) or IgM (open bars) anti- $\alpha(1,3)$ dextran B1355 responses. Culture conditions were as described in Table 1 legend. IgM anti-SRBC responses in parallel control cultures were totally ablated by anti-Thy 1.2 and complement treatment and restored by T cells (not shown).

anti- $\alpha(1,3)$ glucan antibody associated with the B cell or perhaps accessory cells, and not to $\alpha(1,3)$ determinants on dextran B1355.

Considerable evidence in mice substantiates the ability of antibody directed at specific immunoglobulin idiotypes to suppress or stimulate B cells²⁸⁻³². There is less evidence, however, for T-cell help directed towards idiotypic determinants³³⁻³⁶. Previous studies reported that anti-idiotypic T-cell help for the B-cell response was either antigen-indepen-

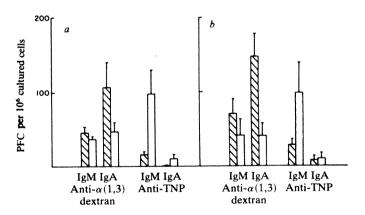


Fig. 3 Specific anti- $\alpha(1,3)$ dextran and anti-TNP responses in spleen or MLN cultures stimulated with TNP-dextran B1355. Cultures containing 2.5×10^6 spleen or MLN cells from 10-17-month-old unprimed BALB/c mice (a) or BALB/c mice primed three times at 1-4-month intervals with TNP-ovalbumin or TNP-keyhole limpet haemocyanin (b) were stimulated in culture with TNP-dextran B1355 (1 ng per culture, hatched bars or 10 ng per culture, open bars). Cultures were assayed 5 days later for specific IgM and IgA anti- $\alpha(1,3)$ dextran B1355 and anti-TNP responses. Data represent the mean±s.e.m. of five experiments with unprimed and six experiments with TNP-primed mice. Culture conditions were as described in Table 1 legend. Control cultures from TNP-protein-primed mice stimulated with TNP on the homologous carrier yielded > 400 IgA anti-TNP PFC per 10^6 cultured cells.

dent³⁵ or required two populations of T cells, one carrier-reactive and the other idiotype-specific³⁴. The striking observation in our study was the antigen-dependence and apparent lack of need for carrier-specific T-cell help for the IgA anti- $\alpha(1,3)$ response in vitro. Thus, T-helper cells are important in the regulation of the immune response to polysaccharide antigens such as dextran B1355. Moreover, anti-idiotypic Thelper cells may play a major part in influencing the induction and regulation of the B-cell repertoire to polysaccharide antigens.

The immune response to such antigens has been difficult to

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- Howard, J. G. & Courtenay, B. *Immunology* **29**, 599-610 (1975). Fernandez, C. & Möller, G. *Immunology* **33**, 59-68 (1977).
- Vicari, G. & Courtenay, B. M. Immunochemistry 14, 253-258 (1977). Howard, J. G., Vicari, G. & Courtenay, B. Immunology 29, 585-597 (1975).
- Howard, J. G., Christie, G. H., Courtenay, B. M., Leuchars, E. & Davies, A. J. S. Cell. Immun. 2, 614-626 (1971).
- Mitchison, N. A. Eur. J. Immun. 1, 18-27 (1971).
 Basten, A. & Howard, J. G. Contemp. Topics Immunobiol. 2, 265 (1973).
- Mayers, G. L., Bankert, R. B. & Pressman, D. J. Immun. 120, 1143-1148 (1978). Kelsoe, G. & Cerny, J. Nature 279, 333-334 (1979).

- Braley-Mullen, H. Immunology 40, 521-527 (1980).
 Baker, P. J., Stashak, P. W., Amsbaugh, D. F. & Prescott, B. Immunology 20, 469-480
- 12. Fernandez, C. & Möller, G. Scand. J. Immun. 10, 465-472 (1979)
- 13. Sharon, R., McMaster, P. R. B., Kask, A. M., Owens, J. D. & Paul, W. E. J. Immun. 114, 1585-1589 (1975)
- 14. Perimutter, R. M., Hansburg, D., Briles, D. E., Nicolotti, R. A. & Davie, J. M. J. Immun
- 15. Der Balian, G. P., Slack, J., Clevinger, B. L., Bazin, H. & Davie, J. M. J. exp. Med. 152,
- 16. Slack, J., Der Balian, G. P., Nahm, M. & Davie, J. M. J. exp. Med. 151, 853-862 (1980).

study, partly because of the lack of a suitable in vitro system for examining the induction and regulation of the antibody response to such antigens. Our study demonstrates the successful application of in vitro culture methods to study the antigen-specific induction of antibody responses to purified dextran Bl355 and seems to represent the first report of an in vitro antigen-induced response by dispersed cell culture to a purified polysaccharide antigen.

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- 17. Mongini, P. K. A., Stein, K. E. & Paul, W. E. J. exp. Med. 153, 1-12 (1981).
- Mishell, R. I. & Dutton, R. W. J. exp. Med. 126, 423 (1967).
 Blomberg, B., Carson, D. & Weigert, M. in 3rd int. Convocation Immun., New York (eds.) Pressman, D., Tomasi, T. B. Jr, Grossberg, A. L. & Rose, N. R.) 285 (1972).
- 20. Blomberg, B. thesis, Univ. California (1977).
- Kagnoff, M. F. J. Immun. 122, 866–870 (1979).
 Lieberman, R., Potter, M., Mushinski, E. B., Humphrey, W. Jr & Rudikoff, S. J. exp. Med. 139, 983-1001 (1974).
- Eichmann, K., Falk, I., Melchers, I. & Simon, M. M. L. exp. Med. 152, 477-492 (1980).
- Gorczynski, R. M., MacRae, S. & Jennings, J. J. Cell. Immun. 45, 276-294 (1979). Kagnoff, M. F. & Campbell, S. J. exp. Med. 139, 398-406 (1974).
- Couthino, A., Möller, G. & Richter, W. Scand. J. Immun. 3, 321-338 (1974). Elson, C. O., Heck, J. A. & Strober, W. J. exp. Med. 149, 632-643 (1979).
- Cosenza, H. & Kohler, H. Proc. natn. Acad. Sci. U.S.A. 69, 2701-2705 (1972). Fernandez, C. & Möller, G. Proc. natn. Acad. Sci. U.S.A. 76, 5944-5947 (1979).
- Trenkner, E. & Riblet, R. J. exp. Med. 142, 1121-1132 (1975). Eichmann, K., Falk, I. & Rajewsky, K. Eur. J. Immun. 8, 853-857 (1978).
- Eichmann, K. & Rajewsky, K. Eur. J. Immun. 5, 661-666 (1975). Bottomly, K. & Mosier, D. E. J. exp. Med. 150, 1399-1409 (1979).
- Woodland, R. & Cantor, H. Eur. J. Immun. 8, 600-606 (1978). Hetzelberger, D. & Eichmann, K. Eur. J. Immun. 8, 846-852 (1978).
- Adorini, L., Harvey, M. & Sercarz, E. E. Eur. J. Immun. 9, 906-909 (1979).
 Wysocki, L. J. & Sato, V. L. Proc. nam. Acad. Sci. U.S.A. 75, 2844-2848 (1978).

Kainate-like neurotoxicity of folates

John W. Olney, Terry A. Fuller & Taisija de Gubareff

Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri 63110, USA

Kainic acid (KA) is one of the most powerful of a group of 'excitotoxic' analogues of the putative neurotransmitter glutamate (Glu) whose neurotoxicity may involve an excitatory mechanism mediated through glutamergic postsynaptic receptors 1-8. The finding that neural membranes have specific sites where KA binds quite firmly and that Glu inhibits such binding very weakly, however, raises the possibility that KA and Glu receptors may be separate and distinct (see also refs 10, 11). It is in any case known that the neurotoxic properties of KA and Glu are not identical. Thus, when injected into the amygdala, both Glu and KA destroy local neurones but only KA induces sustained limbic seizures and an apparently seizure-mediated pattern of extra-amygdaloid brain damage 12-14. Ruck et al. 15, having recently found that the folic acid derivative, methyltetrahydrofolate (MTHF), competes powerfully for KA binding sites on rat cerebellar membranes and mimics KA in depolarizing frog spinal neurones, proposed that MTHF may be an endogenous neuromodulator with both excitatory and neurotoxic properties. We have therefore injected MTHF directly into the amygdala of the adult rat and found that at a rather high dose (300 nmol), it reproduces the specific component of KA neurotoxicity that Glu fails to reproduce, namely the limbic seizure/brain damage syndrome. We have also found that folic acid itself (pteroyl-L-glutamic acid, PGA) and one of its reduced derivatives (N-5-formyltetrahydrofolate, FTHF) are substantially more powerful than MTHF in reproducing this syndrome.

Fifty-five adult Harlan rats (250-300 g) were anaesthetized with ether and Nembutal (45 mg per kg) and injected stereotaxically with KA (3 nmol), sodium MTHF (10-300 nmol), sodium PGA (25-150 nmol) or calcium FTHF (25-150 nmol) into the amygdala (Pellegrino and Cushman coordinates AP -0.4, L5, V9). Each agent was dissolved in sterile distilled water

immediately before use with NaOH added as needed to neutralize pH and improve solubility. Injections were delivered in volumes of 0.5-3.0 µl over a 5-min period through a 30-gauge needle and Hamilton microsyringe. All animals were killed by perfusion fixation 4 h after injection, as by this time the neurotoxic manifestations of KA are clearly evident in histological sections 16,17. Brains were processed for evaluation by light and

electron microscopy as described previously¹⁸.

As in earlier experiments ^{12,13,19}, KA injection (3 nmol) caused staring, mouth movements, head bobbing, eye blinking, wet dog shakes, sialorrhoea and limbic seizures which, in some animals, could be described as 'status' limbic seizures. All KA-injected animals (n = 6) had a conspicuous acute neurotoxic reaction at the local injection site, consisting of massive dilatation of neuronal dendrites, swelling of astroglia and either oedematous swelling or vacuolization and dark cell degeneration of neuronal somata. The local reaction was typically most severe in the region immediately surrounding the injection site but many amygdaloid neurones further from the injection were also damaged, as were neurones in the piriform, entorhinal and temporoparietal cortices, the hippocampus, lateral septum and certain thalamic nuclei. In some animals all these brain regions were involved but in others only a partial syndrome developed. An apparent 'mirror' focus of acute neuronal degeneration was evident in the contralateral amygdala of one animal. This disseminated pattern of damage in KA-treated animals, which several authors have attributed to a seizure mechanism 12,13,19-22, is illustrated in refs 17, 21, 22.

When MTHF was injected into the amygdala at 10, 30, 60, 100 and 300 nmol, tissue and behavioural pathology was observed only at the highest dose. Of four animals injected with this dose, three were affected; each displayed periodic KA-type seizures over a 1-2 h period and had mild brain lesions primarily involving the piriform cortex (Fig. 1a, b). Amygdaloid neurones were less consistently damaged and the tissue zone immediately surrounding the amygdala injection site was sometimes totally free from damage.

PGA and FTHF were both injected into the amygdala at 25, 50, 100 and 150 nmol (four animals at each dose of each agent) and every dose of each compound reproduced a KA-type seizure/brain damage syndrome. At 25 nmol, the seizures

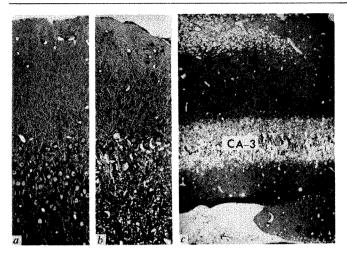


Fig. 1 a, b, The adult rat piriform cortex following injection of 60 nmol(a) or 300 nmol(b) of MTHF into the amygdala. The piriform cortex of a appears normal and this animal exhibited no behavioural pathology. The piriform cortex of b displays the typical acute cytopathological changes seen in animals following an intracerebral injection of KA (illustrated in refs 16, 18, 21); this animal had frequent KA-type seizures for 2 h (\times 106). c, The adult rat hippocampus following injection of 100 nmol PGA into the amygdala; the pattern of acute cytopathological changes is identical to that described and illustrated elsewhere²¹ in the hippocampus of KA-treated rats. Note the extreme oedematous changes in dendritic and glial structures which form a band of rarified tissue in the CA-3 pyramidal layer and the dilated distal CA-3 apical dendrites (arrows) and distal dentate granule dendrites (arrowheads) (×60).

induced by either agent were relatively mild and the brain damage largely confined to the olfactory cortex, although cytopathological changes were also sometimes detected both at the local amygdaloid injection site and in several remote brain regions. At 100 and 150 nmol, animals typically manifested severe KA-type behavioural symptoms including sustained limbic seizures, and their brains exhibited the full pattern of cytopathological changes associated with KA treatment (Fig. 1c). However, the tissue reaction at the amygdala injection site was not identical to that induced by KA in that damage to neuronal groups adjacent to the injection site was sometimes either mild or nonexistent.

When KA (2-10 nmol) is injected into the rat striatum, it destroys many local neurones²³ and, in addition, induces sustained seizures which are accompanied by the disseminated pattern of apparently seizure-mediated brain damage associated with KA injection into the amygdala 17.24. Intra-striatal injection of Glu (500-1,000 nmol) or the potent linear aspartate analogue N-methyl aspartate (40-80 nmol) also results in acute destruction of striatal neurones, but is not accompanied by either seizures or disseminated (distant) brain damage^{7,13,24}. To discover whether folates reproduce both the local and distant neurotoxic actions of KA, we injected PGA (50 and 100 nmol) into the striatum of halothane-anaesthetized rats; all animals (n = 6) manifested a full repertoire of KA-type symptoms, including frequent seizures, and all displayed the KA pattern of distant brain damage, but none sustained damage at the local striatal injection site²⁹. We tentatively conclude, therefore, that PGA and FTHF are partial KA-like neurotoxins which faithfully reproduce the distant (seizure-linked) pattern of KA-type damage while failing to reproduce the powerful local (direct) brain-damaging action of KA. The present finding of folateinduced damage to the amygdala can possibly be attributed to a seizure mechanism, as amygdala neurones are a vulnerable part of the excitatory limbic circuits within which this type of damage occurs. Additional studies are needed to clarify whether folates are totally without local neurotoxicity or merely display local neurotoxic manifestations more weakly than distant ones, and

whether separate mechanisms underlie the seizure-linked neurotoxic properties shared by KA and folates and the direct neurone-necrotizing properties shared by KA and Glu linear analogues.

It is believed that all the metabolic functions of folates are mediated by reduced tetrahydrofolate (THF) forms of PGA and that PGA itself is metabolically inactive. Because PGA was a much more active neurotoxin in our experiments than was its reduced derivative, MTHF, it seems unlikely that PGA neurotoxic activity is dependent on its conversion in vivo to MTHF. To explore the possibility that PGA activity depends on conversion to FTHF, we injected dihydrofolate (DHF) into the amygdala. As this compound is an intermediary metabolite in the PGA - FTHF pathway, it should convert in vivo to FTHF as readily as does PGA and, therefore, should be at least as active as PGA. However, we found DHF, like MTHF, to be quite weak in reproducing KA-like neurotoxicity¹⁴. Moreover, we injected amethopterin, an irreversible blocker of DHF reductase, into the amygdala 15 min before PGA injection¹⁴; although amethopterin presumably blocks conversion of PGA to any of its reduced forms, it did not decrease or prevent the expression of KA-like neurotoxicity. Thus, we suspect that the PGA molecule itself may have KA-like neurotoxic activity, and that FTHF independently has such activity.

A report²⁵ that PGA is not taken up by brain tissue slices is of interest here because the enzymatic conversion of PGA to reduced derivatives probably occurs intracellularly and cannot do so if PGA is not taken up by brain cells. Thus, injection of PGA into brain might be expected to result in its extracellular accumulation, allowing maximum opportunity for it to interact with neuromodulatory folate receptors, if any exist, on the external surfaces of central neurones. The efficient intracellular transport (and thus inactivation) of DHF and MTHF could explain their lower activity. This possibility, and the intriguing alternative that PGA and FTHF differ from other folates in having subtle structural features in common with KA, should be further investigated.

Others have shown that folates are convulsants 26,27, but it has not previously been demonstrated that they are also powerful brain damaging agents. Since folates are naturally present in the central nervous system and the seizure-related pattern of brain damage we describe here in folate-treated rats conforms closely to that frequently observed in autopsied brains of human epileptics²⁸, a possible role for folates in human epilepsy and epileptic brain damage warrants consideration.

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- Shinozaki, H. & Konishi, S. Brain Res. 14, 368-371 (1970). Olney, J. W., Rhee, V. & Ho, O. L. Brain Res. 77, 507-512 (1974). Curtis, D. R. & Watkins, J. C. J. Physiol., Lond. 166, 1-14 (1963). Olney, J. W., Ho, O. L. & Rhee, V. Expl Brain Res. 14, 61-76 (1971).
- Olney, J. W., Sharpe, L. G. & de Gubareff, T. Neurosci. Abstr. 1, 371 (1975).
 Watkins, J. C. in Kainic Acid as a Tool in Neurobiology (eds McGeer, E., Olney, J. W. & McGeer, P.) 37-70 (Raven, New York, 1978).
 Olney, J. W. in Kainic Acid as a Tool in Neurobiology (eds McGeer, E., Olney, J. W. &
- McGeer, P.) 95-122 (Raven, New York, 1978).
 Schwarcz, R., Scholz, D. & Coyle, J. T. Neuropharmacology 17, 145-151 (1978).
 London, E. D., Klemm, N. & Coyle, J. T. Brain Res. 192, 463-476 (1980).

- 10. Lambert, J. D. C., Flatman, J. A. & Engberg, I. Adv. Biochem. Psychopharmac. 27, 205-216 (1981).
- 11. Watkins, J. C., Davies, J., Evans, R. H., Francis, A. A. & Jones, A. W. Adv. Biochem. Psychopharmac. 27, 263-274 (1981). Ben-Ari, Y., Tremblay, E., Ottersen, O. P. & Meldrum, B. S. Brain Res. 191, 79-97 (1980).
- Olney, J. W. Adv. Biochem. Psychopharmac. 27, 375-384 (1981) Olney, J. W. et al. (in preparation).
- Ruck, A., Kramer, S., Metz, J. & Brennan, M. J. W. Nature 287, 852-853 (1980).
- Hattori, T. & McGeer, E. G. Brain Res. 129, 174-180 (1977).
 Olney, J. W. & de Gubareff, T. in Kainic Acid as a Tool in Neurobiology (eds McGeer, E., Olney, J. W. & de Gubareff, T. in Kainic Acid as a Tool in Neurobiology (eas McGeer, E., Olney, J. W. & McGeer, P.) 201-218 (Raven, New York, 1978).
 Olney, J. W., Misra, C. J. & de Gubareff, T. J. Neuropathol. exp. Neurol. 34, 167-177 (1975).
 Collins, R. C., Lothman, E. W. & Olney, J. W. Proc. Univ. Calif. Symp. on Status Epilepticus, November 1980 (Raven, New York, in the press).
 Nadler, V., Perry, B. W. & Cottman, C. W. Nature 271, 676-677 (1978).
 Olney, J. W., Fuller, T. & de Gubareff, T. Brain Res. 176, 91-100 (1979).
 Schwob, J. E., Fuller, T. A., Price, J. L. & Olney, J. W. Neuroscience 5, 991-1015 (1980).

- Coyle, J. T., McGeer, E. G., McGeer, P. L. & Schwarcz, R. in Kainic Acid as a Tool in Neurobiology (eds McGeer, E., Olney, J. W. & McGeer, P.) 139–160 (Raven, New York,
- 24. Olney, J. W. Adv. Neurol. 23, 609-624 (1979)
- Spector, R., Levy, P. & Abelson, H. T. Biochem. Pharmac. 26, 1507-1511 (1977).
- Hommes, O. R. & Obbens, E. A. M. T. J. neurol. Sci. 16, 271-281 (1972). Spector, R. G. Biochem. Pharmac. 20, 1730-1732 (1971).
- Corsellis, J. A. N. & Meldrum, B. S. in Greenfields Neuropathology (eds Blackwood, W. & Corsellis, J. A. N.) 771-795 (Arnold, London, 1976).
- 29. Olney, J. W., Fuller, T. A., de Gubareff, T. & Labruyere, J. Neuroscience Lett. (in the press).

Novel leukaemogenic retroviruses isolated from cell line derived from spontaneous AKR tumour

Finn Skou Pedersen*, Robert L. Crowther†, Donald Y. Tenney[†], Andreas M. Reimold[†] & William A. Haseltine†

* Department of Molecular Biology, University of Aarhus, 8000 Aarhus C. Denmark

Sidney Farber Cancer Institute, Department of Pathology, Harvard Medical School and Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts 02115, USA

Viruses isolated from leukaemic and preleukaemic tissues of the AKR mouse differ in their ability to induce thymic lymphomas. The Gross passage A virus, which originates from the thymus of a leukaemic AKR mouse¹, and the MCF viruses, isolated from preleukaemic and leukaemic AKR tissues^{2,3}, accelerate the onset of disease on injection into newborn AKR mice3,4 whereas the endogenous ecotropic Akv virus, which is expressed in AKR mice from mid-gestation onward, is nonleukaemogenic3,5 What are the structural features of the genome that account for the leukaemogenic activity of these viruses? The observation that the gp70 envelope glycoprotein of the leukaemogenic MCF virus contain ecotropic and xenotropic regions⁷⁻⁹ raised the possibility that the structure of the gp70 gene was the major determinant of leukaemogenic activity2. However, a cloned, in vitro passaged, leukaemogenic isolate of the Gross A virus very closely resembles that of the nonleukaemogenic Akv virus in the gag-pol and gp70 coding regions of the genome¹⁰. Most of the differences in structure of the Gross A and Akv viruses are located within the 3'-terminal 1,000 nucleotides—this raises the possibility that sequences outside the gp70 gene which are proximal to the 3' end of the genome encode determinants of leukaemogenic activity. Due to the complex passage history of the Gross A virus we sought to isolate further leukaemogenic AKR viruses that had not been passaged in other animals to determine whether or not they resembled the Gross A virus. Here we report the isolation and characterization of new leukaemogenic viruses from a lymphoid cell line derived from a spontaneous AKR tumour. These isolates resemble the Gross A virus in the 3' region of the genome. Our results suggest that this region encodes determinants of leukaemogenic potency and that major changes in the envelope glycoprotein are not necessary for the leukaemogenic activity of these viruses.

The source of virus was the SL3 cell line, established from a spontaneous tumour of an AKR mouse⁵, that produces a low titre of an ecotropic, N-tropic, XC virus 5.6. To obtain clonal isolates of this virus, the cell-free supernatant of the SL3 cell line was used to infect a culture of NIH 3T3 cells. Viruses were cloned from the supernatant of the infected culture by two rounds of end point dilution. For each round of cloning, less than one in five wells contained virus at the end point. Two viruses, SL3-1 and SL3-2, were obtained in one such experiment; the SL3-3 virus was obtained in a separate but similar experiment.

The biological properties of the cloned and uncloned SL3 were determined (Table 1). All viruses obtained from the SL3 cell line are ecotropic and N-tropic. The SL3-1 and SL3-3

viruses are positive in the XC fusion test, whereas the SL3-2 virus is not. The ratio of infectious virus to reverse transcriptase activity is much higher for virus grown in NIH 3T3 cells than for virus produced by the SL3 cell line.

The cloned SL3 isolates retain potent leukaemogenic activity (see Table 1): injection of these viruses into newborn AKR mice results in 100% incidence of thymic lymphoma with an average latent period of ~80 days. Mock-inoculated controls do not develop disease before 220 days. The pathology of the accelerated disease is indistinguishable from the spontaneous AKR or Gross passage A virus-induced thymic lymphoma. The cloned SL3-2 and SL3-3 isolates also induce rapid leukaemia in C3H_f/Bi mice (100% incidence, mean latent period of 100 days). Normally, these animals have a negligible incidence of thymic leukaemia. Our preliminary results indicate that the SL3-3 isolate also induces rapid thymic leukaemia in NSF-N mice—these animals show a pathology typical of Gross passage A-induced disease.

The structure of the SL3 viral RNAs was analysed using high resolution methods for analysis of RNase T₁-resistant oligonucleotides^{11,12}. Fingerprints of 70S RNAs of virus produced by the uncloned SL3 virus grown on NIH 3T3 cells and the cloned isolates are shown in Fig. 1, together with schematic interpretations. The fingerprint of the virus produced by the SL3 cell line itself is indistinguishable from that of the uncloned SL3 virus grown on NIH 3T3 cells. The schematic interpretations are based on co-electrophoresis of labelled oligonucleotides with those of the Akv virus, and on complete or partial sequence analysis of each numbered oligonucleotide. The fingerprints of the cloned virus indicate that they are biochemically pure and the molar yield of the unique oligonucleotides is close to unity in each isolate. The fingerprints of the cloned viruses do not change on subsequent cloning or passage of these isolates. The order of the unique oligonucleotides along the genome was determined (see Table 2). The sequence of the oligonucleotides of the SL3 viruses that are not present in the fingerprint of the Akv virus were also determined (see Table 3).

The map of Table 2 shows that oligonucleotides 208B and 36B are located near the 3' terminus of the SL3 viruses. It is striking that identical oligonucleotides are also found in the same relative positions in the leukaemogenic Gross A/NIH virus¹⁰. The sequence of oligonucleotides 208B and 36B differ from sequences in the Akv virus by one or two base changes; except for these two oligonucleotides and a few in the gag-pol region of the genome, the SL3-1 and SL3-3 viruses are identical to the Akv virus within the limits of resolution of the fingerprinting method, yet the SL3 viruses are potent leukaemogenic agents. Evidently, slight changes in the genome structure can affect the leukaemogenic activity of AKR viruses.

The SL3-2 virus differs from those of SL3-1 and SL3-3 cell lines in the region that encodes the gp70 protein. Most of this region is substituted with a heterologous sequence. One explanation for the isolation of two different viruses from the same cell line is that the SL3 cell produces a mixture of two viruses that have a large part of the genome that is identical but differ in the gp70 gene. The observation that, in the uncloned viruses, the oligonucleotides derived from the gp70 region of the genome are present in half the molar yield of the other oligonucleotides in the genome supports this notion (data not shown)

We have also studied viruses produced by eight other lymphoid tumour cell lines of AKR origin. Four of these produce mixtures of virus identical to those produced by the SL3 cell line and which also have the changes in the gag-pol and env regions noted here. Comparison of these viruses will be the subject of a separate communication.

Some of the gp70 oligonucleotides of the SL3-2 virus are identical to sequences found in fingerprints of a xenotropic virus chemically induced from AKR fibroblasts¹³ (see Table 3). As is the case for MCF viruses isolated from AKR tissues, substitution of xenotropic virus-related sequences within gp70 correlates with the XC⁻ phenotype of the SL3-2 virus. Unlike the

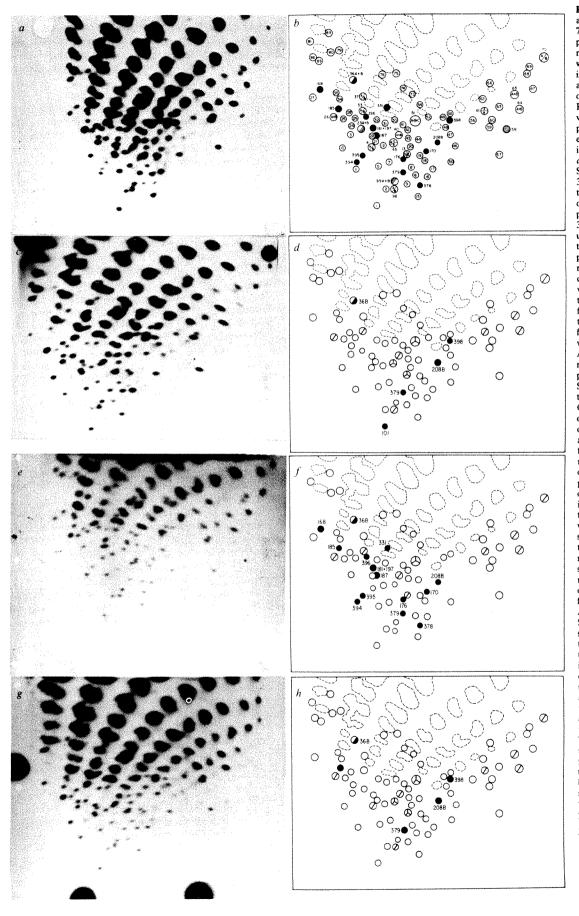


Fig. 1 RNase T_1 fingerprint analysis of SL3 viruses. Viral 70S and 35S RNAs were prepared from cell-free supernatants as described else-where 11,12. The schematic The schematic interpretations are based on (1) analysis of mixtures of labelled oligonucleotides of each viral RNA with those of the Akv virus and; (2) complete or partial sequence analysis of each numbered oligonucleotide in the fingerprints of each virus. a, b, 70S RNA of the uncloned SL3 virus propagated on NIH 3T3 cells. The fingerprints of the virus produced by a subline of the SL3 cell line, and virus produced by two other NIH 3T3 cultures infected with the uncloned SL3 virus were indistinguishable from this fingerprint, shown in a. Open circles represent oligonucleotides common to the Akv and SL3 viruses. The Akv virus used for this comparison was derived from the same mouse colony that gave rise to the SL3 cell line from (from Esther Hays, University of California, Los Angeles). Solid circles oligonucleotides represent present in the fingerprint of the SL3 virus that are not present in the fingerprint of the Akv virus. Cross-hatched circles represent oligonucleotides characteristic of the Akv genome that are absent altogether from the fingerprint of the SL3 virus. The numbering of the Akv oligonucleotides is as described previously12. The numbering of oligonucleotides 208B and 36B is the same as that assigned to the Gross A oligonucleotides which have identical sequence 10. c, d, 70S RNA of the SL3-1 isolate. Open circles oligonucleotides represent shared with the Akv virus; solid numbered circles represent oligonucleotides that are not found in the fingerprint of the Akv virus. e, f, 35S RNA of the SL3-2 isolate; symbols are the same as for d. g, h, 70S RNA of the SL3-3 isolate; symbols are the same as for d. Although not indicated in the schematic drawings, the same Akvspecific oligonucleotides that are missing from the fingerprints of uncloned SL3 virus are also missing from the finger-prints of the cloned derivatives. The molar yields of each of the unique oligonucleotides of each fingerprint were determined in two independent experiments by counting gel fragments corresponding to the position of the oligonucleotides. The molar yield of each of the unique oligonucleotides was the same, within experimental error, in the fingerprints of the cloned viruses. For the uncloned SL3 virus, the molar yield of the oligonucleotides in the gp70 region of genome (see Table 2) was half that of the other oligonucleotides.

AKR-MCF viruses, the gp70 substitution in SL3-2 apparently does not result in an extended host range. More important, the gp70 substitution in SL3-2 is not essential for the leukaemogenic potential of the virus, as the SL3-1 and SL3-3 viruses which lack the substitution are also potent leukaemogenic agents.

What functions relevant to leukaemogenic activity may be

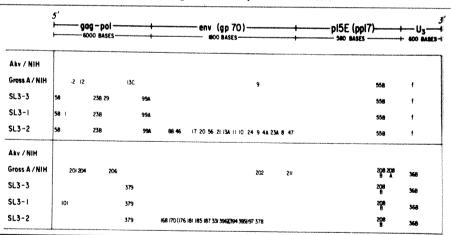
altered in the SL3 viruses? All the SL3 isolates differ slightly from the Akv virus in the gag-pol region of the genome. It is possible to argue that these differences correlate with oncogenic potential. However, the Gross A/NIH virus also contains changes in this region of the genome, but these sequences are not the same as those present in the SL3 viruses. Therefore, we consider it more likely that the variant 3' region of the genome

Table 1 Biological properties of the SL3 viruses

Virus Akv Gross A/NIH SL3	NIH3T3 + + +	BALB/c	CCL64	8155	FLF ₃	c.p.m. per Cell (RT) 2.3×10 ⁻¹ 2.5×10 ⁻¹	IU/ml ⁻¹ end point titre 1×10 ⁷ 2×10 ⁷	S+L- PFU/ml ⁻¹ 2×10 ⁵ 2.5×10 ⁵	XC PFU ml ⁻¹ 1×10 ⁵ 3×10 ⁵	Leukaemogenicity acceleration days post- inoculation No acceleration 102-146 (ref. 10)	Induction in C3H _t /Bi No induction 135-220 (ref. 4)
			_	_	_	2.5×10^{-3}	1	0	0	80-100 (ref. 6)	NT
SL3/NIH	+		-		_	3.3×10^{-1}	2×10^{6}	3×10^{3}	60	69-104	NT
SL3-1	+			***		1.4×10^{-1}	1×10^5	3×10^{3}	7×10^{3}	120-130	NT
SL3-2	+	-			***	2.6×10^{-1}	1×10 ⁴	2.25×10^{5}	0	69-105	84-114
SL3-3	+					2.5×10^{-1}	1×10^{6}	3.5×10^4	6×104	61-82	73-117
AKR-6		-	+	+	+	2.5×10^{-1}	1.10^{6}	NT	NT	NT NT	NT
MCF-247	+		+	***	_	3.5×10^{-1}	NT	NT	NT	90–155 (refs 3, 21)	NT

The biological properties of the viruses were determined using standard methods. The viruses used were Akv and Gross A/NIH described elsewhere ¹⁰⁻¹²; AKR MCF-247; a xenotropic virus, AKR-6, derived from tissues of an AKR mouse propagated on dog 8155 cells, and the SL3 viruses described in the text. The same preparation of each virus was used for all tests. The ability of the viruses to grow on mouse NIH/3T3 and BALB/c cells, mink CCL64 cells, dog 8155 cells, and cat FLF₃ cells was determined by assay of reverse transcriptase activity in cell-free supernatants. The reverse transcriptase activity produced per cell was determined by dividing the c.p.m. incorporated by the virus in 1 ml of culture fluid in a standard reverse transcriptase assay by the number of cells per ml (in the case of suspension cultures) or the number of cells per ml of supernatant (for adherent cells). The end point titre (in Infectious Units, IU) was determined in NIH3T3 cells. The S+L- and XC fusion assays are those described elsewhere ^{22,23}. PFU, plaque-forming units. The leukaemogenic activity of each virus was determined by inoculation of 0.1 ml of cell-free supernatant intraperitoneally into 2-4-day-old AKR and C3H_t/Bi mice. Mockinjected littermates served as controls in all cases. Experimentally induced acceleration of disease in AKR mice was clearly a separate event from that of spontaneous disease in control littermates. All eight animals injected with the uncloned SL3 virus grown on NIH3T3 cells. (SL3/NIH) developed leukaemia in 69-104 days with an average onset of 87 days, whereas seven control littermates did not develop disease before 220 days. All five animals injected with the SL3-1 virus developed disease at 120-14 h.days, average 124 days post-inoculation, whereas three uninoculated littermates did not develop disease before 220 days. All eight animals injected with the SL3-3 virus developed disease between 61 and 82 days whereas sight control littermates remained disease-free at 230 days post-inoculation. Induction

Table 2 Oligonucleotide maps of the SL3 viruses



The order of the large T_1 oligonucleotides of the genome of the SL3-2 virus was determined as described elsewhere 10 . The map order of the Akv and Gross A/NIH viruses are those described elsewhere 10 . The map order of the oligonucleotides of the SL3-1 and SL3-3 viruses are inferred by comparison with the maps of the Akv, Gross A/NIH and SL3-2 viruses. The upper half represents oligonucleotides, present in Akv, which are missing from these viruses, the lower half represents the positions of oligonucleotides which are not present in Akv. For the Akv genome, there are a total of 34 oligonucleotides within the gag-pol region, 16 within gp70, 9 within p15E, and 6 within U_3 . The assignment of oligonucleotides 88 and 46 to the *env* region of Akv, Gross A, SL3-1, and SL3-3 is further supported by alignment of these sequences with the protein sequence of the *env* gp70 proteins of the Moloney and Rauscher murine leukaemia viruses 24 . This alignment is indicated below

An asterisk indicates positions in which the oligonucleotide sequence differs from that predicted by the amino acid sequence. The alignment of oligonucleotides with p15E-R and U_3 is further supported by DNA sequences of the Moloney leukaemia 15 and Akv viruses (W. Herr, personal communication and J. Lenz, R. Crowther, A. Straceski and W. Haseltine, unpublished data). In addition to those shown, the Gross A/NIH virus possesses oligonucleotides numbered 205, 207, 213 and 215, which, by fingerprint analysis, map within the extreme 3'-terminal regions. Their precise locations relative to other oligonucleotides within p15E and U_3 are unknown.

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									Ta	bie 3	Olig	gonuc	leotid	e seq	uence	s of A	KR-r	elate	d viru	ses									
36B f	A G	A A	A A	A A	A A	U	A A	C C	C C	A A	G G					,													
208B 55B	U U	A A	U U	U	C C	U U	C C	A A	A A	U U	C C	A G	C	A	U	G													
197 24	c	U	C C	U U	C C	A A	A A	C C	C C	U U	C C	A A	C C	C C	A A	G G													
378 9	U U	A A	C C	C C	U	A A	U C	U U	C C	C	A A	A A	C C	C C	A A	U U	A A	C C	U	U	C C	U U	G G						
101	U U	C C	U U	C C	U	C C	C C	C C	A A	A A	A A	C C	U	C C	U U	C C	C C	C C	C C	C	U	C C	U U	U C	C C	A A	A A	c	G G
168 170 176 181	A U A	C U	A C C C	A U U	A A U A	A A C	C U A C	C U A A	A U A C	A C C U	G C C C	C C A	U C C	U U C G	A A A	A A G	G A	G											
185 187 331	U U	C U U	U C A	C	C C	C A C	A A C	A A C	C A C	A C A	c c	A C C	A A U	U G	C	A	G												
379 394 395 396	C C A	C C A	U C A A	C C A	U A A A	A C A C	A C C U	υ C C	C U A C	A C A C	U C A A	A C U A	C A A A	C A U G	U C A	C A A	U A A	A C A	A C G	A U	G G								

The complete sequence of the non-Akv oligonucleotides present in the fingerprints of the SL3 viruses was determined as described elsewhere 10-12. Several of these sequences are related by one or two base changes to oligonucleotides present in the fingerprints of the Akv and Gross A/NIH RNAs. Such sequence variations are indicated for oligonucleotides 36B, 208B, 197, 101 and 378 of the SL3 viruses which are related to oligonucleotides 7, 55B, 197, 1 and 9 of the Akv virus, respectively. The sequences of oligonucleotides 208B and 36B of the SL3 viruses are identical to oligonucleotides of the same number in the Gross A/NIH virus. The sequence of the SL3-specific oligonucleotides for which no Akv analogues were found are also listed in the lower half of the table. The sequence of two of the oligonucleotides derived from the gp70 gene of the SL3-2 virus, numbers 170 and 176, are identical to oligonucleotide sequences present in the fingerprint of xenotropic virus induced from AKR fibroblasts (unpublished observations).

U C G

affects the leukaemogenic activity of the viruses, as identical changes in this region of the genome are observed in the genomes of the leukaemogenic Gross A and SL3 viruses. Furthermore, the same variant 3' region, characterized by oligonucleotides 208B and 36B, is also present in the RNA of virus produced by four other independently isolated lymphoma cell lines which were derived from spontaneous tumours of AKR mice that produce leukaemogenic viruses (refs 5, 6, 14, and unpublished observations).

UAUUA

Changes in the 3' region could affect several viral functions. Sequence analysis of recombinant DNA fragments of the Moloney murine leukaemia virus (Mo-MuLV) provirus reveals a continuous coding sequence that runs from the amino terminus of the gp70 protein to the origin of plus strand synthesis at the 5' end of the U³ region¹⁵. The open reading frame extends past the end of the p15E gene. An open reading frame in this region of the Akv genome is also indicated by the preliminary sequence of this region (W. Herr, personal communication, and J. Lenz, R. Crowther, A. Straceski and W. Haseltine, unpublished observations). Comparison of the sequence of oligonucleotide 208B with that of the Akv sequence implies that the protein coded for by the SL3 virus would differ from that corresponding to the Aky protein by a change from an Arg-Leu sequence to His-Met sequence near the carboxyl region of the p15E region as indicated below.

	G	ross A/N	IIH and	SL3 sec	uence	
5'	Cys GT	Ile ATT	Leu CTC	Asn AAT	His CAC	Met ATG
5'	Cys GT	Ile ATT		Asn AAT		
		A	kv seat	ience		

Much of the protein sequence coded for by the p15E region of the genome would not be affected, because the other oligonucleotides derived from this region are identical in the Akv, SL3 and Gross A/NIH viruses. A possible explanation for the leukaemogenic activity of these viruses is that the 3'-terminal region of the virus encodes a protein that is active in the transformation process and that this protein has different structures in the leukaemogenic and nonleukaemogenic viruses.

Oligonücleotide 36B may also serve as a marker for leukaemogenic activity. This oligonucleotide is located in the U₃ portion of the genome and is related to an oligonucleotide in the Akv virus by a single base change. The U3 region of the virus codes for functions that are important for the replication cycle, including initiation of the plus strand of DNA synthesis, initiation of RNA transcription, processing of RNA transcription and possibly integration 16-18. Changes in the structure of the region of the genome that controls this process could alter the physiological effects of virus infection on cell growth and regulation. Recent studies of the recombinants between the avian retroviruses RAV-0 and RAV-2 also suggest that leukaemogenic activity might be associated with structural determinants at the extreme 3' terminus of the viral genome 19,2

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- 1. Gross, L. Proc. Soc. exp. Biol. Med. 76, 27-32 (1951); 78, 342-348 (1951); 94, 767-77
- 2. Hartley, J. W., Wolford, N. K., Old, L. J. & Rowe, W. P. Proc. natn. Acad. Sci. U.S.A. 74, 789-792 (1977).
- 3. Rowe, W. P., Cloyd, M. W. & Hartley, J. W. Cold Spring Harb. Symp. quant. Biol. 44, 1265-1268 (1979)
- Buchhagen, D. L., Pincus, T., Stutman, O. & Fleissner, E. Int. J. Cancer 18, 835-842
- Hays, E. F. & Vredevoe, D. L. Cancer Res. 37, 726-730 (1977).
- Nowinski, R. C. & Hays, E. F. J. Virol. 27, 13-18 (1978). Rommelaere, J., Faller, D. V. & Hopkins, N. Proc. natn. Acad. Sci. U.S.A. 75, 495-499
- Li-Lung, M., Hering, C., Hartley, J. W., Rowe, W. P. & Hopkins, N. Cold Spring Harb. Symp. quant. Biol. 44, 1269-1274 (1979). Elder, J. H. et al. Proc. natn. Acad. Sci. U.S.A. 74, 4676 (1977).
 Buchhagen, D. L., Pedersen, F. S., Crowther, R. L. & Haseltine, W. A. Proc. natn. Acad.
- Sci. U.S.A. 77, 4359-4363 (1980).
- Sci. U.S.A. 71, 4339-4363 (1980). Pedersen, F. S. & Haseltine, W. A. Meth. Enzym. 65, 680-687 (1980). Pedersen, F. S. & Haseltine, W. A. J. Virol. 33, 349-365 (1980). Copeland, N. G. & Cooper, G. M. Cell 16, 347-356 (1979).
- 14. Haseltine, W. A., Pedersen, F. S., Sahagan, B. G., Rosenberg, Z. F. & Koslov, J. F. in Modern Trends in Human Leukemia III (eds Neth, R. & Gallo, R.) (Springer, Berlin,
- 15. Sutcliffe, J., Shinnick, T., Verma, I. M. & Lerner, R. A. Proc. natn. Acad. Sci. U.S.A. 77, 3302-3306 (1980).
- 16. Bishop, J. M. A. Rev. Biochem. 47, 35-88 (1978)
- Coffin, J. M. J. gen. Virol. 42, 1-26 (1979). Gilboa, E., Mitra, S. W., Goff, S. & Baltimore, D. Cell 18, 93-100 (1979).
- Tsichlis, P. & Coffin, J. M. J. Virol. 33, 238-249 (1980).
- Robinson, H. L., Pearson, M. J., DeSimone, D. W., Tsichlis, P. N. & Coffin, J. M. Cold Spring Harb. Symp. quant. Biol. 44 (1979).
- 21. Pedersen, F. P., Buchagen, D. L., Chen, C. Y., Hays, E. F. & Haseltine, W. A. J. Virol. 35, 211-218 (1980).
- Rassin, R. H., Tuttle, N. & Fischinger, P. J. Nature 229, 564-566 (1971).
 Rowe, W. P., Pugh, W. E. & Hartley, J. W. Virology 12, 1136-1139 (1970).
 Orozlan, S. & Gilden, R. in The Molecular Biology of RNA Tumor Viruses (ed. Stephanson, J.) (Academic, New York, 1980).

Identification of E. coli uvrC protein

George H. Yoakum & Lawrence Grossman

Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, Maryland 21205, USA

The excision of pyrimidine dimers from DNA is one of the major mechanisms of repair involving an initial endonucleolytic step followed by excision, resynthesis and ligation¹. The enzymatic mechanism for endonucleolytic cleavage of UV light-damaged DNA by the Micrococcus luteus2 and Escherichia coli bacteriophage T4 (ref. 3) dimer-specific endonucleases involves two steps: cleavage of the N-glycosylic bond of the 5'-pyrimidine moiety of the pyrimidine dimer, followed by cleavage of the phosphodiester bond 3' to the apyrimidinic site produced by the first step. Although E. coli requires functional uvrA, uvrB and uvrC genes to repair UV light-damaged DNA4, and enzymatic cleavage of damaged DNA requires the concerted action of at least three proteins⁵⁻⁸, the mechanism of catalysis by which the uvr-endonucleolytic system cleaves UV light-damaged DNA is unclear. We have constructed hybrid multicopy plasmids containing the uvr genes of E. colis to study the expression of amplified uvr genes. We have physically mapped uvrC on plasmid pGHY4211 (previously referred to as pGY4211 (ref. 9)) by interruption with the $\gamma\delta$ sequence of F⁺ as described by Guyer¹⁰, and determined the minimum size for the uvrC gene to be 1.45 kilobase pairs. SDS-gel electrophoresis of radioisotopically labelled plasmid proteins indicates that the uvrC⁺ gene product is a polypeptide of molecular weight (MW) 68,000. We estimate that wild-type E. coli may have as few as 10 copies of uvrC polypeptide per cell.

We have previously described a set of plasmids, consisting of pBR322 and a 3.4-kilobase pair PstI fragment of E. coli K12 DNA, which complements the uvrC34 mutation in either orientation relative to pBR322 (pGHY4211, pGHY3233)9. To study the role of uvrC in the uvr repair system of E. coli we have physically mapped the uvrC gene, and identified the uvrC protein by SDS gel electrophoresis of plasmid proteins labelled by the maxi-cell¹¹ method.

Inactivation mapping of cloned genes has proved useful in determining the physical size, direction of transcription and identification of gene products¹²⁻¹⁵. The $\gamma\delta$ -insertion sequence of F was used to identify the uvrA and uvrB gene products in a similar manner^{16,17}. This insertion sequence integrates into DNA by a recA-independent mechanism¹⁰, and DNA sequencing indicates that termination signals occur in five of the six possible reading frames near the ends of the $\gamma\delta$ sequence (M. Guyer, personal communication). Thus, insertion of the $\gamma\delta$ sequence into a structural gene terminates translation in almost all cases. The F⁺ recA56 streptomycin-sensitive (Str^s) strain (MG1063) used here to isolate $\gamma\delta$ insertions in plasmid pGHY4211 was provided by M. Guyer¹⁰, and was transformed¹⁸ with pGHY4211. Transconjugants of pGHY4211 containing $\gamma\delta$ insertions were isolated by mating tetracyclineresistant (Tetr) MG1063 carrying pGHY4211 with an F recA56 uvrC34 rpsL strain (SR57, Kendric Smith). To select recipient cells carrying pGHY4211 with $\gamma\delta$ insertions, cultures were plated on Luria agar containing 10 µg ml⁻¹ tetracycline and 50 µg ml⁻¹ streptomycin, and colonies were tested for Tet and Str (ref. 10). About 200 Tet Str colonies were tested for UV light sensitivity as described elsewhere9. Several colonies carrying plasmids unable to complement the uvrC34 mutation of SR57 (recA56 uvrC34) were selected for further study, and small amounts of plasmid DNA isolated by a rapid isolation procedure¹⁹. The recA56 uvrC34 double mutant (SR57) was transformed with the plasmid DNAs isolated above, screened

for Tet', Str' and UV light sensitivity, then stored at -20 °C in glycerol cultures for later use in protein labelling experiments.

The site of integration was determined for 10 $y\delta$ insertions in pGHY4211 which inactivate the $uvrC^+$ function of this plasmid, by measuring the size of restriction fragments after digestion with EcoRI or BamHI. pGHY4211 contains only one EcoRI and one BamHI site in the pBR322 portion20 of this 7.76kilobase pair plasmid9. Therefore, the EcoRI site located at 0.9 kilobase pair from the y end and the BamHI site at 0.4 kilobase pair from the δ end of the insertion sequence 10 allow an unequivocal location of the site of $\gamma\delta$ insertion and its relative orientation, by determining the size of the EcoRI and BamHI restriction fragments on agarose gels. Mapping data yielded the locations of $y\delta$ insertions (shown in Fig. 1), indicating that the minimum size for the uvrC gene transcription unit is 1.45 kilobase pairs. This is enough genetic information to encode an average polypeptide of MW 56,000.

SDS-polyacrylamide gel electrophoresis²¹ of ³⁵S-Metlabelled proteins encoded by γδ-inactivated pGHY4211 plasmids, reveals that a 68,000-MW polypeptide is missing in each case (Fig. 2b). This 68,000-MW polypeptide is present among the plasmid-encoded products of pGHY4211 and pGHY3233 (Fig. 2a, b) which contain the uvrC+ 3.4-kilobase pair Pst1 fragment in both orientations relative to pBR322. As the uvrC gene transcription unit in pGHY4211 maps between 0.85 and 2.3 kilobase pairs (Fig. 1), and a 68,000-MW polypeptide is missing from the plasmid-encoded products of each γδinactivated plasmid, these data indicate that the 68,000-MW polypeptide is encoded by uvrC. However, these data do not preclude the possible disappearance of a low-molecular weight polypeptide, fortuitously similar to a low-molecular weight polypeptide among $\gamma\delta$ plasmid-encoded background bands in Fig. 2. Although identification of the uvrC gene product must also be established by conventional protein purification methods, the 68,000 molecular weight observed here is in the same range suggested for uvrC from complementation activity in E. coli extracts7.

Additional evidence that the 68,000-MW polypeptide is encoded by the uvrC gene was obtained by in vitro mutagenesis of pGHY3233. Mutations in the uvrC⁺ gene of pGY3233 were isolated by irradiation of plasmid DNA with various doses of UV light and transformation 18 of irradiated plasmid DNA into rec⁺ uvrC34 AB1884 (ref. 4). About 800 Tet^r transformants were tested for Tet and UV light sensitivity as previously described9. From these, two colonies were found which carried Tet' plasmids that were unable to complement the uvrC34 mutation. After isolation of a small amount of plasmid DNA, SR57 recA56 uvrC34 was transformed with pGHY7077 and pGHY7064 and cultures tested for Tet and UV light sensitivity and placed in storage as described above. pGHY7077 and

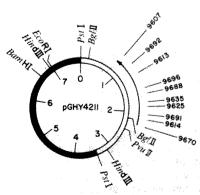


Fig. 1 $\gamma \delta$ insertion map of the *uvrC* gene in plasmid pGHY4211 including the minimum physical size of the uvrC gene transcription unit, and the most probable direction of transcription. The site of $\gamma\delta$ integration in pGHY4211 was determined by restriction mapping as described in the text. The most distant sites of integration which inactivate the uvrC⁺ function of pGHY4211 are at 0.85 kilobase pair (9607) and 2.3 kilobase pair (9670), indicating a minimum size of 1.45 kilobase pair for uvrC.

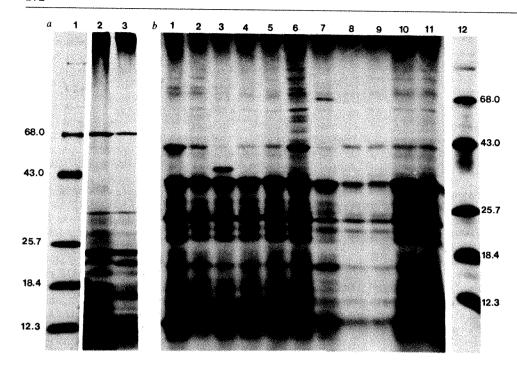


Fig. 2 The 35S-Met-labelled protein products of various plasmids were visualized by fluorography of maxi-cell extracts resolved on 10-20% polyacrylamide gradient2% SDS gels by electrophoresis, as described in the text. The first lane in a contains 14 C-labelled protein standards obtained from Bethesda Research Laboratories. Lane 2 contains ³⁵S-Met-proteins from *uvrC*⁺ pGHY3233 (ref. 9); lane 3, ³⁵S-Met-labelled proteins from pGHY4211. b, Fluorography of a similar polyacrylamide gel used to resolve the 35S-Met-labelled proteins encoded by the plasmid indicated for each lane. The electrophoretic mobility of protein 14Clabelled standards is indicated in the margin. Lane 1, $\gamma\delta$ -9607; lane 2, $\gamma\delta$ -9866; 3, γδ-9613; 4, γδ-9614; 5, γδ-9625; 6, γδ-9670; 7, pGHY4211 (uvrC⁺); 8, pGHY7064 (uvrC⁻ mutation); 9, pGHY7077 (uvrC mutation); 10, γδ-9691; 11, $\gamma \delta$ -9696. The appearance of a number of bands in lane 6 ($\gamma\delta$ -9670) could have resulted from an increase in background due to a low level of contamination for this extract.

pGHY7064 plasmid DNAs were digested with PstI and Hin dIII and compared with pGHY3233 by agarose gel electrophoresis (data not shown). Both pGHY7077 and pGHY7064 contain the 3.4-kilobase pair PstI fragment in the same orientation as their $uvrC^+$ counterpart, pGHY3233 (ref. 9). Radioisotopic labelling of plasmid-encoded proteins from $uvrC^-$ pGHY7077 and pGHY7064 derived by $in\ vitro\ UV$ light mutagenesis lack a 68,000-MW polypeptide (Fig. 2b). This confirms the conclusion drawn from the $\gamma\delta$ -inactivation experiment that the 68,000-MW polypeptide is encoded by the uvrC gene.

The most probable orientation of uvrC transcription was deduced from the polypeptide products and map position of $\gamma \delta$ -9613—this maps at 1.275 kilobase pairs (Fig. 1), and produces a unique polypeptide of MW 35,000 (Fig. 2b, lane 3). The appearance of this polypeptide among the 35S-Met-labelled products of a plasmid that is unable to produce the 68,000-MW polypeptide and inactivated for uvrC complementation, suggests that the 35,000-MW polypeptide is a partial product of uvrC. Because the 1-kilobase pair distance between 9613 and 9670 is adequate to encode an average polypeptide of MW~ 38,300, and the 420 base pairs from 9613 to 9607 could only encode ~16,200 MW, the position of 9613 and the 35,000-MW polypeptide are most consistent with a starting point for the uvrC gene close to 9670, with transcription proceeding towards 9607 (Fig. 1). In addition, we found that $\gamma\delta$ -9692 produces a similar truncated polypeptide (data not shown) to that produced by $\gamma \delta$ -9613, supporting the orientation of transcription deduced from the distribution of $\gamma\delta$ inserts which inactivate the uvrCgene and the 35,000-MW truncated product from $\gamma \delta$ -9613.

To estimate the approximate number of uvrC polypeptide chains synthesized in wild-type $E.\ coli$, we compared the densitometric intensity of the uvrC and ssb bands from the labelled protein products of a $uvrC^+\ ssb^+\ uvrA^+$ composite plasmid $(pGHY4610)^9$. After labelling maxi-cell extracts of pGHY4610 ($uvrA^+\ ssb^+\ uvrC^+$) with ^{14}C -amino acid mixtures consisting of 20 amino acids present in similar specific activity, the labelled products were visualized by SDS-polyacrylamide gel electrophoresis and fluorography as described above. Autoradiographic films were exposed for various lengths of time and densitometric scans made. A comparison of the ssb^+ band, present in 1,200 polypeptide chains per $E.\ coli\ cell^{22}$, with the $uvrC^+$ band, indicates that uvrC may be present in as few as 10 copies per cell (Fig. 3).

The *uvrC*⁺ protein of *E. coli* is identified as a 68,000-MW protein on SDS-polyacrylamide gels. This protein is required for

ATP-dependent incision of DNA containing pyrimidine dimers⁷ and psoralen cross-links²³, as well as incision due to the presence of several other types of DNA damage in *E. coli*. Seeberg^{7.8} reported that $uvrB^+/C^+$ -complementation activity can be detected from Sephadex gel columns corresponding to \sim 70,000 MW—this agrees well with our observation that uvrC encodes a 68,000-MW protein on SDS gels. Previous attempts

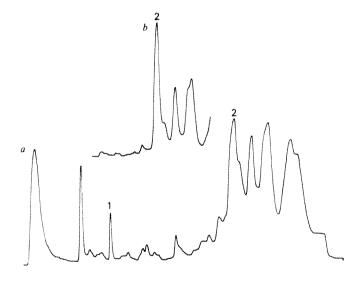


Fig. 3 Densitometric comparison of 14 C-labelled uvrC and ssb protein bands after SDS-polyacrylamide gel electrophoresis of extracts from $uvrC^+$ ssb^+ $uvrA^+$ pGHY4610 (ref. 9). The tracing shown in a includes the entire gel; the film was exposed for 8 days. The uvrC protein band is indicated by 1, and ssb by 2. As the ssb band is overexposed on this film (a), a second film was exposed to this gel for 1.6 days; the result is shown in b. The uvrC peak (1) in a was compared with the ssb peak (2) in b for this estimate. This comparison was based on the area of each peak, the relative size of uvrC and ssb polypeptides, and the relative time of exposure for each film. As ssb is present in 1,200 copies per cell²² and its molecular weight is 18,500 (ref. 22), these data provide an estimate of \sim 10 uvrC polypeptide chains per log-phase E. coli cell, assuming that there is a similar ratio of expression in vivo and in maxi-cells for uvrC and ssb.

to determine the mechanism of dimer-specific endonucleases in E. coli have been hampered by the twofold problem of identification of the elements of a 3-gene system (that is, uvrA, uvrB and uvrC) and the presence of very low amounts of UV dimer-specific activity. Our identification of the uvrC protein as a product from a multicopy plasmid provides a means to purify uvrC protein in sufficient quantities to allow its characterization as a component of the 3-gene product system required for incision of UV light-irradiated DNA.

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- Received 6 January; accepted 26 March 1981.
- Grossman, L., Braun, A., Feldberg, R. & Malher, I. A. Rev. Biochem. 44, 19-43 (1975). Haseltine, W. A. et al. Nature 285, 634-641 (1980).

- Haseltine, W. A. et al. Nature 285, 634-641 (1980).
 Radany, E. H. & Friedberg, E. C. Nature 286, 182-185 (1980).
 Howard-Flanders, P., Boyce, R. P. & Theriot, L. Genetics 53, 1119-1136 (1966).
 Seeberg, E., Nissen-Meyer, J. & Strike, P. Nature 263, 524-526 (1976).
 Braun, A. & Grossman, L. Proc. natn. Acad. Sci. U.S.A. 71, 1838-1842 (1974).
 Seeberg, E. Proc. natn. Acad. Sci. U.S.A. 75, 2569-2257 (1978).
 Seeberg, E. in DNA Repair Mechanism (eds Hanawalt, P. C., Friedberg, E. D. & Fox, C. F.) Seeberg, E. in DIVA Repair Internation (eds Handward, 17.5), Frieddarg, E. 225–228 (Academic, New York, 1978).
 Yoakum, G. H., Kushner, S. & Grossman, L. Gene 12, 243–248 (1980).
 Guyer, M. S. J. molec. Biol. 126, 347–365 (1978).
 Sancar, A., Hack, A. M. & Rupp, W. D. J. Bact. 137, 692–693 (1979).

- Dugan, G. & Sherratt, D. Molec. gen. Genet. 151, 151-160 (1977).
 Dugan, G., Saul, M., Warren, G. & Sherratt, D. Molec. gen. Genet. 158, 325-327 (1978).
 Pannekoek, H., Noordermeer, I. & Van de Putte, P. J. Bact. 139, 54-63 (1979).
- Pannekoek, H., Hille, J. & Noodermeer, I. Gene 12, 51-61 (1980).
 - Sancar, A. et al. J. molec. Biol. (in the press).
- Sancar, A., Clarke, N. D., Griswold, J., Kennedy, W. J. & Rupp, W. D. J. molec. Biol. (in the
- 18. Kushner, S. R. in Genetic Engineering (eds Boyer, H. W. & Nicosia, S.) 17-23 (Eisevier, Amsterdam, 1978).
- Birnboim, H. C. & Doly, J. Nucleic Acids Res. 7, 1513–1523 (1979).
 Bolivar, F., Rodriguez, R. L., Betlach, M. C. & Boyer, H. W. Gene 2, 75–93 (1977).
 Laemmli, U. Nature 227, 680–685 (1970).
- Weiner, J. H., Bertsch, L. L. & Kornberg, A. J. biol. Chem. 250, 1972-1980 (1975).
- 23. Yoakum, G. H. & Cole, R. J. biol. Chem. 252, 7023-7030 (1977).

Positively activated transcription of λ integrase gene initiates with UTP in vivo

U. Schmeissner*, D. Court*, K. McKenney† & M. Rosenberg†

*Laboratory of Molecular Biology and †Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Bacteriophage & requires two viral gene products for lysogenic development-repressor (cI) and integrase (int). Although the genes for these two proteins are located in distinct operons. separated by 9,000 base pairs (bp) of the phage genome, their expression is coordinated by the positive regulation of transcription mediated by another phage-encoded protein, cII (see ref. 1 for review). We have already identified the promoter signal for the establishment of repressor synthesis (PE) and demonstrated that cII protein is required for the initiation of transcription at this site. Here we determine the precise in vivo start site for int mRNA and demonstrate that transcription from this site is also cII-dependent. This start site defines the int promoter P₁. Atypically, transcription from P₁ initiates with UTP. Comparison of the nucleotide sequences of the P_i and P_E promoters reveals a striking homology in identical positions between 26 and 39 bp upstream of their respective start sites.

Our objective was to describe the mechanism of transcription in the xis-int region of phage λ and to study the effect thereon of cII expression (see ref. 2). A 417-bp \(\lambda \) DNA fragment, extending from 280 bp upstream of the xis coding sequence to include 150 bp of the xis gene as far as the structural int gene³ purified and inserted into the plasmid pBR322 (Fig. 1). The DNA of the recombinant plasmid (pUS3) was used to purify RNA made from this region during phage infection by a singlestep hybridization procedure (see Fig. 2 legend and ref. 2). RNA was labelled by exposing cells to ³²P-orthophosphate between 10 and 11.5 min after infection—an interval chosen to minimize degradation of the mRNA. This specific RNA (Fig. 2) was characterized by T1 ribonuclease digestion and separation of the resulting oligonucleotides by a two-dimensional fingerprinting technique⁶. The T1 fingerprints were prepared from RNA isolated from cells infected with either $\lambda c \text{II}^-$ (Fig. 2a) or $\lambda c \text{II}^+$ (Fig. 2b). The major (numbered) oligonucleotides were further digested with pancreatic ribonuclease and the products separated by one-dimensional electrophoresis on DEAE paper at pH 3.5 (ref. 6). The results of these analyses allowed us to determine the composition of each T1 oligonucleotide and to position it unambiguously within the known DNA sequence3 of the 417-bp fragment (Fig. 3).

RNA from $\lambda c II^-$ infection yielded equimolar amounts of oligonucleotides spanning the entire fragment (Fig. 3, oligonucleotides 1-9 and 11-17), indicating uniform transcription throughout the region; transcription probably originates at the upstream promoter P₁ (Fig. 1). In contrast, for RNA from λc II infection, the intensity of oligonucleotides 11-17 was increased some eightfold, whereas the intensity of oligonucleotides 1-9

Fig. 1 Partial genetic map of phage λ. Indicated are the two cII-dependent promoters P_E (responsible for the establishment of repressor synthesis) and P_I (responsible for the production of integrase). Other λ promoters (P_L, P_R, P_O, P_M) also shown, as is the direction of transcription from these sites (wavy arrows). The 417-bp DNA restriction fragment shown below the map was inserted into the plasmid pBR322 by blunt-end ligation. The resultant plasmid (pUS3) was used in the hybridization experiments as described in the text.

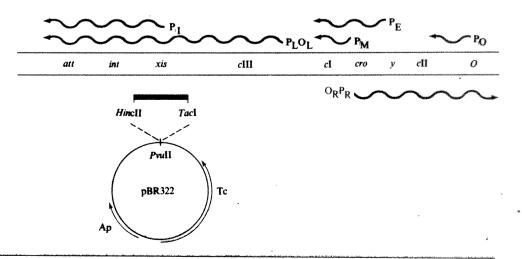
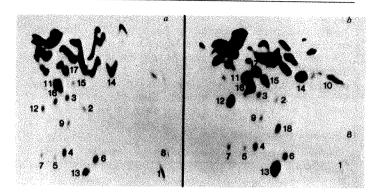
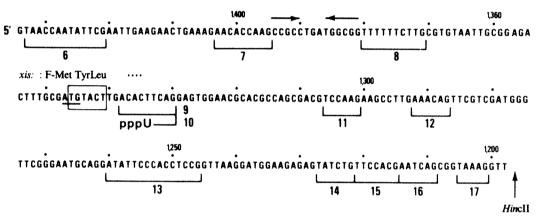


Fig. 2 Two-dimensional fingerprints of ribonuclease TI oligonucleotides derived from RNA transcribed in vivo from the xis-int region of phage λ . Fingerprints were prepared from RNA isolated after infection of Escherichia coli K12 SA500 (his, rpsL) with $\lambda c I_{14} c II_{28} c III_{611}$ (a) or $\lambda c I_{14}$ (b)¹¹. Total RNA was extracted as described by Court et al.¹² and hybridized to plasmid pUS3 DNA bound to filters. Hybridizations were carried out at 67 °C for 6 h as described elsewhere². RNA purified by this single-step hybridization procedure was digested with ribonuclease T1 and the products separated by standard fingerprinting techniques. Horizontal dimension, electrophoresis on Cellogel strips at pH 3.5. Vertical dimension, homochromatography on thin-layer plates of DEAE-cellulose using Homomix C. The numbered oligonucleotides in each fingerprint were further analysed by digestion with pancreatic ribonuclease⁶. The results of these analyses are summarized in Fig. 3. In addition, oligonucleotide no. 10 was treated with nuclease P.



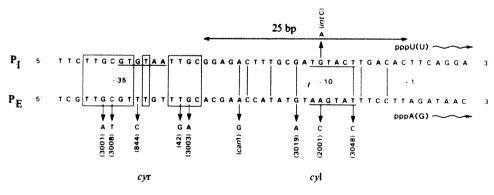
and the resulting mononucleoside-5' phosphates fractionated by two-dimensional chromatography². The results (not shown) indicate that this oligonucleotide contains the triphosphate, identified as pppU. Oligonucleotides 1 to 5 are not shown in Fig. 3; these correlate with the DNA sequence 5' to that shown in Fig. 3 (that is, beyond residue 1,434). Their sequences are: 1, AU₃AU₄AUCUG; 2, AUUA₃UG; 3, CACUUCCG; 4, CCAUUCAACAG; 5, AACAUCAACG. Oligonucleotide no. 18 has the sequence ACUUA₃UCG and derives from the region immediately 3' to the DNA fragment used for hybridization. The presence of this oligonucleotide probably results from the mild ribonuclease trimming conditions used to preserve the 5' end of the cII-dependent transcript during the isolation of the RNA-DNA hybrids.

Fig. 3 Nucleotide sequence of the P_I promoter region³⁻⁵. Only the coding strand of the DNA is shown. The orientation of the sequence is inverted relative to the position of λ genes indicated in Fig. 1. The numbering of the nucleotide positions starts in the centre of the λ attachment site⁴. Numbered brackets designate the responding oligonucleotides in the RNA fingerprints of Fig. 2. The 5' end of the RNA is indicated by pppU, and the -10



region of the promoter is boxed. Note that the transcriptional start point of the *int* mRNA lies within the *xis* coding region. This is consistent with the finding that the expression of xis is not controlled by c II (ref. 13). The arrows define a region of dyad symmetry, which is followed by the sequence T_6C . Such a structure is common to sites signalling termination of transcription 14 . However, the relative molar yields of oligonucleotides 7, 8 and 9 indicate that the transcription does not terminate at this site during our analyses.

Fig. 4 Comparison of the nucleotide sequence of the P₁ and P_E promoters. Only the coding strands of the DNA are shown. The sequences are aligned with respect to the cII-dependent transcriptional start sites used in vivo. The nucleotides in the -10 region of both promoters and in the -35 region of the P₁ promoter, which show some homology with the conserved sequences found in other promoters for E. coli RNA polymerase¹⁴, are underlined. The boxed regions indicate 11 of 14 bp which are identical in both promoters. The various



cyr and cyl mutations which eliminate P_E promoter function are indicated, as in the can mutation which alters the second codon of the cII structural gene but has no effect on P_E promoter function. Also shown is the int mutation which occurs in the -10 region of the P_I promoter and results in cII-independent, constitutive expression of gene int.

was similar to that from $\lambda c II^-$ infection. In addition, a new oligonucleotide (no. 10, Figs 2, 3) was identified as the pentamer pppUUCAG. These results indicate that in the presence of c II, a new transcript starts at a site located 183 bp before the translation initiation codon of *int* and 13 bp downstream of the AUG initiation codon of *xis* (at position 1,334 in Fig. 3 as numbered from the centre of the core of the *att* site⁴). This c II-dependent RNA accounts for over 80% of the *int* gene transcripts made during the pulse-labelling interval.

Identification of the 5' end of this RNA defines the c II-activated P_1 promoter.

Note that the P_I transcript initiates with UTP. This is the first in vivo demonstration of the use of UTP by RNA polymerase to initiate transcription. Recently, it has been shown that purified cII protein causes RNA polymerase to initiate transcription in vitro at the same UTP start. This demonstrates that the positive activation observed at P_I in vitro accurately represents the in vivo situation.

Hoess et al.4 and Abraham et al.5 have determined the base change associated with the mutation intC (Fig. 4) that allows cII-independent, constitutive expression of int. More recent work8 indicates that the intC mutation also allows RNA polymerase to initiate transcription in vitro from the same UTP start as demonstrated here. Our finding confirms the earliest suggestion that intC is a promoter-up mutation affecting the -10 region of the P_I signal^{4,5,8}.

We have compared the nucleotide sequences of P_E and P_I by aligning them with respect to their in vivo start sites. Homology in 11 of 14 bp is observed at identical positions in the -35 region of both promoters. This finding further supports the proposal^{2,8,9} that this region of the promoter is involved in the positive activation mechanism, perhaps as a site of interaction with protein cII.

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- Herskowitz, I. & Hagen D. A. Rev. Genet. 14, 399-445 (1980). Schmeissner, U., Court, D., Shimatake, H. & Rosenberg, M. Proc. natn. Acad. Sci. U.S.A. 77, 3191-3195 (1980).
- Davies, R. W. Nucleic Acids Res. 8, 1765-1782 (1980)
- 4. Hoess, R. H., Foeller, C., Bidwell, K. & Landy, A. Proc. natn. Acad. Sci. U.S.A. 77, 2482-2486 (1980).
- Abraham, J. et al. Proc. natn. Acad. Sci. U.S.A. 77, 2477-2481 (1980).
 Barrell, B. G. in Procedures in Nucleic Acids Research Vol. 2 (eds Cantoni, G. & Davies, D.) 751-779 (Harper & Row, New York, 1971).
- Shimatake, H. & Rosenberg, M. Nature 292, 128-132 (1981)
- Abraham, J. & Echols, H. J. molec. Biol. 146, 157-165 (1981). Wulff, D. B. et al. J. molec. Biol. 138, 209-230 (1980).
- Hershey, A. D. (ed.) The Bacteriophage Lambda (Cold Spring Harbor Laboratory, New York, 1971).
- Court, D., Green, L. & Echols, H. Virology 63, 484–491 (1975).
 Court, D. et al. J. molec. Biol. 138, 231–254 (1980).

- Chung, S. & Echols, H. Virology 78, 312-319 (1977).
 Rosenberg, M. & Court, D. A. Rev. Genet. 13, 319-353 (1979).
 Jones, M. O. & Herskowitz, I. Virology 88, 199-212 (1978).
 Shimada, K. & Campbell, A. Proc. natn. Acad. Sci. U.S.A. 71, 327-241 (1974).

Heterogeneous host DNA attached to the left end of mature bacteriophage Mu DNA

Marie George & Ahmad I. Bukhari

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

Bacteriophage Mu, a highly efficient transposon during its lytic cycle in Escherichia coli, is always associated with the host DNA1. In mature Mu particles, both ends of Mu are heterogeneous in length and sequence owing to the presence of host DNA²⁻⁶. The right end contains host sequences which vary between 500 and 3,200 base pairs (bp), while the left end heterogeneity was proposed to correspond to ~50-100 bp. To understand the mechanism by which a small amount of host DNA at the left end is packaged along with Mu DNA, we have now determined the precise lengths of these host sequences. The minimum size of the host sequences attached to the left end of the mature bacteriophage Mu DNA is 56 bp; host sequences longer than 144 bp are rare. The host sequences do not show a continuum from 56 to 144 bp but are rather packaged in discrete blocks. The first such block is 56-61 bp, the second is 67-72 bp and so on. Thus, each block represents fragments covering a 5-6-bp range, with a space of 5 bp between each block. It seems that Mu DNA is being measured in units of helical turns for packaging.

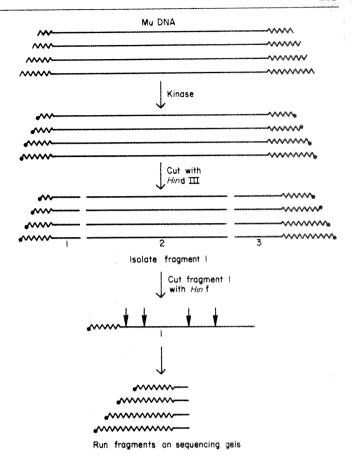


Fig. 1 The experimental design. A single line represents doublestranded Mu DNA. Wavy lines represent host DNA (not drawn to scale). Solid circles represent ³²PO₄ groups at the 5' ends of the DNA strands.

The ends of Mu DNA were labelled with ³²P using [y-³²P]ATP and polynucleotide kinase. Mu DNA was then digested with HindIII, which makes two cuts in the DNA, giving rise to three fragments. The 1,000-bp fragment containing the left end of Mu was isolated and cut with Hinf, which makes four cuts in the fragment, one of which is 7 bp from the left end of Mu. The digest was then run on a sequencing gel to size the molecules. The experimental design is shown in Fig. 1.

Figure 2 shows the results of the experiment. The smallest fragment obtained is 63 bp. As Hinf cuts 7 bp away from the Mu end, the minimum size of the host sequences at the left end must be 56 bp. As the largest fragments are in the range of 150 bp, the maximum size of the host sequences is ~144 bp. However, smaller fragments are much more frequent, and only a small minority of the fragments are larger than 100 bp, while fragments larger than 150 bp are extremely rare. The most striking feature of the size of the host sequences is that they are not present as a continuum from 56 to 144 bp, but rather appear in discrete blocks. Moreover, there is a regular pattern in which these blocks appear: the first block is from 63 to 68 bp; a gap of 5 bp is then seen, after which there is another block from 74 to 79. There are 10 such blocks, each one separated from the next by a 5-bp space, and each containing fragments covering a 6-bp range. This means that DNA cutting begins with a regular spacing of 5 bp but cutting can cover any of the next 5-6 bp. Analysis of the end nucleotides, by labelling of the end and analysis of the 5' nucleotide, showed that all four bases are present in equal amounts at both the left and right ends.

Two general modes of packaging of DNA into viruses have been recognized^{8,9}. One is site-specific packaging with some size constraints, such as the recognition of the ter sites of λ for packaging, and the other is the headful packaging, in which the size of DNA seems to have the predominant role. Packaging by a

headful mechanism was first proposed by Streisinger et al. 10 for phage T4, and has been shown to be a common mode of DNA packaging 5,11,12. However, the molecular mechanism of headful packaging is unclear. In context with Mu packaging, A.I.B. et al.3 discussed two alternative ways in which a small amount of host DNA can be packaged at the left end. In one mechanism, a specific site at the left end is recognized by a DNA cutting protein, the DNA is then cut to the left of the site randomly, and the cleaved DNA condensed into the heads being assembled. In

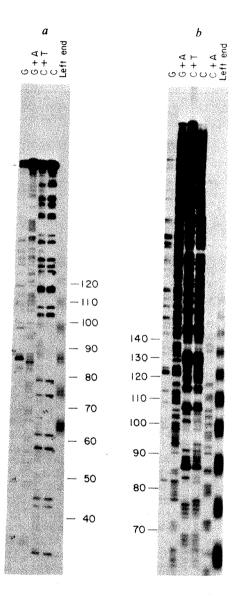


Fig. 2 Determination of the lengths of the left-end host sequences by electrophoresis in sequencing gels. Bacteriophage Mu particles were purified by a caesium chloride density gradient centrifugation. The DNA was extracted with phenol. To label the ends, the 5' phosphates were first removed by treating the DNA with alkaline phosphatase¹⁵. The ends were labelled using $[\gamma^{-32}P]ATP$ (>2,000 Ci mmol⁻¹) and polynucleotide kinase. The DNA was then cut with *HindIII* and the digest run on a 6% 1:40 acrylamide gel. The 1,000-bp left-end fragment was isolated¹⁵ and cut with Hinf. The digest (left end) was run on 8% polyacrylamide gels containing 7 M urea, together with DNA samples that were being sequenced; a and b are autoradiograms of 8% sequencing gels run for different times. The xylene cyanol dye marker was threequarters of the way down (a) or off (b) the gel. Portions of double-stranded DNA, labelled with ^{32}P at one 5' end, were partially cleaved at guanines (G), guanines and adenines (G+A), cytosines and thymines (C+T), cytosines (C), and cytosines and adenines (C+A), respectively, using the method of Maxam and Gilbert¹⁵. The numbers indicate the size of the fragments as seen on the sequencing gels.

the second mechanism, a packaging protein recognizes a specific sequence at the left end, the DNA is then folded and perhaps pushed into the heads being assembled, after which the DNA is cut at the left and right end.

It has been observed that the phage tail is attached at the right end of Mu^{13,14}; presumably, the right end is the last to be packaged, and the first to be ejected, suggesting that the left and right ends are being cut in different time and space. However, the minimum size of the host DNA at the left end is ~ 56 bp. It is difficult to reconcile this observation with the first mechanism in which the first event is cutting of the left end by an enzyme behaving like a type I restriction enzyme. The enzyme would have to recognize a site on the left end but start cutting only after 56 bp. As a working hypothesis, we favour the idea that the DNA is rolled or packaged first, in such a way that the first 56 host base pairs at the left end are not available for cutting. This could either mean that the left end is condensed first, and cut, after which the rest of the DNA is packaged in, or that the whole genome is packaged, and then the left and right end cuts are made.

How can we explain the regular spacing of the cuts? Note that the distance from the beginning of one block of 6 bp to the beginning of the second block is ~11 bp, which is about one turn of the double helix. It seems that the DNA is being measured for packaging, in units of helical turns. This measurement could be brought about by proteins that bind at each turn of the helix. The proteins perhaps cover one half of the turn so that only the other half is available for cleavage.

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- Bukhari, A. I. A. Rev. Genet. 10, 389-411 (1976).

- Bukhari, A. I. A. Rev. Genet. 10, 389-411 (1976).
 Daniell, E., Kohne, D. E. & Abelson, J. J. Virol. 15, 739-743 (1975).
 Bukhari, A. I., Froshauer, S. & Botchan, M. Nature 264, 580-583 (1976).
 Daniell, E., Abelson, J., Kim, J. S. & Davidson, N. Virology 51, 237-239 (1973).
 Bukhari, A. I. & Taylor, A. L. Proc. natn. Acad. Sci. U.S.A. 72, 4399-4403 (1975).
 Chow, L. T. & Bukhari, A. I. in DNA Insertion Elements, Plasmids, and Episomes (eds.) Bukhari, A. I., Shapiro, J. A. & Adhya, S. L.) 295-306 (Cold Spring Harbor Laboratory, New York, 1977).
- Allet, B. & Bukhari, A. I. J. molec. Biol. 92, 529-540 (1975). Murialdo, H. & Becker, A. Microbiol. Rev. 42, 529-576 (1978).

- Earnshaw, W. C. & Casjens, S. R. Cell 21, 319–331 (1980). Streisinger, G., Emrich, J. & Stahl, M. M. Proc. natn. Acad. Sci. U.S.A. 57, 292–295 (1967).

- Stefsniger, G., Edirich, J. & Stafi, M. M. Proc. Rath. Acad. Sci. U.S.A. 57, 292-295 (1967).
 Tye, B. K., Huberman, J. A. & Botstein, D. J. molec. Biol. 85, 501-532 (1974).
 Gill, G. S. & MacHattie, L. A. J. molec. Biol. 104, 505-515 (1976).
 Inman, R. B., Schnös, M. & Howe, M. Virology 72, 393-401 (1976).
 Breepoel, H., Hoogendorp, J., Mellema, J. E. & Wijffelman, C. Virology 74, 279-286.
- 15. Maxam, A. M. & Gilbert, W. Meth. Enzym. 65, 499-560 (1980).

Errata

In the article 'IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts' by P. J. Gearhart et al., Nature 291, 29-34 (1981), Figures 1 and 2 were transposed.

In the letter 'Somatic and behavioural postnatal effects of fetal injections of nerve growth factor antibodies in the rat' by L. Aloe et al., Nature 291, 413 (1981), the received date was given as 7 November 1980. The paper was originally submitted on 7 November 1979; the date of receipt of the revised manuscript was 17 November 1980.

The cover caption for issue no. 5813 (28 May-4 June) of Nature was incorrect. The correct version from the authors is 'Homozygous (pigmented and unpigmented) progeny from heat-shocked eggs of heterozygous mothers'.

ATTERS ARISING

Sensory cues invalidate remote viewing experiments

TART, Puthoff and Targ1 have recently responded to our critical analysis^{2,3} of their evidence in favour of extrasensory remote viewing ability by reporting a rejudging of the original transcripts with all sensory cues removed. This yielded a high correlation between Price's descriptions and the target information. Furthermore. Tart et al. claim that the Marks-Kammann cueing explanation of remote viewing does not apply in principle to any of the replication experiments carried out after the series with Price.

The validity of the re-judging exercise for the Price series can be disputed on two counts. First, it should be noted that the editing of transcripts was carried out by one of the investigators (Tart). As Tart was himself aware of the correct targettranscript pairings, this could have led to biasing. Second, it is not permissible to include material for re-judging which has already been published or which may be available in some other form. A so-called 'blind' judge may have some memory of previously seen target-transcript matchings or have access to the published material. Only five of the series of nine targets and transcripts for which there is no normal or available method of matching except perceived similarity, could validly be used in re-judging. The remaining four transcripts should have been excluded, as in the unsuccessful rejudging exercise reported by us².

A much more serious problem with the response of Tart et al.1 to our report is their claim that sensory cues were not present in later experiments. Unfortunately, I have been unable to obtain a complete set of transcripts from SRI investigators despite frequent requests. However, in June 1977, Dr Arthur Hastings, who was a consultant to the SRI investigations responsible for judging experimental transcripts, allowed me to see six transcripts from the series with the subject H. Hammid, and I was far from satisfied with them as they contained sensory cues. A listing of targets in Hastings' possession correlating 0.83 (P < 0.01) with the order of target usage, together with transcript cues, would have provided an artefactual basis for correct target-transcript matchings.

Although Tart et al.1 conclude that SRI replication studies confirm the remote viewing hypothesis, serious methodological flaws throughout the experiments prohibit any such conclusion. The Targ-Puthoff researches conform to a long history in parapsychology of methodological flaws and mistaken conclusions. Unless proper controls and methods are used by impartial observers, the search for

scientific proof of paranormal and spiritual beliefs remains a futile enterprise.

DAVID MARKS

Department of Psychology, University of Otago, Dunedin, New Zealand

- 1. Tart, C. T., Puthoff, H. E. & Targ, R. Nature 284, 191
- 2. Marks, D. & Kammann, R. Nature 274, 680-681 (1978). Marks, D. & Kammann, R. The Psychology of the Psychic (Prometheus, New York, 1980).

Micrograzers may affect macroalgal density

THE EFFECT of density on plant growth and mortality has been recently discussed by Schiel and Choat1. Studying two species of large brown seaweed, Ecklonia radiata and Sargassum sinclairii, they found that high density had a positive effect, in contrast to data for terrestrial plants. They concluded that density affects marine and terrestrial plants differently, and suggested that these differences were due to protection from wave shock and the difference in plant-arthropod associations between terrestrial and marine environments. Uninjured brown algal thalli 1,700 contained up to copepods, amphipods and isopods.

Terrestrial plant density has a limiting effect on nutrient and water supplies to the individual plant that would not be expected for algal density, but we believe that the data for Sargassum and Ecklonia communities1 are exceptional and subject to reinterpretation. Specifically, conclusions on the general effect of density on marine plants should await data from algae typical of less exposed communities.

Our work on amphipod grazing in the field and in the Smithsonian Institution's coral reef microcosm demonstrates that coarser algae (for example, Hypnea) are protected from amphipod grazing by their size, but most filamentous species are heavily grazed². By eliminating epiphytes on coarser algae, amphipods increase growth rates of macroalgae such as Hypnea by as much as 300%. In subtidal areas, amphipod densities are kept low by fish predation³, but in situations where this is reduced by factors such as turbulence, micrograzer herbivory may be particularly important. Such high energy areas are characteristic habitats for brown algal macrophytes. Up to a point, densely growing Ecklonia and Sargassum plants would shelter more amphipods from predation. This would contribute to a positive effect of high density on these seaweeds, while being equivalent to a locust attack in its effect on many other algal species.

Schiel & Choat suggest that the study of marine plant-arthropod relationships could have important implications for mariculture. In fact, our work on amphipod grazing implies that maintenance of some amphipod species in mariculture facilities could increase yields significantly.

> SUSAN H. BRAWLEY WALTER H. ADEY

Marine Systems Laboratory, W-310, Department of Paleobiology, Smithsonian Institution. Washington DC 20560, USA

- Schiel, D. R. & Choat J. H. Nature 285, 324-326 (1980).
 Brawley, S. H. & Adey, W. H. Mar. Biol. 61, 167-177
- 3. Young, D. K. & Young, M. W. J. mar. Res. 36, 569-593

SCHIEL AND CHOAT REPLY-Our recent paper1 reported two of our findings for large marine algae: (1) individual plants in dense, monospecific stands tend to be larger compared with plants of the same age in sparser stands, and (2) the number of crustagea in algal fronds may be quite high with no apparent adverse effect on algal plants. We contrasted this to terrestrial plant systems, where the opposite seems to apply in both cases. These observations were put forward as hypotheses worthy of further testing.

An experimental demonstration of a link between fish predators, frond-dwelling crustacea and the occurrence of epiphytes on large brown algal plants has proved largely intractable in field situations. Data on the comparisons of arthropod loads between densities and exposures of plants, the fish effect on arthropod loads, and the effects of arthropods on host plants are either lacking altogether or equivocal, particularly over large areas generally. Brawley and Adey² have provided evidence in their coral reef microcosm that such a link exists, and that high numbers of crustacea may be of benefit to larger algae by reducing epiphytism. Their results do not negate our hypotheses. Our argument admits the likelihood of a beneficial effect on plants, or no effect at all, due to crustacean loads. However, before a general case is made more data are required concerning: (1) the general effects of density on growth, survival and size of marine plants; (2) the effects of crustacea on marine plants, and (3) the effects of fish predators on crustacea. Brawley and Adey have provided some information. We welcome further testing of the hypotheses we have put forward.

> DAVID R. SCHIEL J. H. CHOAT

Department of Zoology and Leigh Marine Research Laboratory, University of Auckland, Leigh, New Zealand

- 1. Schiel, D. R. & Choat, J. H. Nature 285, 324-326 (1980).
- 2. Brawley, S. H. & Adey, W. H. Mar. Biol. (in the press).

The glass bead game

THE gap between theoretical and applied ecology seems to be widening. Indeed it is difficult to escape the worrying conclusion that some theoreticians are playing a version of Hermann Hesse's Das Glasperlen Spiel1 or that they have little feeling for or understanding of biological problems. A particularly good example is the recent article by Gurney et al.2 which attempts to provide a theoretical model to explain the oscillatory behaviour of laboratory populations of blowflies.

After reaching the esoteric conclusion that the blowfly cycles are "self-sustaining limit cycles" rather than "driven quasicycles", the authors proceed to use their model to explain the 'double-humped' nature of the cycles in terms of minimum population size (N_{\min}) in relation to the size at which the population achieves maximum reproductive success (N_0) . The mathematics are correct, but in their zeal, the authors fail to notice Nicholson's own explanation of the 'double-hump' phenomenon (see Fig. 3 legend in ref. 3). He says "the lack of a clear inverse relation between the various low adult densities and the number of eggs produced is due to the fact that adults are mostly senile as the adult minima are approached, near the minima many are newly emerged and incapable of laying eggs, and subsequently highly fertile young individuals dominate". In other words, the fact that breeding occurred in 'quasi-discrete' generations is probably almost entirely a consequence of age-specific variation in the reproductive performance of the adult blowflies. As Gurney et al. ignore this variation their model must be seen as artefactual and spurious—a product of the 'game'.

Of less importance, but still worrying, is the promotion of "a satisfying qualitative fit" in the conclusions to "good quantitative agreement" in the abstract, and the failure to refer to similar published work^{4,5}. In contrast, Readshaw and Cuff⁶ have published a biologically realistic model of Nicholson's results which includes readily identifiable parameters. An age-specific version of the model would undoubtedly simulate the 'doublehump' but the data are not yet available. J. L. READSHAW

CSIRO, Division of Entomology, PO Box 1700, Canberra City, ACT 2601,

Australia

1. Hesse, H. Glass Bead Game (Penguin, London, 1972). Gurney, W. S. C., Blythe, S. P. & Nisbet, R. M. Nature 287, 17–21 (1980).

- Nicholson, A. J. Aust. J. Zool. 2, 9-65 (1954).
- Oster, G. Lect. appl. Math. 16, 149-90 (1977). Oster, G. & Ipaktchi, A. Theoretical Chemistry Vol. 4 (eds Evring, H. & Henderson, D.) 111-132 (Academic, New York, 1978)
- 6. Readshaw, J. L. & Cuff, W. R. J. Anim. Ecol. 49, 1005-1010 (1980)

GURNEY ET AL. REPLY-Readshaw raises three scientific objections to the model described in our recent article1. We shall deal with these in turn.

The question of the nature of the mechanism responsible for the observed cycles is very far from 'esoteric'. Any moderately repetitive fine structure exhibited by a limit-cycle type of fluctuation carries readily extractable dynamic information, whereas the fine structure of a driven quasi-cycle is mainly 'noise' which only serves to obscure our view of the underlying population dynamic. Thus our judgement that detailed investigation of the fine structure of the cycles observed by Nicholson is a worthwhile exercise hinges on our unambiguous demonstration that they are limit cycles.

It is clear from our work that if average future recruitment bears any kind of humped relationship to current adult population then cycles which have minima well below the population size at which maximum overall reproductive success is

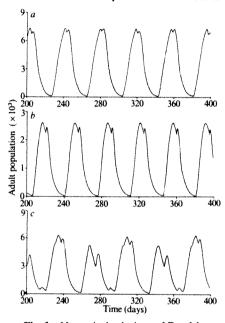


Fig. 1 Numerical solutions of Readshaw and Cuff's model equation⁴: n(t+1) = $0.8n(t) + R(n(t-\tau))$. a, Adult food-limited case: R = 10n when n < 171; R =1,795-0.503n when 171 < n < 3,569; R = 0 when n > 3,569. $\tau = 15$ days. b, Larval food-limited case: R = 10n(1- $\exp \{0.154-109.9/n\}$) when n < 714; R =0 when n > 714. $\tau = 13$ days. c, Adult food-limited case with modified parameters: R = 2.49n when n < 600; R =1,795-0.503n when 600 < n < 3569; R =0 when n > 3.569. $\tau = 15$ days.

achieved, must be accompanied by a 'discrete generation' pattern of breeding activity. A clear implication of Nicholson's^{2,3} batch culture results displayed in Readshaw and Cuff's paper4 is that just such a relationship exists for Lucilia cuprina, and there is thus no shred of evidence for their ex cathedra statement that the double-humped egg-laying rate curves observed by Nicholson are entirely the product of the age structure-dependent fecundity changes noted in Fig. 3 legend of ref. 3. However, such effects do provide a very plausible explanation of the observation that in four out of seven cycles shown the second peak of the double hump is considerably higher than the first.

The final objection of Readshaw is the claim that the fine structure predicted by our model must be 'spurious and artefactual' because their 'biologically reasonable' model predicts a limit cycle with no fine structure. This claim has no sound basis. Their model is effectively identical to ours except in the details of the functional form chosen for the recruitment rate function. In both experimental regimes considered the form chosen has a single hump with a maximum at a population size (N_0) comfortably in excess of the observed minimum population and thus there seems every reason to suppose that careful numerical analysis will reveal that their model predicts population cycles with a fine structure very similar to that shown in Fig. 6 of our paper¹. Figure 1a, b shows that this is indeed the case. Furthermore, in the adult food-limited case (Fig. 1a) note that there is no direct experimental evidence for the value of N_0 implied by the parameters chosen by Readshaw and Cuff (171) and indeed that this value is considerably below the value of 600 that may be deduced from Nicholson's data (see Fig. 3 of ref. 3). If we abandon the attempt to force Readshaw and Cuff's piecewise linear approximation to the recruitment function to fit the behaviour of the population as $N \rightarrow 0$ and instead place the maximum of the curve somewhere near the correct value (Fig. 1c), then the structure predicted by their model becomes very strong indeed.

We conclude that the very simple mechanism proposed in our original article captures much of the spirit of the population dynamics underlying Nicholson's blowfly cycles and is thus a contribution to narrowing (rather than widening) the gap between theoretical and applied ecology. W. S. C. GURNEY

S. B. BLYTHE R. M. NISBET

Department of Applied Physics, University of Strathclyde, George Street, Glasgow G4 ONG, UK

- 1. Gurney, W. S. C., Blythe, S. P. & Nisbet, R. M. Nature 287, 17-21 (1980).
- Nicholson, A. J. Aust. J. Zool. 2, 1-8 (1954).
 Nicholson, A. J. Aust. J. Zool. 2, 9-65 (1954).
- Misiolison, A. J. Aust. J. 2001. 2, 9-05 (1954).
 Readshaw, J. L. & Cuff, W. R. J. Anim. Ecol. 49, 1005–1010 (1980).

Matters Arising

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BOOK REVIEWS

Unravelling the threads of cosmic history

James N. Fry, Craig Hogan and David N. Schramm

CLASSICAL physical cosmology, as exemplified by texts such as Weinberg's Gravitation and Cosmology (Wiley, 1972), deals with an idealized "smooth" Universe taken as a whole, its overall dynamics and mean composition. General relativity describes the universal expansion in an average sense, assuming that the material content is very nearly uniform. All standard calculations, such as the synthesis of primordial helium, rely on this background. Astronomers, on the other hand, have always studied discrete concentrations of matter, the galaxies for example, but until recently many regarded cosmology as a "study of the search for two numbers" - the expansion rate and the deceleration parameter of the smoothed substratum.

However, the detailed study of the distribution of galaxies, their clustering and their peculiar (non-Hubble) motions, forms a fascinating subject in its own right, which offers a great variety of problems, dynamical, statistical and observational, for both the astronomer and the physicist. Here there exists the possibility of unravelling some of the threads of cosmic history. Jim Peebles has written the first textbook to deal almost exclusively with what might more appropriately be described as the "in-between-scale structure of the Universe" - bigger than galaxies, but much smaller than the observable Universe.

On the very largest scales, above about 10 Mpc (30 million light years), the Universe is indeed nearly uniform, as is indicated by such diverse measures as the isotropic distribution of galaxies, faint radio sources, the X-ray background radiation, and especially the microwave background radiation, which of all these comes from farthest away and reflects the oldest parts of the Universe we can reach its last interaction with matter probably occurred when the Universe was about 1,000 times smaller than it is now. On smaller scales, less than 10 Mpc, the distribution of galaxies displays a rich structure, illustrated in the frontispiece of Peebles's book and also in a beautiful poster ("One Million Galaxies" published by Peebles and Stewart Brand of CoEvolution Quarterly). The structure can be quantified by statistical measures such as the galaxy correlation functions, and it is consistent with the model that galaxies are distributed in a sort of self-similar nested

The Large-Scale Structure of the Universe. By P. J. E. Peebles. Pp.416. ISBN hbk 0-691-08239-1; ISBN pbk 0-691-08240-5. (Princeton University Press: 1981.) Hbk \$30, £18.10; pbk \$9.95, £6.20.

hierarchy — galaxies are bound in groups which orbit each other in clusters and so on, up to scales of 3-10 Mpc where the largest aggregations blend smoothly into the uniform background.

From its gravitational effects on luminous galaxies, we now know that most of the mass of the Universe is dark. We cannot tell from these dynamical studies what the mass is made of, although there are some constraints from big bang nucleosynthesis on the total baryonic component. Depending in part on how the total cosmological mass density compares with these constraints on baryon density, the dark matter might be anything from swarms of invisible particles, such as neutrinos, to clusters of very dim, low mass stars, to black holes as large as a million solar masses each. Nevertheless, if the distribution of mass is similar to the visible galaxy distribution then natural mechanisms exist for creating a hierarchical structure. (One must be careful here, since there is some evidence that the dark matter may have a different spatial distribution from the light-emitting matter.) In one picture, as the Universe expands and cools, galaxies (with their dark counterparts) condense out of the primordial matter. Neighbouring galaxies gravitationally pull each other out of the expansion and start orbiting each other, forming groups; neighbouring groups approach each other, and so on. Opposed to this is an alternative view — that the first scales to condense are very large, about 10 Mpc, and that flattened gas clouds of this size ("pancakes") fragment into smaller ones, which break up into still smaller ones, and so on, finally reaching galaxy sizes. Both frameworks seem viable and are intuitively satisfying. It remains a fascinating enigma exactly how a nearly uniform expanding Universe produces the structure we see. This area of cosmology is at an interesting stage of development because, although the expanding Universe option is widely used, various detailed models (to explain the same observations) differ in almost all essential aspects. Fortunately, the differing predictions are on the verge of being tested, primarily by

observations of anisotropy in the microwave background, our best direct probe of the epoch just prior to galaxy formation.

Peebles's book supplements the standard textbook version of cosmology with the results of these structural studies. If you are active in research dealing with cosmological structure, you must have a copy, for it is the most complete source for finding out what has been done. However, for those with only casual interest, the book is much less effective. The approach is that of a pioneer in the field, recollecting calculations and lines of thought from over the years, and it is thus extremely valuable as a reference: but this does not make for ideal pedagogy. Except for the historical introduction, there are few pointers in the 400 pages toward which material is likely to remain important; current fashion and fundamentals are treated with almost equal weight. If you know specifically what you want to know, you can find it in this book - unlike Peebles's previous book, Physical Cosmology (Princeton University Press, 1973), it does have an index; every standard calculation is here (there are four frameworks for deriving the linear growth equations) and there is an extraordinarily complete list of references. But if you are an outsider and want to get a picture of what is going on, what is likely to remain relevant and what may be tossed out in the next ten years, you will probably be lost.

Peebles does not attempt to conceal his biases, and exercises the author's right to present a convincing case — with a fair assessment of the uncertainties. He emphasizes the idea of a "well-regulated", quiescent initial state replacing the formerly popular ideas of primordial chaos, turbulence, magnetic fields; the hierarchical clustering picture emerges as the one simple physical process which can make solid predictions about the galaxy distribution; the Universe contains a lot of unseen matter, possibly enough to make the daring statement $\Omega = 1$.

Despite the possible shortcomings, there is no question that the field has needed this book. It will undoubtedly become a standard graduate extragalactic astronomy textbook. Here is a collection of useful calculations which were not previously available in any one place (although some of the book overlaps with S. M. Fall's excellent review on correlation functions (Rev. Mod. Phys. 51, 21; 1979). Even

though there is no complete universal model, there is a great deal of standard lore—correlation functions, newtonian statistical dynamics, relativistic perturbation theory, scaling laws and so on. Peebles avoids the fundamental uncertainties by focusing on manageable physical problems

which can be solved to yield precise answers to specific questions. That is perhaps the best that anyone could do given our present state of ignorance.

The authors are at the Astronomy and Astrophysics Center of the University of Chicago.

Rabbie Burns and the art of genetics

Tony Searle

Genetics and Probability in Animal Breeding Experiments. By Earl L. Green. Pp.271. ISBN 0-333-27243-9/0-19-520159-0. (Macmillan Press, London/Oxford University Press, New York: 1981.) £20, \$36.95.

IT is well known that even "the best laid schemes o' mice an' men gang aft a-gley", so the need for this book is obvious. It is meant to minimize the "a-gley factor" where the two species undertake joint genetic enterprises, and it should fulfil its purpose if read as conscientiously as the lectures engendering it were doubtless listened to. They were given by Earl Green when Director of the Jackson Laboratory, to each year's new crop of staff members and postdoctoral fellows. No one who is familiar with the high standards of genetic

research and animal breeding at that laboratory can doubt their effectiveness.

Amidst all the marvels of somatic cell hybridization, probes, DNA sequencing and the like, the more humdrum procedures of constructing and maintaining inbred strains for particular purposes, introducing mutant genes into them, studying newly arisen mutants and finding where they belong in the genome must go on. In the past, the necessary expertise has had to be acquired piecemeal and often belatedly; now it is all gathered together in one volume. I could have done with such a vade-mecum 30 years ago, but it (and I) would have been slimmer then. Thus, there is now full coverage of how to produce and use recombinant inbred strains, which have proved so valuable in recent years, and of modern methods of linkage analysis. In fact, the chapters "Linkage, Recombination and Mapping" and "Mating Systems", with associated appendices (for example on maximum likelihood methods) take up nearly half the book.

J.B.S. Haldane used to punctuate his genetics lectures with "Let me give you an example" and Earl Green uses the same idea liberally in this book. Matings of mice with pale ears and dilute coats illustrate probabilities, tests of significance, symbolism, gametic output and so on, other matings illustrate linkage and still others demonstrate the use of Finney's scores for linkage and analysis. These help a lot in what would otherwise be a harder slog. All the examples are based on mice but of course the book as a whole should be a boon to all mammals in search of a genome. The mouse is far ahead of the rest at present, but with modern mutagenic methods the gap could be narrowed quite rapidly.

Among the ten appendices are the complete rules of nomenclature for mouse genes and inbred strains, as well as the author's system of record-keeping and a recommended mouse-room layout. My only regret is that there is so little on how to deal with translocations and other chromosome anomalies. There are a lot of them about nowadays and, with some types of cross, they can play havoc with Mendelian genetics.

A.G. Searle is in the Genetics Section at the Medical Research Council's Radiobiology Unit, Harwell.

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Spectroscopic sound

M. J. Adams

Photoacoustics and Photoacoustic Spectroscopy. Chemical Analysis, Vol. 57. By Allan Rosencwaig. Pp.324. ISBN 0-471-04495-4. (Wiley: 1981.) £19, \$43.75.

THE photoacoustic effect (prior to 1977 more commonly termed the optoacoustic effect) provides the basis for a calorimetric method of measuring the absorption of electromagnetic radiation by a sample under study. The phenomenon may be applied to many spectroscopic and nonspectroscopic studies involving the interaction of radiation with matter. Dr Allan Rosencwaig, internationally recognized as a pioneer in the modern development of photoacoustic techniques, has attempted to bring together in this book a comprehensive review of the theory and current applications of methods employing the effect.

In the first two chapters the author presents a brief summary of the phenomenon and describes its study up to the last decade. Until quite recently the majority of applications of photoacoustic spectroscopy (PAS) have been limited to the study and analysis of gaseous systems, and six chapters (approximately a quarter of the book) are given over to this work. The theory and more common experimental arrangements for gaseous PAS are discussed and a chapter is devoted to a review and evaluation of suitable radiation sources for PAS. Applications of PAS to the study of gases, including multicomponent analysis, de-excitation studies, high resolution spectroscopy, photolysis and non-linear effects, are examined.

Much of this first part of the book is already available in many excellent reviews and serves here only as a background and introduction to the remainder of the text which covers the application of photoacoustic studies to the examination of condensed-phase media. It is in the contents of these 15 chapters that the author's own contributions are predominant and which provide the book with its special interest. A chapter is devoted to the general theory of the photoacoustic effect employing gas-microphone systems and piezoelectric transducers, and another, of five pages, provides a simplified theoretical account for those who might find the more rigorous treatments, derived in previous sections, demanding. Experimental systems and apparatus for the examination of solid and liquid samples are reviewed and a chapter is reserved for the more novel spectroscopic studies, including dichroism and Fourier transform techniques. The areas of condensed-phase sample PAS reviewed comprise chemical studies, surface studies and applications in biology and medicine. De-excitation phenomena in solid and liquid media, including fluorescence

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studies and quantum efficiency measurements, are discussed in detail. There is also a useful account of thermal processes in samples and sample depth profiling, and thickness measurements using the photoacoustic effect are considered. The final two chapters of the book are concerned with more specialized instrumental topics: photoacoustic spectroscopy at low temperatures and photoacoustic microscopy.

During the past decade studies involving the photoacoustic effect have increased dramatically and the range of instrumental methods and applications is now widespread. In producing this book, the first of its kind to deal exclusively with photoacoustics, Dr Rosencwaig has succeeded in including a discussion of all the major topics currently being investigated employing the techniques. With its wide-ranging coverage, the text will be of interest not only to those actively engaged in photoacoustic research but also to spectroscopists seeking a detailed introduction to the technique and its possible use in their own field.

M.J. Adams is in the Department of Spectrochemistry at the Macaulay Institute for Soil Research, and is a past SRC Research Fellow in photoacoustic spectroscopy.

Earliest technology

J.A.J. Gowlett

Premiers Outils Taillés D'Afrique. By Hélène Roche. Pp.263. ISBN 2-901161-146. (Société d'Ethnographie, Paris: 1981.) 80F. Available from C. Klincksieck, 11 rue de Lille, Paris 7.

"FEEL good" wrote Johanson in his diary just before discovering the early hominid remains of "Lucy". It was good too that Hélène Roche, a French co-worker in the same Afar region of Ethiopia, succeeded in finding the earliest stone tools yet known (c. 21/2 million years old), which now gain mention in a book of wider scope. Her book, though, is not so much the popular introduction which the title might imply, but rather a painstaking exercise in the study of early stone technology. English texts on early man abound, but a book in French is rarer, and deserves to find an audience. Central questions are approached thoughtfully: "What is the significance of the acquisition of tools in the process of hominization?" is an issue too often swept aside by currently favoured theories which stress social factors, such as food sharing, in human evolution. Here, in contrast, the flaked stone tools, which preserve sequences of individual decisions from the distant past, are seen as an important aspect in early man's progress, encouraging technical awareness, aptitude

The heart of the book is a statistically

presented examination of the manufacturing routines employed in fashioning "worked pebbles". It seems a pity that some key sites, including those at Afar, are treated only in the useful introductory chapters, and that the main study hangs on just two samples from Olduvai and two from insecurely dated sites in Morocco – but then, nobody has access to material from all the early sites.

There are some debatable points of terminology or fact (Australopithecus africanus has not been found at Baringo), but Roche is surely right to criticize prehistorians for continuing to use terms like "chopper" even though we now intend no functional implication.

Roche concludes that simple tools were fashioned by using only certain preferred flaking sequences, amongst a range of possible routines, thus showing a consistent human desire to "get out" a worked edge from the raw material. But could they have been made differently? Some workers will argue that a worked pebble can only end up in certain forms. Nevertheless, Roche makes perceptive points about early human conceptual abilities, and her book offers much food for thought.

J. A. J. Gowlett is Senior Archaeologist of the Radiocarbon Accelerator Unit, Research Laboratory for Archaeology and the History of Art, Oxford.

Notes on the birth of experimental psychology

O.L. Zangwill

An Education in Psychology: James McKeen Cattell's Journal and Letters from Germany and England, 1880–1888. Edited by M.M. Sokal. Pp.372. ISBN0-262-19185-7. (MIT Press: 1981.) \$30, £18.60.

WILHELM Wundt (1832-1920), an unsuccessful physiologist who held the Chair of Philosophy at the University of Leipzig for many years, is remembered as the founder of the first laboratory explicitly dedicated to the pursuit of experimental psychology. It opened in 1879 and soon began to attract graduate students from all over the world - and in particular from the United States - drawn it would seem less by the profundities of Wundt's thought than by the promise of his laboratory. Accordingly, it was to Leipzig that a 20-year-old American graduate, James McKeen Cattell, the son of a Presbyterian Minister who was also Principal of the College from which his son had graduated, directed his footsteps.

Cattell studied under Wundt for two years. He then returned to America and for a year held a teaching post at Johns Hopkins University, which however he relinquished in order to return to Leipzig and to become, largely at his own initiative. Wundt's assistant. Their work at this time was largely concerned with the measurement of reaction times, then a very popular preoccupation of psychologists and which has in recent decades regained much interest as a result of contemporary concepts of human information processing. Cattell's work was published in both German and British journals (including Mind and Brain). He obtained his doctorate at Leipzig in 1886 and spent the greater part of the next two years at St John's College, Cambridge, which has a long-standing reputation as an institution sympathetic to experimental psychology.

Cambridge had at that time no Department of Experimental Psychology and a proposal set in train by James Ward in 1877

that a psychophysics laboratory be instituted was vetoed as disrespectful to the dignity of mind. While unable to proceed further with his experiments on reaction time and associative processes, Cattell's iournal and letters report exciting discussions with philosophers such as Ward and Henry Sidgwick, neurologists such as Ferrier and Hughlings Jackson and, most significant of all, the polymath Sir Francis Galton. Indeed in his year or two at Cambridge Cattell acquired from Galton a major interest in the measurement of individual differences in mental capacity which owed nothing to his years with Wundt and which did much to shape his work at Columbia in the earlier decades of this century.

This selection from Cattell's journal and letters, mostly to his parents, together with some of their replies, provides an interesting picture of the impact of Europe on a sensitive and intelligent young American psychologist in the penultimate decade of the last century. While some of his judgements, particularly on matters aesthetic, are naive in the extreme, others reflect an intellectual dedication and whole-hearted devotion to science which might well have led to much more important original research had the young author decided to act on his original plan to remain permanently in Cambridge. (As it was, Cattell's later career was largely that of an organizer of research and an academic administrator, and he cannot be rated as in the top flight of American psychologists of his period.) The material has been assembled and edited with scholarship and care, though it is perhaps a pity that many passages labelled as 'gossip'' have had to be excluded. If one's interest lies in the man rather than in his work, which appears to be the case here, his gossip may provide much more interesting clues to his character than the details of his work, his travels and his social encounters with the distinguished.

None the less, some interesting items of social history escape the editor's scissors. For example, it is evident that self-experiments with a whole variety of drugs, including caffein, ether, hashish, laudanum and morphine, were regarded as perfectly in order even in the family of a Presbyterian College President. Such "experiments" appear to have been regarded as healthy explorations of the

range of consciousness and as carrying no social or medical stigma whatsoever. It would be interesting to trace the reasons why such widely disseminated personal idiosyncracies apparently failed to produce anything remotely approaching the student drug culture of today.

O. L. Zangwill is Professor of Experimental Psychology at the University of Cambridge.

Transformations in magma physics

M.J. O'Hara

Physics of Magmatic Processes. Edited by R.B. Hargraves. Pp.585. ISBN hbk 0-691-08259-6; ISBN pbk 0-691-08261-8. (Princeton University Press: 1980.) Hbk \$40, £24; pbk \$15, £9.30.

This companion volume to Evolution of the Igneous Rocks; Fiftieth Anniversary Perspectives (Princeton, 1979) emerges from a conference commemorating the fiftieth anniversary of Bowen's great publication. Written by an impressive list of experts, it contains 11 chapters on a range of aspects of the physics, thermochemistry and trace element chemistry of magmas.

In the opening contribution, P.C. Hess discusses the structure of silicate liquids and indicates the importance of its influence on liquid immiscibility and distribution coefficients for trace elements. His account, though useful, would have been more valuable with the inclusion of the instructive saga of the variations of nickel distribution between olivine and liquid, and if the implications of changes in liquid structure with pressure (and with time in an experiment) for measured distribution coefficients had been surveyed in more detail.

D.F. Weill, R. Hon and A. Navrotsky next illustrate the progress made, despite considerable difficulties, in the prediction of crystal-liquid phase equilibria from thermodynamic data. In this context, it is worthy of note that experimental determination has a more important future than some would have predicted five years ago. Progress in determination of the important physical parameters of magmas is covered by I. Kushiro. He emphasizes the importance of the transition from four-fold to six-fold coordination of aluminium in controlling increase of density, decrease of viscosity and change of liquid structure in the 10-20 kb range. (The mind leaps to the possible effects of the same transition in the coordination of silicon, which should produce yet more dramatic changes of such properties at pressures encountered within the lower part of the upper mantle — it is a pity that Kushiro does not speculate on this.) He does, however, discuss the overall implications of the changes of viscosity and density for rates of magma movement, changes in volatile solubility and the efficiency of magma extraction, crystalliquid fractionation and diffusive processes.

S.R. Hart and C.J. Allegre review the field of trace element and isotope geochemistry, before going on to provide an excellent summary of the "traditional" (or pre-1980) theory of the petrogenesis of mafic and granitic rocks. The more complicated but entirely plausible trace element models (touched on briefly in this chapter), combined with new understanding of the physics of magma mixing, have transformed thinking on the subject; fractionating magma chambers fed by uniform parent liquids can erupt lava suites which must (traditionally) be interpreted as partial melting products of heterogeneous mantle sources. The rehabilitation of assimilation and contamination is at hand, and increasing evidence for diffusive differentiation of trace elements (and isotopes?) in magma chambers may shortly overwhelm all other considerations. These are stirring times!

In his critical review of problems in heat flow determination and interpretation, E.R. Oxburgh concentrates on a survey of partial melting in upward convecting systems. Somewhere in this book it would have been appropriate to refer to the vexed question of density constraints on mantle plumes and to the inverse possibility that partial melting at the base of the mantle precedes and drives the convective motions. An unconsidered possibility is that partial melts may become denser than olivine (but not denser than a bulk residue containing silica-rich garnet rather than pyroxene) near the base of the upper mantle, hence allowing upward motion of low percentage partial melts, downward movement of high percentage melts and complex redistribution of heat-producing elements.

Whereas the first five chapters are models of clarity, H.R. Shaw's contribution is distinctly tough going. In almost mystic prose he explores possible relationships between stress fields, rates and volumes of magma flow, and seismicity. Nevertheless, I shall be reading and re-reading this section in order to understand its implications for the (rather neglected) situations where major magma chambers intervene between source and vent, and changes in magma composition

and density may become key factors in controlling magma transport. In the following contribution, F.J. Spera covers some of the same ground as Shaw but extends the discussion to many other aspects of the segregation, ascent and eruption of magmas. Major evolution of liquid composition probably occurs between source and point of eruption in most volcanic systems, and will complicate further the relationships discussed here.

In a thoughtful and stimulating contribution, T.N. Irvine documents the recent revolution in ideas concerning chemical and physical processes in slowly cooled magma chambers. This will be essential reading for anyone interested in consolidation of magmas. Irvine covers diagenesis of crystal accumulations but unfortunately only touches on fluid dynamics; it is in this latter field that some of the most spectacular progress has been made since the book went to press.

A.W. Hoffman deals with one- and two-way diffusion in magmas, making particular reference to determinative methods rather than specific applications. One of the most important questions which is discussed in detail by Hoffman concerns the possible influence of diffusion through the boundaries between individually wellmixed convecting layers in a cooling magma chamber. While crystal-liquid equilibria apply a constraint on the lowest layer — at least if the chamber is solidifying by bottom-crystallization - it does not follow that the higher layers of the magma from which lava flows are likely to be derived will be in major- or trace-element equilibrium with the "cumulate" assemblage, or even in isotopic equilibrium with it.

E. Dowty deals with the basic theory of crystal nucleation and growth. He reviews a range of factors which may affect the morphology and chemistry of growing (or dissolving) crystals, and thus the development of grain size distributions and textures of igneous rocks, stressing once again the value of experimental work. G. Lofgren continues the story of the experimental study of crystal growth and texture development, especially with regard to lunar and terrestrial basic magmas. This final contribution provides a timely review of recent activities in the field.

Like its companion volume, this book appears at the moment when igneous petrology is making its most rapid advances since Bowen's day. Consequently it, too, suffers from omissions which will prevent it from standing as a milestone on the long road towards our understanding of magmatic processes; rather, it should be appreciated as a speedometer. Nevertheless, I found the book stimulating reading and recommend it to all serious students of igneous petrology.

M.J. O'Hara is Professor and Head of the Geology Department, University College of Wales, Aberystwyth.

BOOKS RECEIVED

Mathematics

BERGER, J. O. Statistical Decision Theory. Foundations, Concepts and Methods. Pp.425. ISBN 3-540-90471-9. (Springer-Verlag: 1980.) DM 45, \$26.60.

HELLEMAN, R. H. G. (ed.). Nonlinear Dynamics. Annals of the New York Academy of Sciences, Vol.347. Pp.505. Hbk ISBN 0-89766-103-6; pbk ISBN 0-89766-104-4. (The New York Academy of Sciences, New York: 1980.) Hbk \$98; pbk np.

NICKEL, K. L. E. (ed.). Interval Mathematics 1980. Proceedings of an International Symposium held at the Institut für Angewandte Mathematik Universität Freiburg i, Br., Germany, May 1980. Pp.554. ISBN 0-12-518850-1. (Academic: 1980.) \$29.50.

PLUMPTON, C. and MACILWAINE, P. S. W. New Tertiary Mathematics. Vol.1; Part 1, Pure Mathematics: The Core. Pp.401. Hbk ISBN 0-08-025031-9; flexi ISBN 0-08-021643. (Pergamon: 1981) Hbk £12.50; flexi £5.

PLUMPTON, C. and MACILWAINE, P. S. W. New Tertiary Mathematics, Vol.1; Part 2, Basic Applied Mathematics. Pp.229. Hbk ISBN 0-06-025035-1; flexi ISBN 0-08-021645-5. (Pergamon: 1981.) Hbk £12.50; flexi £5.

PLUMPTON, C. and MACILWAINE, P. S. W. New Tertiary Mathematics. Vol.2; Part 1, Further Pure Mathematics. Pp.804. Hbk ISBN 0-08-025033-5; flexi ISBN 0-08-021644-7. (Pergamon: 1981.) Hbk £17.40; flexi £5.

ROGERS, C. A. et al. Analytic Sets. Developed from Lectures given at the London Mathematical Society Instructional Conference on Analytic Sets, University College London, July 1978. Pp.495. ISBN 0-12-593150-6. (Academic: 1980.) £48, \$110.50.

ZACKS, S. Parametric Statistical Inference. Basic Theory and Modern Approaches. International Series in Nonlinear Mathematics: Theory, Methods and Applications, Vol.4. Pp.387. (Pergamon: 1981.) £20, \$48.

Physics

BORN, M. and WOLF, E. Principle of Optics. Electromagnetic Theory of Propagation, Interference and Diffraction of Light. 6th Edn. Pp.808. Hbk ISBN 0-08-026482-4; flexi ISBN 0-08-026481-6. (Pergamon: 1980.) Hbk np; flexi £12.

FERRARI, E. and VIOLINI, G. (eds). Low and Intermediate Energy Kaon-Nucleon Physics. Proceedings of the Workshop, Institute of Physics of the University of Rome, March 1980. Pp.428. ISBN 90-277-1183-6 (Reidel: 1981.) Dr. 100

GRIFFITHS, D. J. Introduction to Electrodynamics. Pp.479. ISBN 0-13-481374-4. (Prentice/Hall International: 1981.) £15.55.

MAHAN, G. D. Many-Particle Physics. Physics of Solids and Liquids. Pp.1003. ISBN 0-306-40411-7. (Plenum: 1981.) \$85.

MÜLLER, K. A. and THOMAS, H. (eds). Structural Phase Transitions 1. Topics in Current Physics, Vol.23. Pp.190. ISBN 3-540-10329-5. (Springer-Verlag: 1981.) DM 50, \$29.50.

PERSONICK, S. D. Optical Fiber Transmission Systems. Applications of Communications Theory Series. Pp.179. ISBN 0-306-40580-6. (Plenum: 1981.) Np. RAMOND, P. Field Theory. A Modern Primer. Frontiers in Physics, No.51. Pp.397. Hbk ISBN 0-8053-7892-8; pbk ISBN 0-8053-7893-6. (Benjamin/Cummings, Menlo Park, California: 1981.) Hbk \$26.50; pbk \$14.50.

RAPP, D. Solar Energy. Pp.516. ISBN 0-13-822213-4. (Prentice/Hall: 1981.) \$32.

SAXENA, S. C. and JOSHI, R. K. Thermal Accommodation and Adsorption Coefficients of Gases. McGraw-Hill/CINDAS Data Series on Material Properties, Vol.II-1. Pp.412. ISBN 0-07-065031-4. (McGraw-Hill: 1981.) Np.

SOBELMAN, I. I., VAINSHTEIN, L. A. and YUKOV, E. A. Excitation of Atoms and Broadening of Spectral Lines. Pp.328. ISBN 3-540-09890-9. (Springer: 1981.) DM 75, \$44.30.

Chemistry

BRADY, J. E. and HOLUM, J. R. Study Guide. Fundamentals of Chemistry. Pp.396. Flexi ISBN 0-471-05817-3. (Wiley: 1981.) Np.

DENBIGH, K. The Principles of Chemical Equilibrium. With Applications in Chemistry and Chemical Engineering. 4th Edn. Pp.494. Hbk ISBN 0-521-23682-7; pbk ISBN 0-521-28150-4. (Cambridge University Press: 1981.) Hbk £22; pbk £8.95.

ENGLMAN, R. et al. Bonding Problems. Structure and Bonding, Vol.43. Pp.220. ISBN 3-540-10407-0. (Springer-Verlag: 1981.) DM 98, \$57.90.

INMAN, D. and LOVERING, D. G. (eds). Ionic Liquids. Pp.450. 0-306-40412-5. (Plenum: 1981.) \$49.50.

ISAACS, N. S. Liquid Phase High Pressure Chemistry. Pp.414. ISBN 0-471-27849-1. (Wiley: 1981.) Np.

KATRITZKY, A. R. and BOULTON, A. J. (eds). Advances in Heterocyclic Chemistry, Vol.27. Pp.331. ISBN 0-12-020627-7. (Academic: 1981.) \$49.50.

LOWDIN, P.O. Advances in Quantum Chemistry, Vol.12. Pp.325. ISBN 0-12-034812-8. (Academic: 1980.) \$48.

McGUIRE, S. Y. Study Guide for Chemistry. An Introduction to General, Organic and Biological Chemistry. Pp.307. Pbk ISBN 0-7167-1314-4. (W. H. Freeman: 1981.) \$7.95.

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ZAHRADNIK, R. and POLAK, R. Elements of Quantum Chemistry. Pp.462. ISBN 0-306-31093-7. (Plenum: 1980.) \$29.50.

Technology

CLARK, A. F. and REED, R. P. (ed). Advances in Cryogenic Engineering Materials Vol.26. Proceedings of the 3rd International Conference, the 1979 Cryogenic Engineering Conference, held at the University of Madison, Wisconsin, August 1979. Pp.703. ISBN 0-306-40531-8. (Plenum: 1980.) \$59.

DE SA, A. Principles of Electronic Instrumentation. Pp.280. ISBN 0-7131-2799-6. (Edward Arnold, London: 1981.) \$9.50.

POLLOCK, J. R. A. (ed.) Brewing Science, Vol.2. Pp.628. ISBN-12-561002-5. (Academic: 1981.) £41, \$98.50.

Earth Sciences

DAVIES, P. A. and RUNCORN, S. K. (eds). Mechanisms of Continental Drift and Plate Tectonics. Preceedings of a NATO Advanced Study Institute held at the University of Newcastle upon Tyne, March-April, 1979. Pp.362. Pp.362. ISBN 0-12-206160-8. (Academic: 1981.) £30 \$72.

ELDER, J. Geothermal Systems. Pp.508. ISBN 0-12-236450-3. (Academic: 1981.) £20.60, \$49.50.

GRIFFITHS, D. H. and KING, R. F. Applied Geophysics for Geologists and Engineers: The Elements of Geophysical Prospecting. (A 2nd Edn. of Applied Geophysics for Engineers and Geologists.) Pp.230. Hbk ISBN 0-08-022071-1; flexi ISBN 0-08-02272-X. (Pergamon: 1981.) Hbk £11, \$25; flexi £5.30, \$12.

PANAYIOTOU, A. (ed.). Ophiolites. Proceedings International Ophiolite Symposium, Cyprus 1979. Pp.781. (Republic of Cyprus Ministry of Agriculture and National Resources Geological Survey Department: 1980.) £15.

TUCKER, M. E. Sedimentary Petrology. An Introduction. Geoscience Texts, Vol.3. Pp.252. Flexi ISBN 0-632-00072-0. (Blackwell Scientific: 1981.) £8.50.

Biological Sciences

ADLERCREUTZ, H. et al. (eds). Endocrinological Cancer Ovarian Function and Disease. Research on Steroids, Vol.IX. Pp. 400. ISBN 90-219-0444-6. (Excerpta Medica, Amsterdam: 1981.) \$78, Dfl.160.

ANGELINI, C., DANIELI, G. A. and FONTANARI, D. (eds). Muscular Dystrophy Research: Advances and New Trends. Proceedings of an International Symposium, Venice, Italy, April 1980. Pp.332. ISBN 90-219-0453-5. (Excerpta Medica, Amsterdam: 1980.) \$68.25, Dfl.140.

APPLEWHITE, P. B. Molecular Gods. How Molecules Determine our Behaviour. Pp.262. ISBN 0-13-599530-2. (Prentice-Hall: 1981.) \$10.95.

BACH, F. H. and GOOD, R. A. (eds). Clinical Immunobiology, Vol.4. Pp.198. ISBN 0-12-070004-2. (Academic: 1980.) \$19.50. BHASKARAN, G., FRIEDMAN, S. and RODRIGUEZ, J. G. (eds). Current-

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BOLAND, D. J. et al. Eucalyptus Seed. Pp.191. ISBN 0-643-02586-3. (Division of Forest Research, CSIRO, Canberra, Australia: 1980.) \$18.

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HARRIS, E. and HARRIS, J. The Guinness Book of Trees. Britain's Natural Heritage. Pp.160. ISBN 0-85112-303-1. (Guinness Superlatives, Enfield, Middlesex: 1981.) £4.50.

ANNOUNCEMENTS

Awards

The 1981 recipients of the Harvey Prize of the Technion — Israel Institute of Technology are Prof. Sir James Lighthill (Provost of University College, London, UK) receives the Harvey Prize in Science and Technology; Prof. Hans W. Kosterlitz (Director of the Unit on Addictive Drugs, University of Aberdeen, UK) receives the Harvey Prize in Human Health.

The winners of the 1981 General Motors Cancer Research Foundation Awards are Dr Wallace Prescott Rowe (Chief of the Laboratory of Viral Diseases at the National Institute of Allergy and Infectious Diseases in Bethesda. Maryland) for his contributions to virology; Dr E. Donnall Thomas (Director of Medical Oncology at the Fred Hutchinson Cancer Reseach Center in Seattle, Washington) for developing bone marrow transplantation as a successful treatment for acute leukemia and aplastic anemia: Dr Cesar Milstein (Medical Research Council Laboratory of Molecular Biology in Cambridge, UK) for developing hybridoma technology; Dr Takashi Sugimura, (Director of Tokyo's National Cancer Center Research Institute) for identifying possible cancer causing substances in our food.

The Low Temperature Group of the Institute of Physics has awarded the Simon Memorial Prize to **Prof. A. J. Leggett** (University of Sussex), for work on the theory of superfluid ³He.

The Trustees of the Lady Tata Memorial Trust have made international awards for research in leukaemia to: Dr B. Azzarone (Italy), Dr A.D. Crockard (UK), Dr S. Venuta (Italy) — renewed awards. Dr H.D. Abrams (USA), H.J. Allen (UK) — new awards.

The International Meteorological Organization (IMO) Prize, for outstanding work in meteorology or operational hydrology and international cooperation has been awarded to **Prof. Bert Bolin** (Director of the International Meteorological Institute, Stockholm).

The Beit Memorial Fellowships for medical research 1981 have been awarded to David Leonard Bentley (MRC Laboratory for Molecular Biology, Cambridge, UK); Stephen Eric Kearsey (MRC Laboratory for Molecular Biology, Cambridge, UK); David Ian Vaney (The Physiological Laboratory, Cambridge University, UK); John Vincent Priestley (Dept of Pharmacology, Oxford University, UK); Peter John Richardson (Dept of Clinical Biochemistry, University of Cambridge Medical School, New Addenbrooke's Hospital, UK).

Meetings

21-22 August, Excitation-Contraction Coupling in Skeletal, Cardiac and Smooth Muscle, Banff (Dr George B. Frank, Dept of Pharmacology, The University of Alberta, Edmonton, Alberta, Canada T6G H77).

25-27 August, Life Sciences in the Service of Alaska, Fairbanks (Conferences and Institutes, The University of Alaska, Fairbanks, Alaska 99701, USA).

2-4 September, Modern Aspects of Phase Equilibria, Teesside (Mrs M. Findley, Dept of Chemical Engineering, Teesside Polytechnic, Borough Rd, Middlesborough, Cleveland, UK).

1-7 September, Fundamentals of Microprocessors, London (Course Registrar, Bleasdale Computer Systems Ltd, Francis St, London SW1, UK).

6-12 September, 32nd International Astronautical Congress, Rome (Prof. Luigi G. Napolitano, International Astronautical Federation, 250 Rue Saint-Jacques, 75005 Paris, France).

7 September, Water Quality, London (R. Gardam, Thames Water Authority, New River Head, Rosebury Avenue, London EC1, UK).

7-8 September, Chemical Aspects of Changes in Food on Storage, Norwich (Dr R. Fenwick, Society of Chemical Industry, Food Group, 14 Belgrave Square, London SW1, UK).

7-10 September, **EMAG '81**, Cambridge (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

7-11 September, Chemistry of Carbocations, Bangor (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

7-11 September, An Introduction to Hydrometallurgy, Hatfield (D.B. Firth, Institution of Chemical Engineers, 12 Gayfere St, London SW1, UK).

10-11 September, National Conference on Fitness and Aging, Washington DC (U.A. Wheaton, President's Council, 400-6th Street S.W. Room 3030, Washington DC 20201, USA).

16-18 September, Chemical Engineering Education, London (C.J. Bullock, Chemical Engineering Dept, Teesside Polytechnic, Middlesbrough, Cleveland, UK).

17-18 September, Regulation of Energy Balance, Edinburgh (Dr J.A. Milne, Hill Farming Research Organisation, Penicuil, Midlothian, UK).

19-21 September, Teilhard and Metamorphosis, Arizona, (Arcosanti-Events, 6433 Doubletree Rd, Scottsdale, Arizona 85253, USA).

22 September, Autonomic Nerves of the Gut, London (Dr M. Daly, Glaxo Group Research Ltd, Ware, Herts, UK).

22-24 September, Royal Society of Chemistry Autumn Meeting, Leeds (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1, UK).

23 September, Information Meeting for Nature Protection Societies from the European Community, Brussels (Ms J. White, EEB,31 rue Vautier, B-1040 Bruxelles, Belgium).

23-25 September, Wave and Tidal Power, Cambridge (Conference Organiser, Wave and Tidal Energy, BHRA Fluid Engineering, Cranfield, Bedford, UK).

24-25 September, Platinum Group and Gold Mineralization, Ultramafic Rocks, and Zeolites, Manchester (Dr C.M.B. Henderson, Dept of Geology, The University, Manchester, UK).

24-25 September, The World Conservation Strategy and the European Communities, Brussels (Ms J. White, EEB, 31 rue Vautier, B-1040 Bruxelles, Belgium).

25-28 September, Eocene and Oligocene of the Isle of Wight, Sandown (Dr A.N. Insole, Museum of Isle of Wight Geology, Sandown Library, High St, Sandown, Isle of Wight, UK).

28 September — 2 October, Annual Meeting European Society of Nuclear Methods in Agriculture, Aberdeen (Mr A.H. Knight, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, UK).

28 September — 2 October, **Dynamics of Turbid Coastal Environments**, Nova Scotia (D.C. Gordon, Marine Ecology Laboratory, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2).

28 September-17 October, New Approaches to Plant Genetics and Breeding, Peking (Dr O.L. Gamborg, International Plant Research Institute, 887 Industrial Rd, San Carlos, California 94070, USA).

29 September, Gas Cyclones, London (Mrs E.H. Edwards, Institution of Chemical Engineers, 12 Gayfere St, London SW1, UK).

30 September — 2 October, **Deburring'81**, New Orleans (J. Slaughter, Technical Activities Dept, Society of Manufacturing Engineers, 1 SME Drive, PO Box 930, Dearborn, Michigan 48128, USA).

2-3 October, 21st Anniversary Meeting of Radiologists, Dublin (Miss A. Daly, Faculty of Radiologists, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Ireland).

5-7 October, Wind Energy Conference and Workshop, Washington (Conferences Group, SERI, 1617 Cole Boulevard, Golden, Colorado 80401, USA).

5-8 October, Pollution Control, Brighton (NSCA, 136 North St, Brighton, Sussex, UK).



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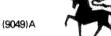
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The Parasitology Department numbers some 40 people including 10 Scientists and is concerned with basic research, biological screening and drug development in fields relevant to human and veterinary medicine. The department is part of the experimental biology division which is also concerned with research into molecular biology and immunology.

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For this important post, we offer a substantial salary which will fully reflect the responsibility of the role plus a valuable range of fringe benefits including 5 weeks holiday, pension and sick pay schemes and assistance with relocation to the London or Kent area, where appropriate.

Please send a detailed curriculum vitae quoting ref WRL/132 to The Pesonnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR33BS.



Wellcome

POSTDOCTORAL POSITION

available. Research on hepatic microsomal electron transport and fatty acid elongation and desaturation.

Contact: Dr Dominick L Cinti, University of Connecticut Health Center, Department of Pharmacology, Farmington CT 06032.

An Affirmative Action/Equal Opportunity Employer. (NW734)A

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY Picosecond pulsed raman studies of adsorbates

Applications are invited, from chemists or physicists, for a postdoctoral

SENIOR RESEARCH ASSISTANT

for three years from as soon as possible, to study the applications of picosecond pulsed lasers in obtaining Raman spectra of strongly fluorescing adsorbed species or adsorbents. This is a new technique and a new picosecond laser system will be available. Some experience is lasers is desirable.

Initial salary in the range £6,070 to £6,880 on Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by 31 July, 1981 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars are available. (9055)A

POSITION of "Full Professor of Physical Chemistry" is open at the Technical University of Graz (succession Prof. Dr. Karl Torkar). Applicants (German speaking) should submit their résumé by October 31, 1981 to Dean, Technisch-Naturwissenschaftliche Fakultät, Technische Universität, Graz, Petersgasse 16, A-8010 Graz (Austria). (W376)A

BROCK UNIVERSITY DEPARTMENT OF BIOLOGICAL SCIENCES GENETICIST

Tenure stream position in the Department of Biological Sciences, Brock University, at the Assistant Professor level is available from January 1, 1982 or earlier. The appointee will be expected to teach molecular, transmission and population genetics at the undergraduate level, to develop an independently funded strong research program, to supervise graduate students and to interact with the faculty over several levels of biological organization. Although specific research speciality is open, research areas of particular interest include microbial genetics, eucaryotic genetics (including mammalian molecular genetics) and population genetics. Canadian applicants will be considered first.

Submit complete curriculum vitae by October 31, 1981, with a statement of research and teaching experience, reprints of published papers, names of three referees to Dr MS Manocha, Chairman, Department of Biological Science, Brock University, St Catharines, Ontario, Canada L2S 3A1. (NW733)A POSTDOCTORAL POSITION—Enzyme mechanisms and models, design of transition state analog inhibitors. Candidate should have experience in two of the following: enzyme purification, immunology and organic synthesis. R V Wolfenden, Dept of Biochemistry, University of North Carolina, Chapel Hill, NC 27514. UNC is an equal opportunity/affirmative action employer. (NW742)A

QUEEN ELIZABETH COLLEGE Kensington

(University of London)
DEPARTMENT OF MICROBIOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

Required for an SERC-funded project tenable for three years which involves an investigation of the reactions of bacterial cytochrome oxidases with oxygen and other ligands using low temperature dual-wavelength and flash photolysis techniques. Applicants should be recently qualified PhD graduates. Previous research experience in bioenergetics, microbial biochemistry and/or enzymology would be advantageous, but not essential.

Starting salary: £7,073, inclusive of London Weighting. Starting Date: 1 October 1981, or as soon as possible thereafter.

Further details can be obtained from Dr R K Poole, Department of Microbiology. Applicants should send a curriculum vitae and names of two referees to Mrs J Staight, Queen Elizabeth College, Campden Hill Rd, Kensington, London W8 7AH.

(9034)A

Apply Your Microbiological Expertise in Cosmetics

Surrey

As one of the U.K.'s largest and best-known perfumery and cosmetics companies, Lentheric Mornay's extensive range of popular products has a considerable weight of research, testing and analysis behind it.

Our well-equipped microbiological laboratory makes a considerable — and continuous — contribution to these investigations, and we are currently looking for a qualified man or woman with appropriate industrial experience to assume responsibility for all routine aspects of sampling and analysis associated with microbiology. In addition the Microbiologist will undertake ad hoc assignments in close liaison with our Research and Development, Marketing and Production departments, especially challenge testing for all new products.

Facilities at our centralised premises are first class, and you will enjoy a competitive salary together with a full range of benefits including pension scheme and the use of our subsidised canteen and staff shop.

To find out more contact Paul O'Bryan, Personnel Department, Lentheric Morny Limited, Vale Road, Camberley, Surrey. Tel: Camberley 62181.

(9025)A

·LENTHÉRIC MORNY

THE UNIVERSITY OF ADELAIDE WAITE AGRICULTURAL RESEARCH INSTITUTE

invites applications for a LECTURER (LIMITED TERM) (S8481)

in the Department of Agronomy for up to two years from 1 January 1982 to undertake research and to lecture in aspects of crop physiology/agronomy. Preference will be given to applicants with a field orientation and who would interact with present staff undertaking research in crop physiology, crop nutrition, pasture utilization and plant breeding. Applicants with interests in grain legumes, continuous cropping, minimum tillage or use of agricultural chemicals are encouraged to apply.

Further enquiries about the duties attached to the position may be directed to Professor C J Driscoll, Department of Agronomy, Private Bag, Glen Osmond, South Australia 5064.

Salary: Lecturer $$A19,182 \times 7 - $A26,037$ with optional superannuation provision.

The Conditions attached to the appointment may be obtained from the Registrar.

Applications, in duplicate, giving full personal particulars, details of academic qualifications, (including citizenship) and names and addresses of two or three referees, should reach the Registrar of the University, GPO Box 498, Adelaide, South Australia, 5001 not later than 15 August 1981. (9036)A

UNIVERSITY OF BRISTOL

SRC supported

SRC supported POST-DOCTORAL RESEARCH ASSISTANT

to work on characterisation of the light requiring enzyme NADPH:Protochlorophyllide oxidoreductase. Experience in protein purification and sequencing or DNA isolation and characterisation desirable but not essential. Post to commence 1st September, 1981.

Details from Dr W T Griffiths, Department of Biochemistry, University of Bristol BS8 1DT. (9026)A

THE UNIVERSITY OF MANCHESTER FOETAL EPITHELIAL PHYSIOLOGY

Applications are invited for the post of

TEMPORARY LECTURER

held jointly between the Departments of Physiology and Child Health. Research will be on ion transfer across the placenta mainly in sheep or pigs. Teaching duties will be in the Department of Physiology. This is a new venture and the initial appointment will be for a term of three years. The possibility exists for the appointment to be made substantive if the joint appointment is a success. Initial salary range pa: £6,070 — £7,290.

Particulars and application forms (returnable by September 1st) from the Registrar, The University, Manchester M13 9PL. Quote ref. 127/81/N. (9004)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF HISTOLOGY & CELL BIOLOGY

TECHNICIAN (GRADE 3)

to assist with a research project. Work concerns cell biology, primarily preparing material for electron microscopy. Minimum qualifications ONC, but might suit graduates in an appropriate discipline.

The post is available for approximately 18 months. Salary in a range £4,672 — £5,473 per annum.

Application forms available from the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref. RV/842/N. (9023)A

UNIVERSITY OF LONDON Institute of Obstetrics and Gynaecology

A vacancy exists for a RESEARCH ASSISTANT

with Biochemical training or experience, for a project on placental transfer and metabolism of amino acids in relation to normal and abnormal foetal growth.

The starting salary on the 1B scale will be £5,285 plus £969 London Allowance.

Applications, with CV and the names of 2 referees to: Dr D L Bloxam, Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W60XG. (9007)A

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF MICROBIOLOGY LECTURESHIP

Applications are invited from men and women for a Lectureship in Microbiology, financed initially by the SRC under their Special Replacement Scheme. Conditions of service, including probation and tenure, as for other University lectureships.

Initial salary up to £7,290 a year on scale rising to £12,860 a year. Expected age of candidate would be up to 27 years but older candidates not precluded.

Starting date will be as early as can be arranged. The successful candidate will be expected to fulfil all the normal duties of a lecturer and to have an enthusiastic commitment to research.

Preference to candidates with experience in molecular biology or microbial genetics.

Further details available from the Registrar and Secretary, the University, Sheffield S10 2TN, to whom applications (including) curriculum vitae, publication list, teaching experience, research interests and three referees) should be sent by 31 July 1981. Quote ref: R 604/G. (9044)A

UNIVERSITY OF NOTTINGHAM

FACULTY OF
AGRICULTURAL SCIENCE
RESEARCH ASSISTANT IN
APPLIED PHYSICS

Applications are invited for a Research Assistant to work in the Food Science laboratories of techniques for measuring the viscosity of food and biopolyme systems at high temperatures. This will be a challenging project in which there is considerable industria interest

The post, which is funded by the SRC will be available for a three year period. There will be an opportunity to register for a higher degree.

Applicants should possess or hope to obtain a degree in Physics of Chemical Engineering.

The salary will be in the range £5,285 to £6,070. Application: including a curriculum vitae and the names and addresses of two referees should be sent to Dr J R Mitchell Food Science Section, Dept o Applied Biochemistry and Nutrition School of Agriculture, Suttor Bonington, Nr Loughborough. (9048)A

Please mention

nature

when replying to

these advertisements

Director, Research & Development

Consider this unique opportunity to apply—and develop—your expertise with an exciting and growing company in biomedical research, development and production.

You'll provide creative and organizational direction to biochemistry, biology and bioengineering groups conducting a wide variety of research and development activities in support of the creation of collagen-based medical products. You and your organization will explore the fields of cell biology, extracellular matrix biochemistry and mitogen physiology with the ultimate business goal of developing products used in repairing, replacing or regenerating human body tissue.

To accomplish these ambitious goals you'll provide direction to the development of unique biomaterials, analytical test methods and instruments which will lead to a wide range of product candidates. You will be expected to maintain a continuing relationship with the scientific community through the generation and presentation of technical papers, and to direct an active program designed to produce invention reports.

The ideal candidate is expected to be a well published scientist with the ability to inspire and maintain scientific excellence. An important requirement is a PhD in biochemistry or biology or an MD with a research orientation. Approximately ten years of related experience including research managerial assignments is required. Experience with protein biochemistry, cell biology or immunology is highly desirable.

We offer an exciting and creative environment and our salary and benefits are highly competitive. We are confident you will also find our equity participation program attractive.

For consideration please submit a comprehensive resume to Dr. Bruce Pharriss, Vice President, Scientific Affairs, Attn: Department D, Collagen Corporation, 2455 Faber Place, Palo Alto, CA 94304. An affirmative action employer.



A(967WN)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PLANT SCIENCES

Applications are invited for a post of

POSTDOCTORAL RESEARCH FELLOW

1 the Department of Plant Sciences or work on the isolation and haracterization of fertilization eceptors from marine algal gametes. Experience in immunological techiques will be a particular advantage. The appointment will be made for a ixed period of up to one year and six nonths.

Applicants should have, or shortly spect to receive, a PhD, preferably in the area of cell surface biohemistry (plants or animals).

Salary within the range £6,070 — 7,290 on the IA scale for Research nd Analogous Staff.

Informal enquiries may be made to or L V Evans or Dr J A Callow telephone 0532 31751 ext. 6576 or 580).

Application forms and further particulars may be obtained from the tegistrar, The University, Leeds LS2 JT, quoting reference number 53/7/D. Closing date for applications 0 July 1981. (9014)A

University of Edinburgh RESEARCH TECHNICIAN

required to work in the Department of Molecular Biology, Kings Buildings, on a project concerning the molecular genetics of insertion sequences in bacterial plasmids. An HNC or equivalent qualification in an appropriate subject is required. Salary on scale £5,695 — £6,650. Appointment to take effect from September 1981 for a period of up to three years. *Ref: NT794*.

Application forms may be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS. Tel: 031-556 2930. (9030)A

ARMAGH OBSERVATORY Armagh, Northern Ireland Applications are invited for the post of RESEARCH ASTRONOMER

tenable from 1st January, 1982.

Salary will be at an appropriate point on the University Lecturers' scale £6,070 to £12,860 pa according to qualifications and experience.

Applications stating qualifications, publications, research proposals, and names of three referees should be received not later than October 31, 1981 by the Secretary, Armagh Observatory, Armagh BT61 9DG, N Ireland, from whom further particulars may be obtained. (8994)A

ROYAL FREE HOSPITAL POST DOCTORAL FELLOW Salary on scale: LAI requir

£6,472 — £10,562 p.a. inc. Applications required from biochemists to work on DNA synthesis associated with leukaemia research.

The appointment is for a postdoctoral fellow for one year in the first instance, which may be extended to three years.

Applicants should apply in writing to Professor A. V. Hoffbrand, Department of Haematology, Royal Free Hospital, Pond Street, Hampstead, London NW3.

(9005)A

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

required to start in September in busy, small histological research laboratory. Ideal post for school leaver wishing to embark on career in medical laboratory technology. In addition to histology, varied duties include photography, illustrations, filing and general laboratory running. Day release granted for relevant studies. Minimum requirements: English, Maths and 2 relevant sciences at GCE 'O' levels.

Application forms from Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP. (Tel: 01-352 8121 Ext. 4164).

(9009)A

POSTDOCTORAL INDIVIDUAL with cell biological and biophysical interests for work on cellular factors controlling translational mobility of molecules on surfaces and/or within the cytoplasm of living cells. The work will involve the following techniques: fluorescence recovery after photobleaching (FRAP or FPR), fluorescence microscopy and image analysis, and biochemical and cell biological methods. Contact: Dr Ken Jacobson, Laboratories for Cell Biology, Department of Anatomy, University of North Carolina, Chapel Hill, NC 27514. Tel: 919-966 5703. An Equal Opportunity/ Affirmative Action Employer.

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) A TECHNICIAN

is required to work in the Academic Department of Surgery in the Royal Free Hospital at Hampstead. The successful candidate will assist with teaching duties and research work and experience of biochemistry or tissue culture would be essential. Qualifications: HNC or Degree.

Salary on scale £5,204 — £7,124 inclusive (under review). 34 days leave including public and customary days. 37 hour week.

Application forms (and further particulars) are available from the School Secretary, RFHSM, 8 Hunter Street, London WCIN 1BP or telephone 01-837 5385 ext. 10. Closing date: 24 July 1981. Reference T/S. (9012)A

Attention: Biomedical Researchers

FUNDING HELP OFFERED

We represent major pharmaceutical companies willing, under certain circumstances, to provide three kinds of funding for promising biomedical innovations:

- Development funding. Provided in return for an option to purchase or right of first refusal.
- 2) Marketing funding. Provided under a suitable licensing/royalty agreement.
- 3) **Venture capital.** Provided under highly selective, special conditions.

PRI serves as a clearing house for these companies, evaluating innovations on their behalf and recommending those we consider worthy of action. The companies pay our fees; there are no fees charged to innovation sources.

For full details, please write Marion C. Gold, Product Resources International, Inc., 800 Third Avenue, New York, New York 10022. Make no disclosures (except for issued patents) until you have read the material.

(NW730)A

City Hospital

SENIOR CYTOGENETICIST Full-time. BASIC GRADE CYTOGENETICIST

Full-time.

The above appointments are in the South-Trent Sub-Regional Cytogenetics Service currently housed in the Maternity Unit at the City Hospital, Nottingham, but will shortly be moving to more extensive accommodation on the same site.

The successful applicants will be expected to share in all standard laboratory procedures including culturing, harvesting, slide making, staining and appropriate analysis of preparations from blood, amnistic fluid, bone marrow and other tissues. The diagnostic workload is heavy but it is hoped that, with these two new appointments all members of staff will have some time for personal work. One post is available immediately and one from 1st October, 1981.

For the Senior Grade post, preference will be given to those candidates with a wide range of experience in diagnostic chromosome analysis.

For the Basic Grade post, a graduate with a reasonable degree in one of the Biological Sciences is required. Preference will be given to candidates whose degree course or postgraduate work includes a large Genetics component and/or tissue

Further information about these posts can be obtained by ringing Dr Pat Cooke on Nottingham (0602) 608111 Extension 2785 or by visiting the Department.

Application forms are available from Personnel Services, Valebrook House, Sherwood Hospital, Hucknall Road, Nottingham. Tel: (0602) 625459 anytime.

Closing date: 30th July, 1981. (9040)A

NORTH NOTTINGHAM HEALTH DISTRICT(T)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF
VETERINARY ANATOMY
Re-advertisement
Applications are
invited for the post of
RESEARCH ASSOCIATE

in the Department of Veterinary Anatomy to work on an ARC funded project on postnatal muscle development in the pig. The project is for three years starting on 1st September, 1981.

Applicants should have a good degree in Veterinary Medicine, Zoology, Agriculture or a related subject. The successful applicant will have the opportunity to register for a higher degree.

Applications, before 24th July 1981, together with a curriculum vitae and the names of two referees, should be sent to Dr N C Stickland, Department of Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh EH9 1QH, from whom further particulars may be obtained. Please quote Reference 5022.

(9041)A

THE UNIVERSITY OF AUCKLAND

New Zealand

COMPUTER SCIENCE — ASSOCIATE PROFESSORSHIP

Closing Date: 4th September 1981

Applicants must be suitably qualifie with a higher degree in Compute Science or a closely allied subject anhave had considerable research anteaching experience. Application from those qualified to teac Computer Architecture, Operatin Systems or Data Base Managemen Systems will be particularly welcome

Present salary scales: Senio Lecturers NZ\$24,110 — \$27,589 pe annum. Conditions of Appointmen and Method of Application are available from the Assistant Registra (Academic Appointments), University of Auckland, or from the Association of Commonwealth Universitie (Appis.), 36 Gordon Square, Londo WC1H 0PF. Applications, in accordance with 'Method of Application should be forwarded as soon a possible, but not later than the closing dates stated. (9037)A

POSTDOCTORAL POSITIONS AVAILABLE

Research on hepatic microsomal electron transport, active oxygen in microsomal reactions, and hepatic cytochrome P-450 mechanism of action.

Contact: Dr. John B. Schenkman, University of Connecticut Health Center, Pharmacology Department, Farmington, Conn. 06032. An Affirmative Action/Equal Opportunity Employer. NW729)A

KING'S COLLEGE London

DEPARTMENT OF BIOPHYSICS POSTDOCTORAL RESEARCH ASSISTANTS

- 1) Biochemist with experience in nucleic acid and protein chemistry or enzymology to study the mechanism of chromatin transcription in vitro. Post tenable for 2 years from 1 September 1981. Salary in the range £6,070 to £8,515 plus £967 London allowance. USS.
- 2) Cell Biologist or Biochemist with experience in tissue culture and recombinant DNA techniques to study the structure and expression of cloned genes in eukaryotic cells. Post tenable for 2 years from 1 September 1981. Salary in the range £6,070 to £9,335 plus £967 London allowance, ISS

Please reply as soon as possible, with a curriculum vitae and the names and addresses of two referees, to Dr H J Gould, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL. (9051)A

MLSO specialising in Microbiology required in the Aerobiology Unit Cardiothoracic Institute, Bromptol Hospital, Frimley, Surrey. Wori involves investigation of occupational hazards associated with microorganisms and includes preparation of antigens and other immunological procedures. Applicants should hold HNC MLSA or equivalent. Experience in isolation and identification methods for fungi and knowledge of microbial biochemicatechniques desirable. Starting salar £4,677. Application forms availably from the Secretary, Cardiothoraci Institute, Fulham Road, Londo SW36HP. (9019)A

QUEEN ELIZABETH COLLEGE Kensington

(University of London)

MICROBIAL BIOELECTROCHEMISTRY POST-DOCTORAL RESEARCH ASSISTANTS

Applications are invited for two SR supported post-doctoral Researc Assistantships to work in a bit technology group on the development of microbial fuel cells an reactors. One post is for an electric chemist and the other for a microbia physiologist. Salary £6,070 p (minimum) plus £967 London Allow ance. The posts are available from October 1981, or as soon as possible and are tenable for 3 years.

Enquiries to Dr H P Bennette Department of Chemistry. Applications with curriculum vitae an names of two referees should be ser to Mrs J Staight, Assistant Secretary Queen Elizabeth College, Campde Hill Road, Kensington, London W 7AH. Closing date for application 31 August 1981. (9033)A

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH BIOSTATISTICIAN

pplications are invited from MSc aduates for the above post.

The incumbent will be involved in atistical aspects of research projects id surveys covering a wide field inuding, inter alia, epidemiology, incer, nutrition and immunology.

Computer facilities are available at the opportunity exists for obtaining a higher degree.

We offer: a competitive salary plus rvice bonus equivalent to 93% of ne month's salary, subsidised medal aid, Pension fund and 30 days ave per annum.

Address applications, including irriculum vitae, to: Head, Person-il Services, SAIMR, PO Box 1038, hannesburg 2000, Republic of buth Africa. (W374)A

WO POSTDOCTORAL positions e available immediately in an active NS-pharmacology laboratory. Onping research projects include: Mechanisms of modulation of ansmitter release; 2) Pre- and postnaptic mechanisms of action of etylcholine, neuroactive peptides nd other putative transmitters; Spinal mechanisms in pain and nalgesia; 4) Synaptic mechanisms in euronal plasticity. The first position suitable for a well-trained and athusiastic neurophysiologst/ europharmacologist capable of irrying out intracellular studies on ammalian central neurones. The cond position is for an imaginative surochemist who is interested in a++ and transmitter release, alysis of presynaptic membrane ructure, Ca++ binding proteins, c. Salary is negotiable. Send irriculum vitae and names of three ferees to: Dr B R Sastry, Departent of Pharmacology, Faculty of ledicine, The University of British olumbia, 2176 Health Sciences lall, Vancouver, BC, V6T 1W5, anada. (NW737)A

INIVERSITY OF ABERDEEN DEPARTMENT OF ENGINEERING RESEARCH ASSISTANT

pplications are invited from raduates in chemical or mechanical agineering, chemistry or physics for two-year post as a Research ssistant to work in a group engaged a materials behaviour. The accessful applicant will be oncerned with the erosive/prrosive effects of hot, (about 90°C) quid flowing through nozzles made f various types of steels and nickel.

The work is sponsored by the K Atomic Energy Authority, ounreay. Consideration will be ven to new or recent graduates. arting salary on Scale IB — £5,285 - £7,700.

Further particulars from The scretary, University of Aberdeen, ld Aberdeen with whom applicaons (2 copies) should be lodged by I July 1981. Ref. No. JAM/1.

(9013)A

VICTORIA UNIVERSITY OF WELLINGTON

New Zealand

LECTURESHIP IN PHYSICS (2 Posts)

Applicants for these positions should have proven excellence in research and must be able to contribute effectively to the Department's undergraduate teaching programme, which covers all the main branches of physics.

The appointees will be required to participate in the Department's research activities. These are concentrated in three fields: Condensed Matter Physics (experimental and theoretical studies of interfaces and of optical and transport properties of metal alloys and amorphous materials), Geophysics (geomagnetism, plate tectonics, seismology, vulcanology, marine geophysics, physical oceanography) and Nuclear Physics (low-energy nuclear Physics (low-energy nuclear techniques applied to material, medical and environmental studies).

For the first post the Department is seeking to appoint a person who, in addition to satisfying the above criteria, has experience in the use of microprocessors and computers in experimental physics, and could contribute to the development of a course in the physics and applications of micro-processors. Additional preference would be given to an applicant who could help establish links between existing research groups.

For the second post preference will be given to those with research interests in Geophysics who would help establish links with other research groups. An appointee in Geophysics may also become a member of the University's Institute of Geophysics.

The salary range for lecturers is NZ\$19,140 to \$23,520 per annum.

Conditions of appointment may be obtained from the Appointments Officer of the University or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 15 October 1981. (9045)A

SWISS INSTITUTE FOR EXPERIMENTAL CANCER RESEARCH

Applications are invited for an associate scientist appointment to participate in an established research project in experimental hematology/oncology, with particular emphasis on regulation of cell differentiation and drug action on cells in culture. Some years of post-doctoral experience in the field, as evidenced by published work, are desired.

This staff position is non-tenured with a 3 to 5-years contract. Starting date: January 1st, 1982 + 2 months.

Applications with curriculum vitae and names of two references to be sent to: Chief of personnel, Swiss Institute for Experimental, Cancer Research, Ref: O/N. 1066 Epalinges/Lausanne, Switzerland.

Closing date: September 10, 1981. (W372)A

Biochemists

Smith Kline & French Research Limited is the U.K. based research group of the international SmithKline Corporation. We are currently undertaking a phased expansion of our research activities as part of the move to a new 39-acre site at The Frythe near the village of Welwyn. This new facility, which is nearing completion, represents a major investment and will be totally dedicated to pharmaceutical research and development.

We have two vacancies for biochemists to join a group working on interesting and varied projects investigating the role of histamine on the brain.

The first position is suitable for a person of graduate or equivalent status, and will involve measurement of enzyme and neurotransmitter levels. Applications are encouraged from newly qualified graduates and from persons with previous experience in this area.

The second position is suitable for a recently qualified Ph. D. or equivalent. The successful applicant will have a strong interest and previous experience in the study of enzymes, not necessarily in the brain. The position is concerned mainly with investigation of aspects of neurotransmitter synthesis, catabolism and turnover.

We offer competitive salaries on incremental scales; commencing salaries will be for newly qualified graduates £5800 per annum and for Ph. D's £7500 per annum. We operate flexible working hours, and benefits include free life assurance amounting to three times annual salary, an excellent contributory pension scheme, discretionary annual bonus and membership of BUPA. A generous relocation grant is available if appropriate.

Please telephone for an application form, quoting the Ref. No. NAT/166 to:

Marie Alpar, Recruitment Administrator,

SMITH KLINE &FRENCH RESEARCH LIMITED

Mundells, Welwyn Garden City, Hertfordshi⊪e AL7 1EY. Tel: Welwyn Garden 25111 Ext 4651.

(9054)A





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UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

LECTURER/SENIOR LECTURER IN CIVIL ENGINEERING

(Geotechnology)
LECTURER/SENIOR LECTURER IN GEOLOGY
(Geochemistry and Isotope Geology)
LECTURER/SENIOR LECTURER IN GEOLOGY
(Metamorphic and Structural)
LECTURER/SENIOR LECTURER IN GEOGRAPHY AND
GEOGRAPHICAL EDUCATION.

Salary Scales:

Senior Lecturer £9,688 × 364 — 10, 780 × 373 — £12,645 Lecturer Grade I £8,777 × 364 — £10,233 Lecturer Grade II £4,836 × 348 — £6,574 × 364 — £8,396

Appointments on these scales according to qualifications and experience. Both permanent pensionable terms and short-term contracts are offered for academic posts.

Further particulars on the posts, on conditions of service and on method of application should be obtained, prior to submitting an application, from the Director, Appointments and Personnel, University of Zimbabwe, PO Box MP 167, Mount Pleasant, Salisbury, Zimbabwe, or from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of applications for all posts 31 July 1981.

Post Graduate/Post Doctoral Research Geologists/Environmental Scientists

The Universities of Cambridge and East Anglia hope soon to be able to appoint two Geologists/Environmental Scientists (1st or upper 2nd class degree) to post-graduate/post-doctoral research positions in connection with a 2-3 year joint research project on Global Palaeonvironments sponsored by the British Petroleum Company Limited Research Centre, Sunbury-on-Thames.

Applications should be submitted as soon as possible both to Professor B. M. Funnell, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, and to Dr. A. G. Smith, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ.

Further particulars may be obtained from either Professor Funnell, tel: Norwich (0603) 56161 or Dr. A. G. Smith, tel: Cambridge (0223) 355463.

(9047)A



Technische Hogeschool Delft

The Faculty of Chemistry and Chemical Engineering of the Delft University of Technology invites applications for the position of

Full Professor of Biokinetics

Biokinetics, other bio-sciences and technology are being integrated to establish a biotechnology group and thus teaching and research in biokinetics have to be directed to biotechnological application. Close cooperation with colleagues working in biotechnology will be required.

Teaching: The professor will be responsible for lectures and/or courses in Systems Analysis, and Kinetics of Microorganisms and their Active Compounds, possibly Irreversible Thermodynamics of Biological Systems. The supervision of experimental work carried out by undergraduate and graduate students will also be involved.

Research: The research task will be concerned with the development and testing of mathematical models to describe enzyme systems and the growth of and product formation by microorganisms This research will be performed in cooperation with colleagues working on biotechnology and will be directed to industrial processes and environmental control.

Administrative duties: The successful applicant will be expected to participate actively in the administrative tasks of the Biotechnology group and the Faculty.

Additional information will gladly be supplied by the Chairman of the Appointing Committee.

Applications, including a curriculum vitae, full detail of qualifications, experience and the names of at least two referees should be addressed to the Chairman of the Appointing Committee, Prof. N. W. F. Kossen, c/o Bureau van de Afdeling der Scheikundige Technologie, Postbus 5045, 2600 GA Delft, The Netherlands. Closing date for applications is 15th August 1981. (W375)A

ST JUDE CHILDREN'S RESEARCH HOSPITAL

DIVISION OF **IMMUNOLOGY**

MOLECULAR AND CELL **BIOLOGY**

Postdoctoral research traineeships are offered in a multidisciplinary research institute. (1) Biochemistry: structure and functions of biological membranes; biological regulation mechanisms with emphasis on hormonal action; receptor functions of normal and neoplastic growth and cellular messengers; and control (W Y Cheung, M Morrison and G Schobaum); (2) Immunology: regulation of antibody formation; macrophage functional heterogeneity and characterization of macrophage receptor functions and structures (F L Adler, M Fishman and W S Walker); (3) Pharmacology: human and animal cancer chemotherapy; drug-induced changes in DNA replication and repair synthesis; genetic, biochemical and physiological approaches to analysis of mechanisms of drug resistance in human cells (T P Brent, A Fridland and A Welch), and (4) Virology: molecular biology and epidemiology of viruses; eukaryotic gene expression and regulation (A Granoff, D Kingsbury and R G

Applicants must be citizens or permanent residents of the US. Please send curriculum vitae, names of three references and a brief statement of career objectives to Dr M Fishman, Division of Immunology, St Jude Children's Research Hospital, 332 N Lauderdale, PO Box 318, Memphis TN 38101

(NW732)A

UNIVERSITY **OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY**

Applications are invited for a postdoctoral research position to investigate the molecular genetics of bacterial plasmids. Experience ir this area, and particularly in plasmic DNA manipulation, is desirable. The appointment becomes available this autumn, and will be for one to three years. Salary will be on Research Range 1A.

Applicants should send a CV, references and a letter describing their research interests to Dr N S Willetts. Department of Molecular Biology, University Edinburgh, Edinburgh EH9 3JR. Please quote Reference 5031.

Postdoctoral Research Associates BROOKHAVEN NATIONAL LABORATORY

Two Postdoctoral Research Associate positions will be available in October, 1981, in the Biology Department at Brookhaven Laboratory.

One position is for study of photosynthetic mechanisms of nitrogen reduction heterocysts of cyanobacteria. Optical and EPR spectroscopic techniques are applied to isolated heterocysts and photosynthetic membrane pre-parations. A PhD in plant physiology or biochemistry, with experience in photohydrogen production, nitrogen fixation or hydrogen assimilation research, preferably in cyanobacteria, is desirable.

The other position is for study of electron flow and energy conversion mechanisms in higher plant photosynthesis. Techniques used include optical and EPR spectroscopy of intact chloroplasts and isolated membrane complexes. A PhD in biochemistry, plant physiology or biophysics, with experience of bioenergetic and electron transport systems, preferably in photosynthesis, is desirable

Initial appointments will be for one year, at a minimum salary of \$14,000 per year, with possibility

Candidates should send résumé and names of three referees to Dr Geoffrey Hind, Biology Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, NY 11973.

An Equal Opportunity Employer m/f (NW731)A

BROOKHAVEN NATIONAL LABORATORY



UNIVERSITY OF EAST ANGLIA Norwich

Applications are invited for the post of

POST-DOCTORAL RESEARCH ASSOCIATE IN SYNTHETIC ORGANIC CHEMISTRY

n the School of Biological Sciences under a Cancer Research Campaign grant. The work will be on the synthesis of novel derivatives of antileukaemic drugs. The appointment will be for one year initially but may be renewable for up to a further three years. Salary on the 1A scale for Research and Analogous Staff (£6,070 -£10,575 p.a.) according to age and experience.

Applications with full curriculum vitae and the names of 2 referees should be sent before 20th July 1981 to Dr. D. M. Tidd, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ

(9010)A

UNIVERSITY OF **PENNSYLVANIA**

DEPARTMENT OF **HUMAN GENETICS**

THREE TENURE TRACK **POSITIONS**

o begin at the Assistant Professor evel are available in the Department of Human Genetics. Candidates (MD or PhD) trained in modern molecular enetic techniques and with a record of research in genetics are urged to

One position is to be filled by an MD researcher who would be qualiied to hold a joint clinical appointment. Areas of interest in molecular approaches include: evolution, levelopment, gene regulation, molcular cytogenetics, polymorphism and molecular mechanism of disease.

Send résumé to: Dr R Schmickel, Dept of Human Genetics, Room 195 Med Labs Bldg/G3, University of Pennsylvania, Philadelphia, PA 19104. An Equal Opportunity/ Affirmative Action Employer. (NW726)A

ROBERT GORDON'S INSTITUTE OF TECHNOLOGY

Aberdeen

SCHOOL OF MECHANICAL AND OFFSHORE ENGINEERING **RESEARCH FELLOW**

Materials scientist for major research project on marine corrosion and fouling of North Sea oil and gas installations, financed by SERC Marine Technology Directorate. RGIT's marine corrosion research group collaborates with University of Aberdeen marine biologists and zoologists and with offshore industry. The appointment will be for one year in the first instance.

Salary range £6,070 to £6,880 with appropriate placing according to qualifications and experience.

Details from Secretary, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 (9043)A



Yale University

DIRECTOR

PEABODY MUSEUM OF NATURAL HISTORY

Applications are invited for the position of Director of the Museum at the rank of full professor, and with appointment (according to field) to the Departments of Biology, Geology and Geophysics or Anthropology. Yale University is seeking a scholar of international reputation who will develop interdisciplinary research and teaching programs in the natural sciences through the Peabody Museum and the affiliated academic departments.

Applications, including a curriculum vitae and the names of three references, should be sent to Professor Keith S. Thomson, Dean of the Graduate School of Arts and Sciences, Yale University, P.O. Box 1504A, Yale Station, New Haven CT 06520. Closing dates for applications - September 30, 1981.

Yale University is an equal opportunity, affirmative action employer.

(NW744)A

POSTDOCTORAL POSITIONS available for study of mechanism of linear DNA replication. Send CV and names of to references to Dr Junetsu Ito, Department of Molecular and Medical Microbiology, College of Medicine, The University of Arizona, Tucson, Arizona 85724. (An Equal Opportunity/Affirmative Action employer). (NW740)A

University of Saskatchewan CELL BIOLOGIST

Applications are invited for a position at the level of assistant professor/associate professor in either the Department of Micro-biology or the Department of Biochemistry to be sponsored by a National Cancer Institute of Canada development program for cancer re-search at the University of Saskatchewan. The program provides for sal-ary support for up to 5 years and funds for research and equipment. The appointee would be expected to work in collaboration with a multidisciplinary team who research areas include tumor metastasis, mem-brane biochemistry, chemotherapy and tumor immunology.
A PhD with at least 2-3 years post-doctoral training is required.

Applicants should send curriculum vitae, research interests, re-prints and names of three references to Dr I Ramshaw, Department of Microbiology, Univer-sity of Saskatchewan, Saska-toon, Saskatchewan S7N 0WO, Canada. (NW738)A MOLECULAR BIOLOGIST

SmithKline Corporation, a diversified multinational health care firm, located in

Philadelphia, PA, is seeking a person to develop host-vector systems for cloning and expression of eukaryotic genes. This position is located at our suburban facility, 17 miles west of Philadelphia.

The successful candidate for this new position should be capable of directing a research program on molecular genetics and become a leader in cloning and expression of eukaryotic genes. This person should have a PhD in Biological Sciences with experience in modern DNA technology as applied to one of the following areas: yeast host-vector systems, gene expression, or synthesis of cDNA.

SmithKline offers an excellent compensation program and opportunity for professional growth. Please send resume to: SMITH KLINE & FRENCH LABORATORIES, A Division Of,

SMITHKLINE CORPORATION. 1522 Spring Garden Street, Philadelphia, PA 19101. We are an equal

opportunity employer, F/H/M/V.

(NW728)A

SmithKline

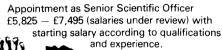
British Museum (Natural History) Electron Microscopist

... to join a team involved in taxonomic research covering the whole field of natural science. Work includes preparing material for both scanning and transmission microseopy: monitoring quality and recording results; first line maintenance of microscopes and high

vacuum units and maintenance of a small

Candidates normally under 27, should have a degree in biological sciences.

Experience as a technician in an electron microscope unit would be advantageous.



For futher details and application form (to be returned by 31 July 1981) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please auote ref: SB/68/DK.

(9016)A

UNIVERSITY OF STRATHCLYDE DEPARTMENT OF COMPUTER SCIENCE

Professor of Computer Science

Applications are invited for a second Professorship in the Department of Computer Science. To preserve the balance of interest in the Department, candidates with a knowledge either of Data Bases or the Theory of Computer Science are particularly encouraged to apply

The post will be remunerated within the professorial range for universities with USS benefits.

Application forms and further particulars (quoting 27/81) can be obtained from the Registrar, University of Strathclyde, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by 31 July 1981. (9028)A

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WE HAVE THE CAPITAL TO DEVELOP THESE NEW DRUGS

We are not a pharmaceutical firm, but a swiss based independant financial group, and we are prepared to let you participate in a capital venture for the exploitation of your

Our object: the development of new biological or chemical products (which have previously had a pharmacological screening) up to the stage of clinical tolerance and effectiveness required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to: DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W368)A

ASSOCIATESHIPS

THE UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY **POSTDOCTORAL**

RESEARCH ASSOCIATESHIP

Applications are invited for a Postdoctoral research associateship to study the composition, spatial organisation and reconstitution of the methanol oxidising respiratory chain of the methylotrophic bacterium Methylophilus methylotrophus. This position, which is supported by an SRC/ICI Cooperative Research grant, is tenable for up to three years and will carry an initial salary of up to £6,880 per annum at age 26 (with USS benefits). Experience in bioenergetics and/or enzyme purification is desirable.

Applications, with a curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr C W Jones, Department of Biochemistry, University of Leicester, Leicester LEI 7RH (please mark envelope "Postdoctoral Associateship").
(9052)O



STUDENTSHIPS

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF PHYSICS

Applications are invited for an SRC CASE STUDENTSHIP in the above department in conjunction with the British Antartic Survey. Involves studies of the Magnetosphere and Ionosphere at Halley, Antarctica, using the Sheffield ELF/VLF Goniometer and the recently installed advanced Ionosonde.

Tenable for three years during which time the recipient will analyse and interpret data acquired in Antarctica with a view to submission of a PhD thesis and publication. A good degree in Physics required.

Details from Professor T R Kaiser, Department of Physics, the University of Sheffield, The Hicks Building, Hounsfield Road, Sheffield S10 2TN, to whom applications should be sent together with the names of two referees. Quote ref: R606/G (9053)F

NOTTINGHAM UNIVERSITY

PHARMACY DEPARTMENT

RESEARCH STUDENTSHIPS The Pharmaceutics Section of the

Pharmacy Department has available SRC Research Studentships for studies on the physico-chemical properties of pharmaceutical systems and their relevance to the biological availability of drugs.

Pharmacy and Chemistry graduates interested in further details should apply to Professor S S Davis, Department of Pharmacy, University of Nottingham, University Park, Nottingham. Tel: 0602-56101 Ext (9001)F

BRISTOL **POLYTECHNIC** SCIENCE DEPARTMENT SRC RESEARCH **STUDENTSHIP**

Two SRC CASE awards have recently been approved for the following projects in the Department of Science. Duties to commence October 1981.

(1) "A study of the physiological and biochemical aspects of the development of seaweed spores" in conjunction with the Admiralty Marine Technology Establishment, Naval Dockyard Laboratories, Portsmouth.

(2) "A study of the role of ATPase enzymes in potassium transport in the midgut of caterpillars"—in conjunction with Shell Biosciences Laboratory, Sittingbourne.

Applications are invited from persons holding (or expecting to hold) a First of Upper Second Class Honours Degree in an appropriate subject. The successful candidates will be nominated to the SRC for the award of studentships. It is anticipated that the students will register for research degrees. Applications and enquiries should be sent as soon as possible to Dr T Green, Head of the Science Department, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol BS161QY. (9022)F

STUDENTSHIPS continued

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BIOLOGY

SRC CASE STUDENTSHIPS

pplications are invited for two SRC ase Studentships in collaboration ith Glasshouse Crops Research stitute, Rustington, Sussex for search leading to the degree of hD. The projects are concerned ith sugar uptake by tomato fruit otoplasts and vacuoles in relation sink strength (Professor J L Hall ad Dr L C Ho) and with hormonal antrol of inflorescence development tomato (Dr D A Morris and Dr Menhenett).

Applicants should have, or expect obtain, a first or upper second class mours degree in a related subject.

Applications, including the names f two referees, should be sent as son as possible to Professor J L Hall ad/or Dr D A Morris, Department Biology, University of Southampon, Highfield, Southampton SO9 NH, from whom further particulars ay be obtained. Please quote Ref: N. (9011)F

UNIVERSITY OF READING

DEPARTMENT OF PHYSIOLOGY & BIOCHEMISTRY

SRC CASE STUDENTSHIP

pplications are invited for an SRC ASE Studentship tenable from 1 ctober, 1981. Candidates should we a good honours degree in ochemistry or a related discipline. he project is in collaboration with IRC Toxicology Research Unit, arshalton, and concerns the etabolism of 6-chloro-6-deoxylars and their action as male intraceptives.

Applications should be sent to: rofessor G M H Waites, Departent of Physiology & Biochemistry, he University of Reading, /hiteknights, Reading RG6 2AJ. (9021)F

UNIVERSITY OF LIVERPOOL

SRC CASE STUDENTSHIP

Mössbauer studies of the Active te of Benzene Dioxygenase"

Applications are invited for an RC CASE studentship to be held in e Department of Physics in connection with the Biosciences aboratory of Shell Research Ltd. he reason, which is expected to take ree years and lead to the award of a 1D degree, will involve the pre-tration of samples of the enzyme id investigation of these samples ider a variety of conditions using ossbauer spectroscopy.

In addition to the usual SRC grant supplement of £200 pa will be paid.

Applicants should have, or expect obtain, a first or upper second class mours degree in biochemistry, bio-ysics, chemistry, physics or related hierts

Applications with a brief rriculum vitae and the names and ldresses of two referees should be nt as soon as possible to The gistrar, The University, PO Box 7, Liverpool L69 3BX, from whom rther details may be obtained. uote Ref RV/847/N (9050)F

UNIVERSITY OF

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

LEAF MICROCLIMATE AND THE PERFORMANCE OF FOLIAR HERBICIDES SRC 'CASE' STUDENTSHIP IN CO-OPERATION WITH

SHELL RESEARCH LTD

A studentship is available to investigate microclimatic factors which influence the action of chemicals applied to foliage of crop plants. The student who will probably have a first degree in agricultural, biological or environmental science will spend part of the time at Shell Research Laboratories, Sittingbourne, Kent. Experience in environmental measurement, or in measuring properties of leaf surfaces would be advantageous. The usual SRC studentship grant will be supplemented by £250 pa.

For further details contact Dr M H Unsworth, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD. Telephone (05097) 2386. (9002)F

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY POST GRADUATE STUDENTSHIPS

Two SRC (CASE) post-graduate studentships are available in Neurophysiology/Neuropharmacology. The experimental work will investigate the responses of single central neurones in vivo to: a) the benzodiazepines and; b) substance P/5HT interaction on nociceptive neurones

Curriculum vitae to Dr M H T Roberts, Department of Physiology, University College, PO78, Cardiff CF11XL. (9031)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF SURGERY
BDH RESEARCH
STUDENTSHIP

A 3 year research studentship is open to candidates with a first or upper second class honours degree in Biochemistry. The value of the studentship is in line with current MRC, SERC Research Studentships. The successful candidate will form part of a multi-disciplinary research team involved in the investigation of the regulation of intermediary metabolism in the post-surgery period and the effect of postoperation metabolic support. This work will be carried out in the Surgical Metabolism Unit, Department of Surgery, University of Aberdeen, under the supervision of Dr J Broom. This unit has close ties with both the Departments of Biochemistry and Chemical Pathology, University of Aberdeen.

Applications, including the names of three referees should be made to Dr J Broom, Department of Surgery, Foresterhill, Aberdeen AB9 2ZD.

Rainbow Research Scholarship

Brewing Research

'Regulatory Mechanisms Influencing Production of Alcohols by Yeasts'

Bass Brewing Limited, in association with the University of Birmingham, invite applications for the Rainbow Research Scholarship which has been founded in memory of the contribution made to brewing science by the late Dr. C. Rainbow, D.Sc.

Applicants, male or female, must possess a first or top second class honours degree in biochemistry, microbial genetics or microbiology.

The successful candidate will spend a period of 3 years in the Research Laboratories of Bass Brewing Limited at Burton-on-Trent with short periods at the University of Birmingham. Research on the above topic will lead to submission to the University of Birmingham for the degree of Ph.D.

For the duration of the scholarship, the successful candidate will be a temporary member of the staff of Bass Brewing Limited, who will meet the costs of the project and the associated university fees.

Comprehensive applications by letter with curriculum vitae should be submitted to:

Research Manager, Bass Brewing Limited, High Street, Burton-on-Trent, Staffs. DE14 1JZ.

Bass Limited



H(950)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES

POSTGRADUATE SCHOLARSHIP

Applications are invited from United Kingdom graduates with a 1st or II(i) Honours degree in Agricultural Science, Biology, Physiology or Zoology for a 3 year postgraduate study concerning endocrine abnormalities causing embryo mortality in cattle.

Applications, including curriculum vitae and names of two referees should be sent as soon as possible to Professor G E Lamming, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE12 5RD.

(9003)H

The Sports Council SPORTS SCIENCE SCHOLARSHIPS

As part of its continuing effort to improve scientific services to sport the Council is once again offering 2 Scholarships for full-time PhD research. The awards will run from October 1981, initially for 2 years, renewable for a third. The subject-matter should be in the field of medicine, physiology, biochemistry/nutritien, motor learning, or social psychology. Projects leading to practical applications are likely to receive preference.

Departments with suitable candidates are asked to submit applications specifying the research topic and the methods proposed for tackling it, the name and background of the Supervisor, and details of any minor equipment/travelling/other expenses. The candidate's full CV should be enclosed.

3 copies of the application should be sent to the Principal Research Officer, The Sports Council, 70 Brompton Road, London SW3 1EX, by July 31, 1981. (9032)H

WANTED



Beckman 890 Sequencer - Call collect R. Kutny (302) 772-1442 or write Du Pont Experimental Station, CR&D, Building 228, Wilmington, DE 19898

(NW743)L

CONFERENCES and COURSES

University of London
British Postgraduate Medical Federation

CARDIOTHORACIC INSTITUTE (In association with the Brompton Hospital)

ANNUAL PHARMACOLOGY OF ASTHMA COURSE

23rd — 27th November, 1981

This Course is of interest to scientists involved in research relating to asthma and to clinicians with an active interest in the pharmacology of asthma and the mode of action of anti-asthma drugs.

Topics to be included:

- * Leucotrienes
- * Prostaglandins
- * PAF acether
- cAMP and cell activation
- Receptor control of cell activation and receptor expression
- * Provocation testing
- Cell accumulation, Oedema, Muco-ciliary function in asthma

FEE: £150 inclusive of coffee, lunch and tea.

Places are restricted to 50. Early registration is strongly recommended.

Application forms may be obtained from: The Dean's Secretary, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

(9020)C

POSITION WANTED

PhD MARINE sciences, 25 publications. English/Spanish speaker. Long experience in South America and UK. University Lecturer, research worker. Serve anywhere. Highest references. Write Box 9057 c/o Nature, 4, Little Essex Street, London WC2R 3LF (9057)B

FELLOWSHIPS

POSTDOCTORAL Fellowship: To study hormonal regulation of vitamin D metabolism in mammals. Theoretical and/or practical experience required. Contact: Dr E M Spencer, Children's Hospital of San Francisco, 3700 California Street, San Francisco, Calif. 94118. Equal Opportunity Employer, M/F/H. (NW723)E

THE QUEEN'S UNIVERSITY OF BELFAST

Department of Pure and Applied Physics

POSTDOCTORAL FELLOWSHIP

Applications are invited for a Post-doctoral Research Fellowship funded by SERC in the Reactive Scattering Group for a period of up two years. The successful candidate will be involved in the further development of UV laser induced fluorescence techniques and their application to studies of energy disposal in chemical reactions.

Salary range: £6,072 — £6,882 pa, initial placing depending on age and qualifications. Applications, which should include CV and names and addresses of two referees, should be sent to the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. Closing date: 31 August 1981. (Please quote Ref. 81/N.) (9027)E

CITY OF LONDON POLYTECHNIC

SIR JOHN CASS SCHOOL OF SCIENCE AND TECHNOLOGY DEPARTMENT OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a research fellowship available from 1 September 1981 to work with Dr J North on the genetics and biochemistry of ribosomal mutants in the basidiomycete fungus Coprinus cinereus. Applicants should have obtained or be about to obtain a PhD. A background in genetics is required; experience of the biochemical genetics of fungi and/or in vitro protein synthesis would be preferred.

Salary (Researcher B) £6,464 per annum in the first year, rising to £6,867 in the second year and to £7,260 in the third year plus London Allowance of £759 per annum.

Further information can be obtained from Dr North at the Department of Biological Sciences, City of London Polytechnic, Calcutta House Precinct, Old Castle Street, London E1 7NT to whom applications, including a curriculum vitae and the names and addresses of two referees, should be sent to arrive within three weeks of the appearance of this advertisement. (9008)E

THE UNIVERSITY OF AUCKLAND New Zealand POSTDOCTORAL FELLOWSHIP

The Haemoglobin Group in th Departments of Zoology and Bic chemistry is studying the physiolog and biochemistry of haemoglobin i lower vertebrates. A fellowhip available for a physiologica zoologist or biochemist to participat in this work. Those with experienc of respiratory physiology and/c haemoglobin separation technique are particularly welcome to apply Applicants must have completed PhD or its equivalent before takin up the Fellowship, which is available for 12 months from February 1982. monthly allowance of NZ\$1,519 wi be paid and there is provision for assistance with fares. Further pair ticulars are available from Dr R M (Wells, Department of Zoology, Un versity of Auckland, Private Bag Auckland, New Zealand. (9038)

UNIVERSITY OF SUSSEX

School of Molecular Sciences SERC POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from sui ably qualified persons for a pos doctoral research Fellowship to it vestigate the feasibility of catalyt systems for the production corganonitrogen compounds fron N₂. Candidates should have cor siderable experience of preparative coordination or organometallichemistry, and a knowledge of electrochemical technique would also buseful.

The appointment, under the super vision of Dr G J Leigh and Dr C Pickett, would be for a maximum of years, starting 1st October 1981 or a soon as possible thereafter. Th starting salary will be towards th lower end of the scale for th Research Fellow Grade 1A, £6,070 - £10,575 pa.

Candidates should submit a C and the names of three referees a soon as possible to the Sub-Dear School of Molecular Sciences, Un versity of Sussex, Brighton BN1 9Q. (9035)E

ASSISTANTSHIP

LEICESTER POLYTECHNIC School of Chemistry RESEARCH ASSISTANTSHIP

Applications are invited from graduates to work on "Film Formation by PTFI Dispersions", a collaborative project with the Petrochemicals and Plastic Division of ICI Limited. The appointments will be made for 3 years on a salar scale starting at £5,268 per annum.

Suitable candidates will be encouraged to register with CNAA for a highe degree, and will probably have a first degree in chemistry, physics or polyme science. The project will involve several aspects of physical chemistry.

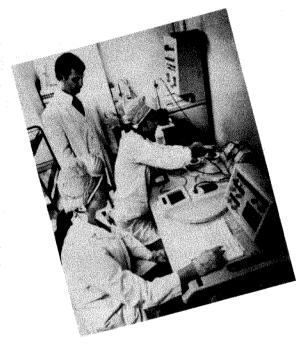
Application forms and further details available from Staffing Officer Leicester Polytechnic, PO Box 143, Leicester LE1 9BH. Tel: (0533) 55155 Ext. 2303/1. (9029)P

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Contact For Further Details



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AMERICAN JOURNAL OF PRIMATOLOGY

Volume 1, 1981, Four Issues

The rapid growth of interdisciplinary primatology within recent years has dictated the need for an efficient domestic journal devoted to publication of high quality manuscripts spanning the entire spectrum of primatology.

The journal publishes original research reports, scholarly reviews, brief reports, and book reviews on all topics relevant to the study of primates, including all aspects of their anatomy, behavior, development, ecology, evolution, genetics, nutrition, physiology, reproduction, systematics, conservation, husbandry, and use in biomedical research. Manuscripts demonstrating a strong comparative perspective are especially welcomed.

The American Journal of Primatology is published quarterly. Should the volume of manuscripts demand it, the number of issues per year will be increased. Manuscripts are reviewed within one month of receipt and are typically published within four to eight months of final acceptance. The results of reviews, along with acceptance, suggestions for revision, or fully justified rejection, are rapidly communicated to authors. Instructions for contributors can be obtained from the editor or the publisher.

Manuscripts for consideration should be sent to: J. Erwin, Ph.D., Editor • American Journal of Primatology • P.O. Box 96 • Honeydew, CA 95545

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Proceedings of the Fourth Meeting of the American Society of Primatologists, June 2-5, 1981, San Antonio, Texas.

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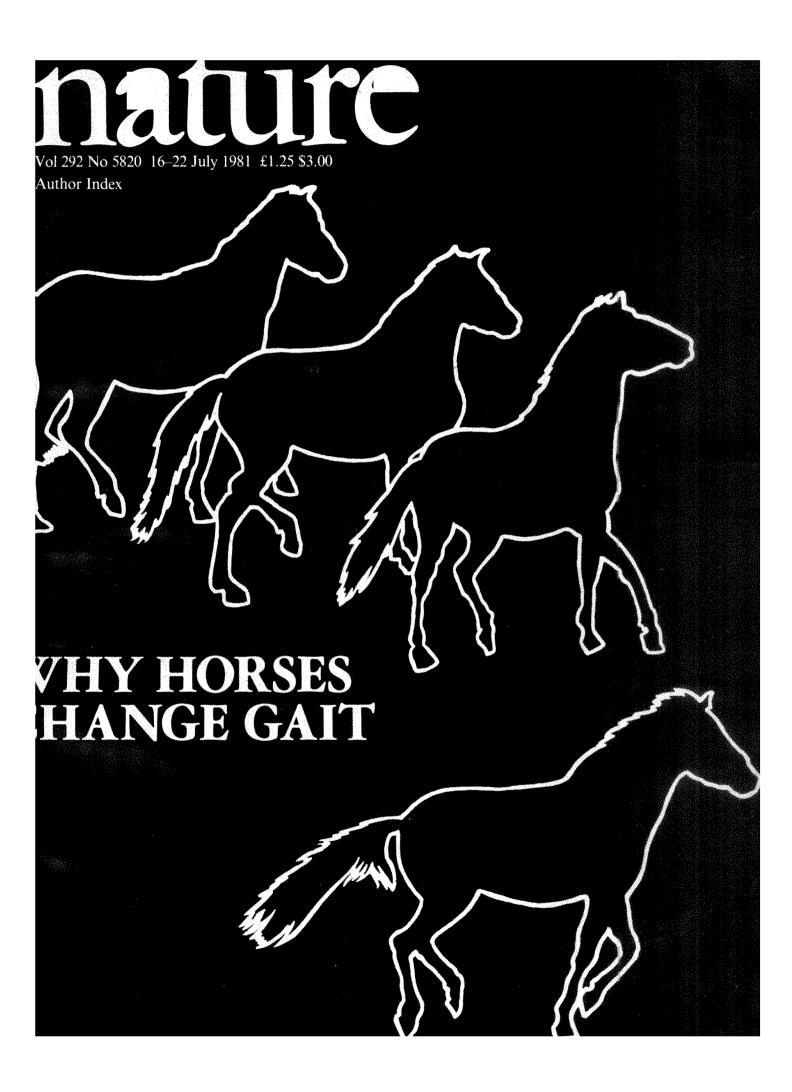
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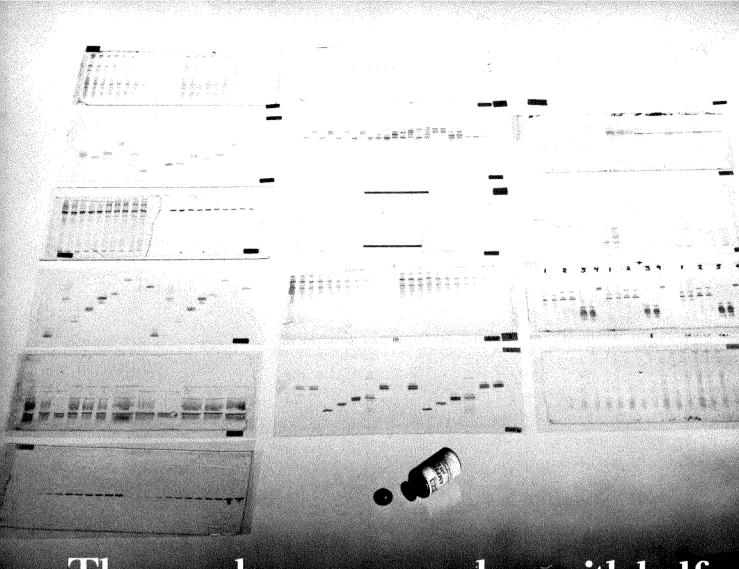
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Tu, C.-P.D. and Cohen, S.N., Gene, 10: 177, 1980

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Hinf I Digest of Plasmid DNA, pBR322 (control to check system

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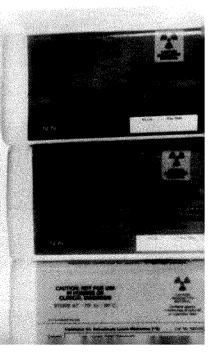
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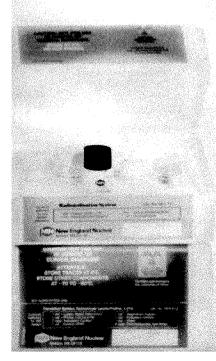
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axam, A.M. and Gilbert, W., Methods in zymology, 65 (1980)



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³Manley, J.L., Fire, A., Cano, A., Sharp, P.A., and Gefter, M.L., PNAS (U.S.A.), **77:** 3855 (1980)

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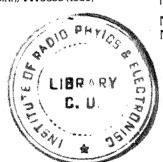
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Wells, R. D., Hardles, S. C., Horn, G. T., Klein, B., Larson, J. E., Neuendorf, S. K., Panayotatos, N., Patlent, R. K. and Seising, E. (1980) Methods in Enzymol. 65, 372-347.

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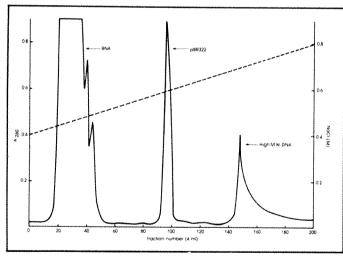


Figure 1. RPC-5 ANALOG chromatography of a total nucleic acid extract of a (pBR322). The nucleic acids were added to the column in 0.2 M NaCl, 10 mM Tris-HCl (pH 7.2) and 10 mM EDTA and eluted with an increasing linear salt gradient (0.4 - 0.8 M NaCl).

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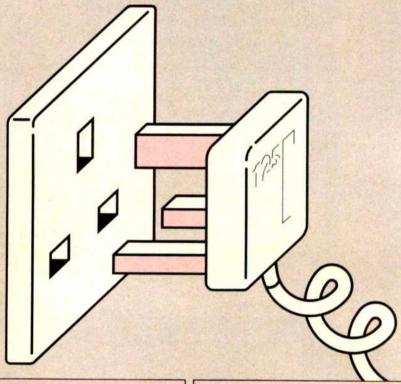
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EDITORIAL OFFICES

London

4 Little Essex Street, WC2R 3LF Telephone: (01) 836 6633 Telex: 262024 Telegrams: Phusis London WC2R 3LF

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff

Alun Anderson Sara Nash Philip Campbell Peta Pickering Isobel Collins Konrad Guettler Judy Redfearn Miranda Robertson Tim Lincoln
Naomi Molson
Robert Walgate
Charles Wenz Nigel Williams

Washington News Bureau 801 National Press Building, DC 20045 Telephone: (202) 737-2355 Telex: 64280 David Dickson (Washington News Editor)

Publisher: Elizabeth Hughes Marketing Director: Ray Barker International Advertising Manager: Andy Sutherland

Features Advertising Manager: Marion Delaney
Promotion Manager: Jonathan Earl

New York 15 East 26 Street, New York, NY 10010 Telephone: (212) 689-5900 American Publisher: Robert Ubell American Advertising Manager: Henry Dale Marketing Manager: Sheila Kane

> Classified Advertising
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Nuclear issues in the Middle East

The conclusion that the Israeli raid on the Iraqi reactor at Tamuz on 7 June will have caused more diplomatic than physical damage is strengthened as the weeks pass. The organization now most on the defensive is not the government of Israel but the International Atomic Energy Agency in Vienna, which has had the invidious task in the past month of defending itself against the charge that its technical safeguards intended to prevent the diversion of fissile materials to military purposes are inadequate to the need. At the same time, many readers have taken offence at the leading article of 18 June which, among other things, argued that the Israeli attack was unjustifiable and that steps should now be taken, chiefly by the United States, to ensure that Israel signs the Non-Proliferation Treaty. The letters of protest have several themes in common — that the Tamuz raid was not a breach of international law because Iraq and Israel are technically in a state of war; that Iraq's motive for embarking on a nuclear programme cannot have been pacific, given its substantial reserves of petroleum; that Iraq's membership of the Non-Proliferation Treaty is irrelevant because the safeguards procedures are ineffective; and that in any case it is no business of a journal such as Nature to discuss political questions such as those prompted by the raid on Tamuz.

The real world, unfortunately, is neither as tidy nor as simple as these protests imply. Those who complain that a scientific journal has no place in politics are in general correct. It would, for example, be inappropriate (and deeply offensive to the bulk of readers) if a journal such as this were to take sides in a political election, "endorsing" this or that candidate for, say, the United States presidency (which is not, of course, to say that candidates' election promises about support for research are of no interest). There are, however, exceptions to the general rule. To the extent that complaints about the British government's policy on the universities chime in with those of the government's political opponents, they are inescapably political. And there is hardly any aspect of the Non-Proliferation Treaty, a matter of inescapable interest to the scientific community, that is strictly technical. The treaty, founded on the bargain between non-nuclear and nuclear states that the former would refrain from making weapons and that the latter would help with the development of civil nuclear technology, is intrinsically as much political as technical. More generally, it has been plain since the early 1960s that the widespread wish to inhibit the spread of nuclear weapons cannot be attained by strictly technical means; as the environmentalists used to say, there is no technological fix. Neither the treaty nor the safeguards elaborated since it came into force eleven years ago are a cast-iron assurance that weapons will not spread. International pressure and the fear of strategic countermeasures are essential ingredients in the now elaborate but untidy system of restraints that has developed. Those who consider that the reality is different should ask themselves why the first Indian nuclear explosion has not been followed by a second even after the passage of seven years.

But why should the scientific community have an interest in arrangements for inhibiting the spread of nuclear weapons? To quote J.R. Oppenheimer's remark at Alamogordo in 1946 that "the physicists have known guilt" would be to over-dramatize the case. Rather, the development of nuclear energy is the most striking illustration so far that research and development is rarely a benefit unalloyed. For the past three decades, the scientific community has been the chief source of policies for the sensible control of nuclear energy, both domestically and internationally.

Individual scientists have had a decisive influence on diplomatic innovations whose effect is to inhibit the spread of nuclear weapons. Thus the Partial Test-Ban Treaty is more a monument to Dr Jerome Wiesner's flair and persistence than to the good sense of his colleagues in the White House in the early 1960s. The safeguards system now operated by the International Atomic Energy Agency, although imperfect, is one of many illustrations that technical devices (and people) can serve wider interests. But to suppose that in such matters technical people should be inarticulate servants of the diplomats is not merely to ask too much of flesh and blood but to ignore the constructiveness of what the technical community has to offer. As it happens, the present is a time of exceptional anxiety about the future development and deployment of nuclear weapons. Can it seriously be suggested that technical people should help to work out and implement agreements of the kind for which people like Chancellor Helmut Schmidt have been asking for the past several months in strict innocence of the underlying issues? That may be a more substantial dereliction of responsibility.

So what are the issues provoked by the raid on Tamuz? The efficacy of the international agency's safeguards systems is certainly one of them. And it is proper openly to acknowledge that the safeguards system is not leak-proof. It never has been and never could be, which is why those who now pretend that the raid on Tamuz has for the first time revealed the truth are guilty of disingenuousness. For the system depends on the collaboration of national governments, which must disclose their activities in nuclear energy (and which could, in theory, keep some hidden). It supposes that inspections of, say, a reactor will reveal all, when some parts of all nuclear installations must be inaccessible on safety grounds. It relies to some extent for the detection of violations on the accumulation of information about a nuclear installation over a period of time, while on paper the treaty permits the resignation of any member state on three months' notice. It requires (but unnecessarily) that inspectors should keep the information they gather confidential, although the appearance of Dr Roger Richter before the Senate Foreign Relations Committee in Washington three weeks ago on the introduction of Senator Alan Cranston (who is not, as erroneously described last week, the chairman but only a member of the committee) shows that even that stipulation can be circunvented.

But so what? The Non-Proliferation Treaty is not the only treaty of its kind that relies on less than perfect arrangements for the detection of violations. Who, for example, would pretend that the requirements of the Threshold Test-Ban Treaty (which restricts the yield of underground nuclear tests to the equivalent of less than 150,000 tonnes of TNT but which relies on seismic measurements of actual yield that may be in error by a factor of two) are cast-iron? For at least two decades it has been accepted that no arms control treaty can embody cast-iron arrangements for verification. The objective is to ensure that the risk of detection is great enough to deter violation - and that the diplomatic risks of abrogation are equally forbidding. It was irresponsible of Senator Cranston to pretend that Dr Richter's evidence amounted to the first discovery of these truths, just as it was reprehensible of Dr Richter to give the Foreign Relations Committee a partial account of the internal discussions of the Iraqi reactor within the international agency. (Dr Hans Gruemm's account on page 187 is inherently more credible.)

Two other issues have been raised by correspondents, of which

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the argument that the raid on Tamuz was legal because Israel and Iraq have never signed a peace treaty with each other is the least substantial. The delicacy of the military and political problems of the Middle East is notorious and several states, supporters of Israeli independence prominent among them, have spent much of their energy in recent years (no doubt partly from self-interest) seeking devices (of which Israel has known and approved) for making life less dangerous for Israelis and Iraqis. Mrs Jeanne Kirkpatrick, the United States representative at the United Nations, told the Security Council on 19 June that Israel had "hurt, not helped, the peace and security of the area". The same meeting of the council went on unanimously to adopt a resolution condemning the Israeli raid on Tamuz, asserting that Iraq should be compensated and demanding that Israel's nuclear facilities should be subject to international safeguards. Legality does not often excite such strictures.

What, then, happens next? The new government of France, which originally agreed to the contract under which the reactors at Tamuz were built, has fortunately reafffirmed its curious quasiadherence to the Non-Proliferation Treaty - although not a signatory itself, it requires its nuclear customers to sign. Indeed, President Mitterrand, no doubt in reaction to recent bellicosity about nuclear weapons by President Hussein of Iraq, has made plain his intention of being even tougher in the future. In the United States, there is also now a prospect of a more rational antiproliferation policy — the new Administration is apparently to announce this week its acceptance of the explicit bargain in the treaty that nuclear powers must be dependable sources of nuclear technology and materials if non-nuclear powers are to continue with their undertaking not to make nuclear weapons. (The promise does not distinguish between states with and without reserves of petroleum, nor should it.) There is much that could be done to improve the safeguards procedures of the international agency, but to the extent that non-nuclear powers may be required to assume obligations not originally contracted for, these questions should be raised at the next review conference of the treaty four years from now (which, as these things go, is almost tomorrow). Some attention should also be paid to recent Israeli advocacy of a nuclear-free zone in the Middle East - the stumbling block so far is that such a treaty would entail recognition of the state of Israel by its Arab neighbours and thus a resolution of the whole problem of the Middle East, but that is not a sufficient reason for ignoring the subject. The most immediate need, however, is that the nuclear powers and especially the United States should give whatever help they can to the international agency that has the invidious task of supervising the working of the Non-Proliferation Treaty. Behaving as if the treaty means what it says, which it does, is the most urgent need. Beyond that, it is essential that the nuclear powers signatory to the treaty should recall the reasons why they were given a drubbing at the second review conference in August last year, and that they should do what they can to implement those provisions of the Non-Proliferation Treaty that promise further steps (beyond Salt I) towards strategic arms control. Is it possible that Mr Eugene Rostow, now confirmed as director of the United States Arms Control and Disarmament Agency and a hawk in President Kennedy's administration, will be a dove in Mr Reagan's?

Lightning without cause

The British university system, more than ever alive with rumour when it is not already on vacation, is buzzing with a curious canard; is it possible, people are asking, that the way in which the cuts announced last week by the University Grants Committee have fallen has been surreptitiously guided, even invited, by stoolpigeons within the institutions concerned? This fanciful notion seems in many places to be the simplest explanation of how, without apparent consultation, the University Grants Committee has been uncannily perceptive in its suggestions of how individual universities should trim their sails. A university whose members have for years been complaining among themselves that too many

biological departments teach their own individual introductory courses is likely now to have been told to cut back on the teaching of "conventional biology". Chemistry departments here and there, whose members from time to time hang their heads with shame about the quality of their collective research, have found that their universities (against the general trend) have been advised to cut back on physical science. But who can have spilled the beans about this supposedly secret knowledge? Academics are understandably looking with suspicion at their vice-chancellors, those people who travel most often to London.

The truth is simpler. Supposedly secret knowledge is usually not as secret as its holders suppose. Many of the grants committee's negative decisions - "cut this, cut that" intelligible. For more than a year, for example, the committee has been urging that too many British universities have been making too much provision for the teaching of Russian; is it surprising that Russian departments were conspicuous among those marked out for oblivion two weeks ago? It is a misfortune that so few would-be students at British universities seek to be Russian experts. The grants committee's recommendations in other fields, and in the sciences especially, are harder to understand. Although some of the universities now complaining that they have been told to close departments superficially in the national interest know secretly that their case is only weak, others are genuinely (and rightly) puzzled. At least part of the explanation seems to be that the grants committee has relied on the public research councils for advice on the health and strength of research in departments of this or that at particular universities, discounting the importance of industrial support. But even more machiavellian calculations may have been involved; is it possible that the committee has assumed that the universities most seriously affected by its cuts are few enough in number to be able to capture support from industry? If so, the calculation is in error. Industrial companies will seek to back the winners in the committee's sweepstake, not the losers. And the chance of forcing a few British universities to make a new (and industrially-linked) way for themselves has been lost.

Science and technology in the new pattern of British universities will also be affected by a previously unremarked feature of the grants committee's general letter to universities the absence (for the first time for many years) of "advice" about the way in which total student numbers should be partitioned between undergraduates and graduate students. Briefly, it will be left to universities individually to decide what balance should be struck. Those universities most seriously deprived of funds will decide, other things being equal as the economists say, to take in more undergraduates and fewer graduate students (who are more expensive). This simple calculation will, however, be muddled by calculations of the costs of dispensing with tenured faculty members. In the interests of the British university system, it would be a great advantage that postgraduate education should be more concentrated than it is, but it is unreasonable that universities that would benefit from following such a course should be expected to do so if the immediate consequence is that there is even less to spend on immediate needs — and no mechanism for making sure that bright undergraduates have a means of becoming graduate students themselves.

What this implies is that the grants committee's declarations about the immediate prospects for British universities cannot in themselves become a policy for the universities. They are at best a challenge to the other interested parties — the research councils, the universities themselves but also the government — to hammer out some kind of policy for the future. On present form, and that of the past two weeks, nothing much will happen. Most of those most closely concerned will be on vacation between now and when the cuts begin to bite. By then, it will be apparent that it is too late even for invigorated administrators of the British university system to exert much influence. The danger, as things have turned out, is that the academics most threatened by what the grants committee has decreed will be on holiday when crucial decisions must be made about the institutions for which they work. Too bad, people will be saying.

Row over nuclear safeguards continues

IAEA official denies evidence to Congress

The evidence given by Dr Roger Richter in the past few weeks to the foreign relations committees of the United States Congress was distorted, partly for effect and partly out of ignorance, according to Dr Hans Gruemm, the Austrian physicist who is head of the safeguards division of the International Atomic Energy Agency (IAEA). Dr Gruemm was speaking at a conference of the Institute of Nuclear Materials Handling in San Francisco on Monday this week.

In his address, Dr Gruemm acknowledged that the attack on the Iraqi reactor at Tamuz on 7 June, and the public comment that had followed — as well as Dr Richter's removal of confidential papers from the agency — had undermined the credibility of the safeguards system. He said, however, that the agency's internal memorandum dated 10 March, made public during the Senate hearings, was only one of several documents circulating within the agency and concerned with the improvement of safeguards on large research reactors.

The memorandum of 10 March, addressed to Dr Gruemm by Dr T. Shea of the agency's safeguards division, is a minute of a staff meeting on 12 February called to consider the feasibility of closing two loopholes in the agency's safeguards procedures — the possibility that a state covered by inspection might not have declared all the nuclear material used in its operations, and the possibility that reactors under safeguards might be used for making plutonium (or uranium-233) by the clandestine irradiation of natural uranium (or of thorium).

The meeting concluded that it would not be feasible to detect undeclared stocks of nuclear materials except by means of country-wide surveillance not covered by the safeguards provisions. On the use of reactors for the production of fissile material by neutron bombardment, the meeting apparently acknowledged that routine inspection would not always detect the use of uranium or thorium in this way because of the frequency with which samples for irradiation would normally be loaded and withdrawn (as in the production of short-lived isotopes), listed five technical devices that might serve continuously to monitor such illicit uses, agreed that none had been "identified" as "effective and efficient" and suggested further study. Dr Richter, in his account of these proceedings, did not say that the

agency was concerned.

Dr Gruemm said in San Francisco this week that the meeting in Vienna in February (at which Dr Richter had not been present) was only one of several technical meetings called to discuss improvements of safeguards procedures made possible by the recruitment of extra inspectors. He complained that Richter had not mentioned that studies of the feasibility of detecting the use of research reactors for manufacturing fissile material had been begun in 1980.

Dr Gruemm also said that detailed studies of the Tamuz reactor as a means of producing clandestine fissile material were begun at the end of 1979, when the agency was first informed of the transfer of natural and depleted uranium to Iraq. The agency's calculations showed that it might be possible to produce between one and two "significant quantitics" (bomb units) of fissile material each year, but that this would be possible only by replenishing the

reactor core "several times a year" with enriched uranium, presumably from France. To make optimum clandestine use of the reactor, it would also have been necessary to add cooling circuits to the reactor, which would have been visible on inspection.

Dr Gruemm's statement in San Francisco is, however, unlikely soon to still the argument about the efficacy of the agency's safeguards, even though the agency appears to have resolved its previously chronic manpower problems—its professional safeguards staff increased fourfold (to 206) between 1970 and 1980.

Meanwhile, interest in the capacity of Israel to manufacture nuclear weapons has been revived by the report of a panel appointed by the United Nations, including Dr George Quester of Cornell University as its United States representative, that Israel is probably now in a position quickly to produce a substantial number of nuclear weapons.

Chevènement still battling for control

The nouvelle politique of science in France is off to a shaky start. Jean-Pierre Chevènement, Minister of State for Science and Technology, is still struggling with the Ministry of Industry for the control of certain key institutions; while on the other wing the more radical unions are calling for the resignation of certain research directors of the old regime.

The minister's contribution, announced last week, to the new 1981 budget — an adjustment to that of the previous government's — also falls a long way short of what he must aim for to bring French research and development spending up to the promised 2.5 per cent of gross national product by 1985. He will ask the National Assembly for an additional FF 154.9 million (around £15 million) compared with a total 1980 expenditure of FF 14,500 million, excluding defence research. That amounts to a 1 per cent increase; M. Chevènement will need ten times as much next year

Of this year's extras, the minister will ask for FF 68.5 million to pay new salaries at the Centre National de la Recherche Scientifique (CNRS) — the main basic research agency - and at the Institut National de la Recherche Agronomique. Chevenement foresees the creation of 525 new posts, most of them for technicians and administrators. Then FF 25 million would prop up the contribution to the European centre for nuclear research (CERN) against the recent fall in the French franc. And FF 61.4 million would increase running budgets — but not of the basic research agencies. The Agence Nationale pour la Valorisation de la Recherche, which helps to convert discoveries in French laboratories into

innovations in French industry — and is rather a pet of M. Chewenement's — would get FF 60 million, and the remaining FF 1.4 million would go to the Centre National pour l'Exploitation des Océans to create fish farms.

Meanwhile Chevènement is struggling to establish his authority over the nonmilitary research activities of the atomic energy and space authorities (CEA and CNES), and of the Agence Nationale pour la Valorisation de la Recherche itself, all of which at present belong to the Ministry for Industry. Chevènement's determination to control these agencies, in one way or another, and so to have - through the management of innovation — a major say in the socialist transformation of French industry, is not going down well among industry ministry bureaucrats, and they are now briefing their second minister in a few months, M. Pierre Dreyfus, to resist the scientific upstart.

However, Chevenement will almost certainly gain control of the Centre National de la Recherche Scientifique. where the unions - to whose interests Chevènement is sensitive — are becoming active. Most dramatically, the communistaffiliated Syndical National des Chercheurs Scientifique last week called for the resignation of both the president and director of CNRS, Professor Charles Thibault and M. Jacques Ducuing. The director-general of the medical research agency, INSERM, M. Philippe Laudat, should also resign, said the union. They stand in the way of progress, according to the union secretary-general M. Michel Gruselle. Chevènement is cryptic; when asked recently if heads would roll, he said "we shall see". Robert Walgate

Fusion research

Where to next?

Brussels

The European Economic Community and its partner countries, Sweden and Switzerland, should go ahead with research into thermonuclear fusion — according to the European fusion review panel. The road to commercial fusion will, however, "be long and costly" and it appears unlikely that commercial fusion power will be in general use within the next 50 years. These are the main conclusions in the review panel's report published last week.

The European Commission has also released the proposed programme for the next five years (1982–86) which will now be discussed in the committee of the member states' permanent representatives (Coreper). It says that a major landmark in fusion research has almost been reached. There is a good prospect that the full objectives of JET (Joint European Torus) may be achieved and that the results could go beyond the stated objectives and attain thermonuclear ignition.

The construction of the basic JET device is now expected to be completed by the end of 1982 and the first discharge could take place in April 1983. A problem in going further, the review panel's report points out, is that the device will become radioactive and hence inaccessible after experiments with tritium take place. But unless this step is taken, JET cannot be used to answer the crucial question of how the plasma behaves when alpha particles from the deuterium-tritium reaction are produced in large quantities. This knowledge is essential for progress towards the stage after JET.

This next step is called, with refreshing logic, NET (Next European Torus) and would demonstrate the technical feasibility of a commercial tokamak. The concept of an international commercial demonstration tokamak (INTOR) is being studied under the auspices of the International Atomic Energy Authority (IAEA). The review panel recommends that the Europeans need to make preparations for studies of the newtechnologies.

"But the Community will only be able to contribute to and benefit from INTOR if it develops in parallel its own design of a next step device and the technological know-how necessary to undertake its construction. Both the Japanese and Americans are planning their own next step devices" warns the report.

The panel also weighs up the pros and cons of fusion research apart from the tokamaks and, not surprisingly at this early stage in the art, feels that options should be kept as wide open as possible. The three new specialized tokamak projects (TORE SUPRA, FTU, ASKEX-UPGRADE), which are at various stages of design, are also considered well justified.

Both the European Commission and the review panel favour the Reverse Field Experiment (RFX) proposed by laboratories in Culham, Padua and Los Alamos. An agreement has been drawn up under which the United States contributes \$8 million out of the total cost of \$48 million, but this awaits approval from the member states. The United States is also likely to participate in the two-stage development of the Advanced Stellarator. This would mean rebuilding the existing device in Garching, West Germany, and a new and much bigger stellarator later on.

All this research is likely to cost around 1,500 million European Units of Account (£740 million) over the next five years, according to the report: 400 million for JET, 560 million for the running costs of the associated laboratories, 125 million for investments in new supporting tokamaks, 260 million for the technology programme and NET and about 40 million for investments in the new alternative devices. This agrees with what the Commission has outlined.

The review panel's report highlights several issues likely to give rise to political problems. One of these is the future of Culham. Should it be the site of the next step project and what should be done to prevent the tokamak becoming useless once it is radioactive? Also questioned is the belief that fusion is as unpolluting as has been argued - the radioactive waste will have to be stored. Finally, there is a danger that the duration of nuclear fusion research will outstrip the mortality of its acolytes. The average age of Community staff is 45 and the increase of average age is very nearly one year per year. Thus in about 15 years time when these staff retire, most of the know-how acquired in 30 to 40 years of research "will disappear rather Jasper Becker suddenly.'

Clinch River reactor

Start again folks

Washington

After four years of delay caused largely by President Carter's reluctance to make a full-scale commitment to the "plutonium economy", plans for the construction of a liquid metal fast breeder reactor at Clinch River in Tennessee have been put squarely back on the rails by the US Congress.

Despite support from President Reagan, it was never a foregone conclusion. In May, the Science and Technology Committee of the House of Representatives voted against funds for the initial construction work on the 350 megawatt reactor, which has been on the drawing board since the early 1970s, and for which over \$500 million worth of components have already been delivered.

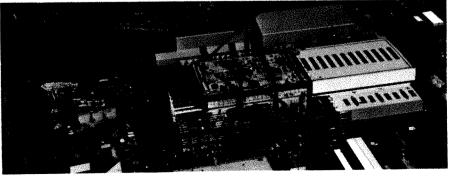
In previous years the same committee had spearheaded congressional efforts which succeeded in preventing President Carter from killing the project; this time, cost-conscious Republicans added their voices to the previous minority which had argued that no further federal funds should be committed.

But larger political forces were at work. In particular, strong support for the construction of the Clinch River reactor came from Senate Leader Howard Baker, who represents Tennessee, and for whom the reactor means both jobs and votes. Reflecting Mr Baker's wishes, the House accepted a Republican amendment to the budget bill at the end of last month which overturned the recommendations of the Science and Technology Committee and inserted \$230 million for preliminary construction of the reactor as proposed by Mr Reagan.

Similar approval has already been given by the Senate (which, ironically, has in past years voted to terminate the project). And although the decision to go ahead with construction must now pass through the appropriations process, it seems that after the crucial House vote this will be little more than a formality.

Opponents of the fast breeder maintain their opposition on several grounds. One is that the declining price of uranium over the past three years has reduced the need for a rapid breeder programme. The pressure has also been eased by declining predictions of future demand for electricity. And even many of those who support fast breeders in principle feel that the Clinch River design is now out-of-date.

The critics appear to have a covert ally in the new director of the Office of Management and Budget, Mr David Stockman. Long opposed to heavy federal subsidies for energy demonstration projects, Mr Stockman wrote to fellow Congressmen in 1977 stating explicitly that the federal government's 90 per cent support for the Clinch River reactor was "totally incompatible with our free-market approach to energy policy".



JET nears completion

Yet with over \$100 million of their own money already spent, the 733 utilities involved have been pushing for construction to go ahead. The level of jingoism has been high. Visitors to the Clinch River site are given a free coffee mug inscribed "we are fighting for energy independence". And in a letter expressing their support, 17 members of a group known as Scientists and Engineers for Secure Energy, headed by ex-president of the National Academy of Sciences, Frederick Seitz, gave as one of their reasons that "in view of recent political developments in certain Western countries, particularly France, the Clinch River Project may become the only reliable technological undertaking of its kind in the free world".

But in the end the personal support of Senator Howard Baker has been decisive. As characterized in the House budget bill, the liquid metal fast breeder reactor (LMFBR) will be one of a series of steps designed to bring US breeder technology in line with that of other industrialized nations.

The most recent of these steps has been the successful operation last December of the Fast Flux Test Facility (FFTF) at the Department of Energy's Hanford Reservation in Washington State. Late in March of this year, the 400 megawatt test facility emerged with flying colours from a safety test in which the reactor was shut down from full power, and the main coolant-circulating pumps were turned off. Construction of the Clinch River reactor, say its supporters, is the logical next step.

In approving the Clinch River funding (and therefore channelling support away from solar energy and conservation research which the Science and Technology Committee in the House of Representatives had preferred), the House authorized an initial \$15 million for the planning of a 1,000 megawatt reactor.

How much future support for the breeder programme will, in fact, be forth-coming from the Reagan Administration remains uncertain. In his formal presentations, budget director David Stockman has forsworn his earlier statements and repeated the Administration's support for LMFBR. In private, however, Mr Stockman and his officials at the Office of Management and Budget are said to be strongly opposed to further substantial government subsidies of the nuclear industry, including its fast breeder plans, preferring that the utilities should pay.

Meanwhile opponents have not given up the fight. They are giving wide publicity to the findings of a congressional investigation team that some of the contractors may have been overcharging for components already supplied. In the wings is a debate about whether the reactor meets the new siting requirements introduced by the Nuclear Regulatory Commission after the Three Mile Island accident. Congress may have made up its mind, but the public debate is far from over.

David Dickson

Research ethics and safety

Changing the guard

Washington

In a small but symbolic way, last Thursday may turn out to be a significant turning-point in the history of public controls on genetic engineering. Meeting in Bethesda, Maryland, an advisory committee to the National Institutes of Health (NIH) decided to recommend to its parent body, the Recombinant DNA Advisory Committee (RAC), a further substantial relaxation of the safety controls applied to recombinant DNA research.

Meanwhile 50 miles away, in the depths of the Virginia countryside, a presidential commission established to look at the ethical problems raised by biomedical advances has suggested the establishment of a new body — possibly at an international level — charged to seek a social consensus on the various dilemmas which the expanding clinical use of genetic engineering techniques will raise.

The RAC subcommittee was set up at a meeting of the full committee in May to discuss proposals for a significant relaxation in the safety guidelines made by Dr David Baltimore of the Massachusetts Institute of Technology and Dr Allan Campbell of Stanford University (Nature 7 May, p.3).

The subcommittee, whose recommendations will now be discussed at the next full meeting of RAC in September, did not agree that NIH guidelines should be made voluntary. However, they did suggest that detailed rules for the composition of local institutional biohazards committees (IBC) be removed.

If eventually approved by NIH, this would mean that research institutions would no longer be required to include "public interest" representatives, for example, on their IBC (although many would probably continue to do so). It could also mean that the responsibilities of the IBC to ensure compliance with the guidelines could be delegated to a single institutional biosafety officer.

The subcommittee also supported a proposal to eliminate from the guidelines a detailed listing of containment procedures, and its replacement by a statement that such procedures should follow recommendations being developed by the Center for Disease Control for experiments using the host or the vector separately.

In other cases the subcommittee proposed that P1 containment levels be used, and that a statement be included about donor DNA, saying that if there is clear evidence that the donor DNA will significantly alter the pathogenicity of the host, then the appropriate containment level will be applied.

Some of the suggestions approved by the subcommittee — for example that all prohibitions requiring special permission from the director of NIH, including work with

Ziman speaks out

Professor John Ziman on Monday strongly criticized the Royal Society, of which he is a fellow, for sluggishness on human rights issues. He was addressing the All-Party Parliamentary Committee for Soviety Jewry at a special award ceremony in the House of Commons, at which he received on behalf of Dr Viktor Brailovskii, who last month was sentenced to five years' Siberian exile, a Henry Moore lithograph entitled "for courage in defence of freedom".

Professor Ziman earlier this year had received, in conjunction with Dr John Humphrey (until recently deputy director of the National Institute of Medical Research) and lawyer Paul Sieghart, the second annual Airey Neave award, which will finance a study of freedom in science. He was therefore an obvious proxy for Dr Brailovskii who, until his arrest last November, had acted as host and organizer of the Sunday seminar for Jewish "refusnik" scientists denied emigration visas but dismissed from their academic posts after applying for them.

Professor Ziman said it would have been more appropriate that Dr Brailovskii's proxy should have been not a private scientist such as himself but the president of the Royal Society in his official capacity. He recalled that Dr Aleksandr Voronel', the founder of the Sunday seminar, said in Britain shortly after being allowed to emigrate in 1974 that "the seminar is the only true representative of free and independent science in the Soviet Union". The Royal Society, whose official aim is "improving human knowledge", should therefore, said Professor Ziman, give the fullest possible support to the seminar - support which, so far, it has been reluctant to afford.

Vera Rich

cultures over 10 litres, be eliminated from the guidelines — went further than Dr Baltimore and Dr Campbell had proposed to RAC. Others, such as the continuation of local biosafety procedures and the recommendation that the guidelines remain mandatory for scientists working with NIH funds, are more conservative.

Any decisions by RAC at its next meeting will be subsequently published in the Federal Register for public comment. After that, the matter will rest with Dr Richard M. Krause, director of the National Institute of Allergy and Infectious Diseases, who was given full responsibility for ensuring compliance with the guidelines by Dr Donald Fredrickson when he resigned as NIH director on 30 June.

As the safety debate is being wound down at NIH, a complementary discussion about the steps necessary to prevent undesirable clinical applications of genetic manipulation techniques has been getting under way with the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research.

The commission was asked last year to look at the ethical issues raised by genetic engineering by President Carter's science adviser, Dr Frank Press, following a letter to the President from three church groups expressing concern that recent advances in genetic research meant that "those who would play God will be tempted as never before". Since then the churches involved have not demonstrated a particularly close interest, but public concern has been stimulated by various press reports of potential new surgical techniques.

At last week's meeting the members discussed a draft report on the ethical and social aspects of genetic engineering. And although reluctant to raise unnecessary fears, they agreed that the implications were likely to be significant — for example in terms of the potential ability of an individual to alter the genetic characteristics of his or her descendants.

Most commission members agreed that there was a need for a wider public dissemination of information about the potential effects of new clinical techniques. Also that it might be appropriate for some type of advisory body to indicate areas in which caution should be used.

There was less of a consensus on whether it was desirable that such a body should suggest that certain types of experiments—for example the cloning of a human being—should be prohibited. Some, for example, suggested that any attempt at what the draft report described as the "control of evolution" should be proscribed; others pointed out the phrase was so broad as to include many currently-accepted practices, such as the treatment of diabetes with insulin.

The commission also debated whether discussions should take place at an international level. There was general agreement, however, that achieving international consensus on the boundary between acceptable and unacceptable practices would be even more difficult than at a national level.

David Dickson

New substance regulations

Industry complains

The British Chemical Industries Association is protesting vigorously at the draft on the notification of new chemical substances drawn up by the Health and Safety Executive. The association claims that the draft regulations would mean the end of research and development in the British chemical industry.

The draft regulations were published last February, when comments from interested parties were invited by the end of this month. They are an attempt to bring British practice into line with a directive of the European Commission, whose aim is to protect "man and the environment" from the potential hazards of new substances. Although the directive deals chiefly with the protection of the consumer, the Health and Safety Executive is (given its remit) primarily concerned with the protection of workers' health.

Thus the British regulations would require industry to notify not only all new manufactured substances but also all new intermediates in chemical processes. The Chemical Industries Association complains that the extra costs involved will drive research and development away from Britain. The dilemma is, however, real. The Health and Safety Executive says that intermediates must be tested if existing regulations to protect workers from potential hazards are to be put on a more formal basis than required by the Health and Safety at Work Act.

The consultative document is precise about the tests required for new chemicals. Manufacturers would be required to assess the toxicity of substances by LD₅₀ tests, provide data on skin and eye irritability, tests for mutagenicity and possibly carcinogenicity and teratogenicity. They would also have to provide data on biodegradability. The executive estimates that the total cost would be about £45,000 per substance.

The objections of the chemical industry appear to centre more on the range of chemicals covered than the direct cost. As well as chemical intermediates, the draft regulations cover pharmaceuticals, foodstuffs and pesticides, all of which are excluded from the European directive on the grounds that they are covered by other regulations. The Health and Safety Executive's argument is that such assessments relate only to specific uses, and are not necessarily sufficient.

After the July deadline, the chemical industry will also be arguing for a strengthening of the provisions for preserving confidentiality. The association is concerned that valuable data, especially those on intermediates which would provide information on novel process routes, could fall into competitors' hands.

So far, few other bodies have put in comments, but the trade unions and environmentalists will also be having their say. The controversy aroused by the chemical industry's response, however, suggests that further negotiation will be needed before the regulations are in a final form and that the European Commission's 18 September deadline for the implementation of legislation will not now be met.

The European Commission is at the same time going ahead with its plan to compile a catalogue of all chemicals manufactured in Europe. Thereafter, industry will be required to notify the commission of all new chemicals manufactured during the past ten years that are not included, a ruling that will apply even in countries that will not have introduced their own legislation.

Judy Redfearn

UK pharmaceutical industry

Keeping up

"Britain's medicine makers have brought out an 'unfashionable' annual report — it tells a success story." So says the cheery publicity blurb announcing the 1980–81 report of the Association of the British Pharmaceutical Industry (ABPI). The claimed success is an increase in the value of exports of pharmaceuticals to £745.4 million in 1980, 16.7 per cent up on the sales in 1979, and representing a £523 million surplus of exports over imports.

Evidence of success is rare enough in British industry and the bouncy confidence affected by ABPI is likely to please a government eager for good news. And the industry seems to be getting its views across with some aplomb. Already this year the industry has had a victory in the form of new regulations governing the granting of the clinical trial certificates which must be obtained before new drugs can be tested clinically. From March, the certification process requires simpler documentation and data requirements have been reduced.

In another area of concern to ABPI, Mrs Sally Oppenheim, Minister of State for Consumer Affairs, has said that the government will try to include a "state of the art" defence in the EEC directive on product liability. The objective is that manufacturers should not be held liable for injuries to health caused by a product which could not be termed "defective" in the light of scientific knowledge when the drug was put onto the market.

The supposed main benefits to be gained by the simpler clinical certification rules are a reduction in the 10 to 12 years now needed for a new drug to reach the patient (a debatable improvement, especially after Fisons' withdrawal of Proxicromil when it was all but on the market, see *Nature* 12 March, p.81), and a stimulus to encourage development of drugs to treat less common diseases.

However, the most tangible effect of relaxations in control of drugs in clinical trials is likely to be an increase in the numbers of trials conducted in the United Kingdom rather than in other, less restrictive, countries. This is one factor to be considered by multinational companies when deciding whether to invest in Britain or go elsewhere. At present investment in research in the United Kingdom is holding up well, with £16 million to be invested by Merck, Sharp and Dohme in a neurobiology research centre near Harlow, £5 million by Upjohn in expanding facilities at West Crawley, £10 million by the Wellcome Foundation in a chemical research laboratory in Beckenham, and £3.3 million by Roche in improving research facilities at Welwyn Garden City. But competition between the developed countries for the favours of the researchbased companies can only increase.

Charles Wenz

Industry and research

Dupont joins in

Boston

Reflecting the growing eagerness of industry and universities to find ways in which both can profit from advances in biotechnology, the chemical company Dupont has promised \$6 million for genetic engineering research to a group of workers at Massachusetts General Hospital, one of the teaching hospitals associated with Harvard University. This announcement comes just as scientists, politicians and university administrators are completing their examination of a ten-year, \$50 million grant to another group of Harvard geneticists proposed by the German pharmaceutical house Hoechst-Roussel (see Nature 18 June, p. 525).

The Dupont grant will support the new genetics department at the Harvard Medical School under Dr Philip Leder who is moving to Harvard in September from the National Institutes of Health. The conditions of the Dupont deal are similar to the Hoechst arrangement; the grant recipients retain patent rights on discoveries made with corporate funds, but the companies are given exclusive licences to develop any products that result. One difference in the Dupont grant is that the company has not requested training positions for its scientists.

Although most university officials applaud these agreements, in the past month committees in both the Senate and the House of Representatives, officials of the National Institutes of Health and high ranking university officials from the United States and Canada have met independently to review Harvard's new affiliations with industry and to question whether the quality and independence of research will be compromised by corporate support.

These hearings have attracted wide attention since Senator William Hatch complained that the Harvard-Hoechst grant fostered a "technology leak" by giving a German company first crack at exploiting discoveries at institutions supported by public funds. The victims of such a situation, he suggested, would be the US taxpayers who have indirectly supported basic research that will benefit foreign investors.

This concern will not be a problem with the Dupont grant, because the money will be coming from a US chemical and drug conglomerate. Dupont plans to give Harvard the \$6 million in annual instalments, with \$2 million immediately and \$1 million over each of the next four years.

University officials say that they will examine all patentable discoveries very carefully to determine whether they were made with federal funds or portions of the Dupont grant. Only the latter will bring Dupont exclusive licensing rights to

university-owned patents.

The toughest scrutiny, however, may come from the local city councils. The Cambridge City Council has officially ordered an investigation of Harvard genesplicing experiments in tax-exempt laboratories. Council members intend to rescind tax exemptions where private corporations will reap financial benefits.

Harvard officials do not anticipate an investigation of the Dupont grant similar to the House investigation of the Massachusetts General Hospital-Hoechst agreement.

Michael D. Stein

Chinese Academy of Sciences

Scientist takes over

China's Academy of Sciences once more has a scientist at its head. A few weeks ago the academy's Scientific Council met for the first time for 21 years and elected as its president Dr Lu Jiaxi, a physicist, who at one time worked at University College London under Professor S. Sugden and at the California Institute of Technology under Linus Pauling.

Dr Lu takes over the presidency from Fang Yi, the minister responsible for the State Commission for Science and Technology. On resigning, Minister Fang stressed that the presidency should be an elected post (he had been a government appointee) and should go to a scientist.

At the same meeting, the Scientific Council accepted a draft constitution which states that the supreme decision-making power for the academy will be vested in the 400-strong council, which from now on should meet every two years. Between council meetings, the academy will be administered by a presidium of 30 members, of whom 20 will be members of the council and 10 from the Communist party and government departments.

These changes reflect an overall policy of increasing the professionalism and prestige of Chinese science, which suffered considerably during the Cultural Revolution of 1966-1976. As well as revitalizing the Academy of Sciences, recent developments include the conversion of a teacher's training college in Hainan into a university, which will commence undergraduate enrolment in autumn 1983 and major discussions on how to develop vocational and technical secondary education. This latter need is so pressing that one government spokesman suggested that in addition to the state system, private projects for training technical workers should be encouraged. And to help young people develop their interest in science, a new "National Association of Science Coaches for Juveniles" was established in Peking, with Jiang Nanxiang, the Minister of Education as Honorary Chairman, and Wu Zhonghua, executive chairman of the Presidium of the Academy of Sciences as chairman of the Board of Directors.

Vera Rich

Spanish research

Painful reform

Barcelona

The Spanish Higher Research Council (Consejo Superior de Investigaciones Científicas (CSIC) is nearing the end of a process aimed at giving it a fresh start. The reformers, the present directorate of CSIC, intend to demonstrate to public opinion and to the government that CSIC can renew itself and become an efficient instrument in the economic and cultural development of Spain.

CSIC is the main public body devoted to research in Spain. It has a permanent staff of 5,000 (1,500 of them scientists) in about a hundred institutes. Since its foundation and haphazard development during the government of General Franco, CSIC has maintained an enormous diversity of research interests of variable quality. There are groups devoted to local studies and to theology, to textile technology and fisheries and also to history, biology and physics. Only a few of these institutes can be compared with advanced European or American laboratories, and most of them suffer from limited and ageing staff and poor funding. This has provoked criticism from both inside and outside CSIC. directed chiefly at the low scientific level of many institutes and their lack of relevance to the general needs of the country. During the past five years of democratic government, even the aboltion of CSIC has been openly discussed.

A move to reverse this trend from inside CSIC began after the appointment of a new directorate last year. CSIC's Scientific Commission, consisting of scientists from different areas of research, was asked to suggest important areas of research and to advise laboratories how they might best pursue them. A number of commissions are now working on detailed programmes based on these suggestions. As CSIC's budget cannot finance all the programmes chosen, it is hoped that outside agencies will be able to provide additional funds. It is hoped that groups will join forces to produce plans that are likely to be financed.

CSIC scientists are anxious to give the proposed changes a chance of success, but there is some doubt about the speed at which the scheme is being carried out, particularly in the absence of any long-term decision at government level.

Increases of staff are unlikely because of cuts in public expenditure and there is doubt as to whether the proposed Law of University, including to provide guidelines for recruiting teaching and research staff, will be passed before the next elections in 1983. In the meantime, the need of people and the low level of salaries are increasing tension inside CSIC and could lead to the resignation of the present directorate. A welcome reform lies on a knife-edge.

Pedro Puigdoménech

CORRESPONDENC

Unfair on DeVita

Sig — The recent US Senate hearings in which Dr Vincent DeVita was scourged for the failure of the NIH-NCI bureaucracy to vitiate promptly an accused laboratory cheat leaves one appalled and apprehensive. Dr DeVita is the first NCI director who is a clinical oncologist, an occupation faced with the unenviable repetitive experience of patients who become progressively worse, mortify and die. In no small part due to the efforts of Dr DeVita and the organization he has assembled, this nó longer takes the invariable course it once did and the concept of a cure has become realistic for many cancer patients. He has tried to redirect the cancer research industry back to the human cancer patient and away from highvisibility science for science's sake, while still juggling the all important basic research that must be done as well. To hear of his being chastized in such a silly way can be best compared to rejecting Socratic reasoning because Socrates may have been bald.

CECH H. FOX

Silver Spring. Maryland, USA

Serbian exchange

Sir - We would like to report on the great value of a recent exchange visit between British and Yugoslav geologists. In May we took part in a ten-day field trip to Yugoslavia, organized jointly as part of an agreement between the Royal Society of London and the Council of Academies of Science and Arts of the Federative Socialist Republic of Yugoslavia. It followed a visit in autumn 1980 when Yugoslav geologists Professor V. Majer and S. Karamata came to the UK, the first time that such an exchange had occurred between geologists of these countries.

Our time was spent in Yugoslavia mostly on a geological excursion through Serbia by minibus organized by Professor S. Karamata of the Faculty of Mining and Geology at Belgrade University. A round trip of over 2,100 km was made, starting from Belgrade and passing through the Carpatho-Balkan chain, the Serbo-Macedonian massif and the Inner and eastern Outer Dinarides. Stops were made at selected localities of interest enabling the international team of British, French, Hungarian and Yugoslav geologists to exchange ideas and collect rock samples. The lithology observed ranged from Palaeozoic basement, Mesozoic lavas and sediments and Cenozoic calc-alkaline flows to ultramafic, mafic and metamorphic components of ophiolites and related olistostromes.

This traverse enabled us to obtain an excellent introduction to the geology of Yugoslavia — an essential part of studies in the Earth sciences where it is necessary to observe at first hand natural phenomena in the field. We hope this initial exchange will lead to further visits and joint research in the near future.

S.O. AGRELL A. G. SMITH J. G. SPRAY

Department of Earth Sciences, University of Cambridge, UK

Human protection

Sir — An imprecise reference in a recent article, "NIH censure for Dr Martin Cline," (Nature 4 June, p.269) prompts us to delineate the distinction between the UCLA School of Medicine Human Subject Protection Committee which reviews research protocols involving human subjects for risk-benefit ratio and the UCLA Human Subject Policy Committee which, based on federal regulations, develops university policy governing research involving human subjects.

ESTHER F. HAYS

Human Subject Protection Committee, School of Medicine. University of California, Los Angeles, USA

Fight the obscure

Sir - Recognizing the present popularity of various forms of anti-scientific obscurantism, both here and still more in his own country, Dr Ralph Lewis was dead right to conclude his letter (Nature 11 June, p.448): "We must find and state clearly our fundamental agreements, so that our differences in presentation cannot be misconstrued and distorted".

I suggest that one first necessary step is to insist always that the contrary of any evolutionary account of the origin of species is a doctrine of special creation. Evolution in this understanding would be decisively refuted were palaeontologists to discover fossil remains of creatures morphologically indistinguishable from the higher mammals in rocks which are much too old - J.B.S. Haldane used to specify human remains in a coal seam. Darwinian and any alternative accounts of the mechanism of such evolution, or of its mechanisms - the Lamarckian inheritance of acquired characteristics, for instance - must all be equally inconsistent with any doctrine of special creation. And, although I will not myself pretend to understand cladistics, we can be sure that its Marxist adherents are not rooting for special creation as opposed to evolution. Their point against Darwin is, surely, that while he defended "that old canon in natural history, Natura non facit saltum", they themselves are committed to the opposite contention, that both Nature and humanity sometimes take 'A great leap forward''

A second step is to dispose of certain philosophical muddles and misconceptions such as those propagated by the British Museum (Natural History). Barry Cox quoted several in his report (Nature 4 June, p.373). One is that the assertion of the survival of the fittest is vacuously tautological: "The Survival of the Fittest is an empty phrase, it is a play upon words". Certainly, since the criterion of fitness to survive is here actual survival, this assertion is not to be construed as an assurance that all is for the best. It is, nevertheless, not a tautology. For clearly it denies that the survivors survive at random, while asserting that they have some sort of competitive edge over the non-survivors.

Again, Cox quotes the statement: "The idea of evolution by natural selection is a matter of logic, not science, and it follows that the concept of evolution by natural selection is not, strictly speaking, scientific". Certainly it is possible formally to deduce the conclusion

that some natural selection must occur from two or three general propositions about multiplicative reproduction, variation, and the finitude of resources. But this neither disqualifies that conclusion as science nor turns it into an empty truth of logic Substantial truths can be and are deduced with perfect formal validity from premises stating other similarly substantial truths.

ANTONY FLEW

Department of Philosophy, University of Reading, UK

Defining a lectin

Sir - The Nomenclature Committee of the International Union of Biochemistry has discussed the objections raised by Kocourek and Hořejší to the definition of lectins proposed by Goldstein et al. 2 which was also published by the Nomenclature Committee in its 1981 Newsletter3.

According to both definitions, "a lectin is a sugar-binding protein of non-immune origin' (glycoproteins are a class of proteins and there is no need to specify them in the definition). The emphasis in the definition of Goldstein et al. is an operational one, so the definition is easy to apply experimentally by the criteria of agglutination of cells (not necessarily of erythrocytes, which carry a limited variety of sugars on their surface) or precipitation of glycoconjugates. By contrast, the criterion of Kocourek and Hořejší of lack of enzymatic activity is difficult to apply; for example, glycosyltransferase activity might be present with an acceptor different from those tested. Moreover, there is now evidence that certain proteins hitherto known as lectins possess glycosidase activity4

The definition of Goldstein et al. implies the presence of more than one carbohydratebinding site. Strictly speaking, it excludes proteins such as ricin that are closely related to lectins in certain properties (for example, acid composition and possibly primary structure) and derived from the same plants, and this may be a disadvantage. Nevertheless the definition of Kocourek and Hořejší is so close to meaning "carbohydrate-binding protein" that a special name seems unnecessary, and the definition is too broad to be useful, since it includes substances such as sugar-transport proteins, chemotaxis receptors, certain bacterial toxins, hormones and interferons.

As long as we do not know with certainty the role of lectins, whether in plants, animals or microorganisms, it seems preferable to focus the definition of these substances on positive and easily testable properties. We therefore plan to continue to use the word as proposed by Goldstein et al.

H.B.F. DIXON (Secretary)

Nomenclature Committee of International Union of Biochemistry, Cambridge, UK

- Kocourek, J. & Hořejši, V. Nature, News & Views 290, 188
- Goldstein, I.J., Hughes, R.C., Monsigny, M., Osawa, T & Sharon, N. Nature 285, 66 (1980)
- & Sharon, N. Nature 283, 90 (1900).
 Nomenclature Committee Archs Biochem. Biophys. 206, 458–462 (1981); Eur. J. Biochem. 114, 1-4 (1981); Hoppe-Seyler's Z. physiol. Chem. 362, 1-IV (1981); J. biol. Chem. 256, 12-14 (1981). Hankins, C.N. & Shannon, L.M. J. biol. Chem. 253,
- 7791 -7797 (1978); Pt. Physiol. 65, 618-622 (1980).

NEWS AND VIEWS

Hydrothermal processes on ridge flanks: the *Challenger*'s return to the Costa Rica rift

from Roger N. Anderson

This coming November the drilling vessel Glomar Challenger will reoccupy a site which has already produced some of the most spectacular scientific discoveries of the highly successful International Phase of Ocean Drilling of the Deep Sea Drilling Project. The drill site, located on the south flank of the Costa Rica Rift in the eastern Equatorial Pacific, is being used to investigate a paradox within plate tectonics.

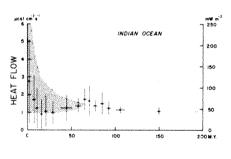
Ten years ago it was discovered that regardless of its location or tectonic setting, the depth of any normal sea floor is only a function of its age^{1,2}. This elevation change is due solely to thermal contraction accompanying cooling as crust spreads from mid-ocean Theoretically, the heat flow measured along the top surface of the lithosphere should decrease exponentially from a maximum at spreading centres to low values in old ocean basins. Instead, as is well illustrated in a compilation prepared by M. Hobart (Lamont-Doherty Geological Observatory), observations (see the figure) show an entirely different form of cooling is taking place. Conductive heat flow is actually lowest near spreading centres, and increases to that predicted by plate tectonics as the lithosphere ages³. The 'missing heat' near midocean ridges is carried away by the hydrothermal convection of seawater through the oceanic crust and sediments4. With ageing, however, each plate somehow becomes sealed from this convective hydrothermal circulation and from then on the measured conductive heat flow matches that predicted by conventional lithospheric plate models.

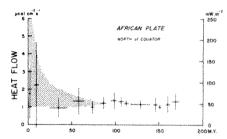
This is no minor event since more than one-third of the entire sea floor of the

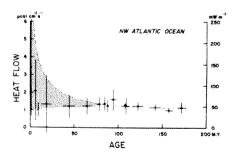
world's oceans contains a currently active convection system⁴. Every drop of the vast volume of ocean water passes through the oceanic crust in a few million years⁵. The crustal basalt consequently buffers the composition of the ocean water and is a more important source of marine chemicals than the input from rivers⁶. In addition, metallogenesis accompanying the discharge of these convective fluids from the crust into the ocean probably forms one of the Earth's major ore generation mechanisms⁷. The ocean water captured chemically within the oceanic

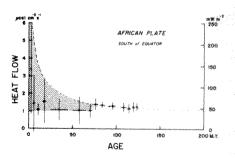
crust even reappears to affect man's day to day life. Volcanicity above lithospheric subduction zones is directly caused by the catalytic effect of seawater released by chemical reactions deep within the mantle⁸. Water vapour accompanying the eruption of Mt St Helens originally circulated through and was caught up in oceanic crust in the Pacific Ocean as part of a ridge flank convection cell before its eventual subduction beneath Washington and Oregon.

In 1979, during drilling legs 68-70, the Glomar Challenger investigated the Costa









Solid dots are regional averages of observed heat flow over the age intervals spanned by the horizontal bars. Vertical bars are ± 1 standard deviation of the data from the mean. Indian Ocean is for all plates in that ocean³. Northwest Atlantic is American Plate only. African Plate north of equator is in the Northeast Atlantic and African Plate South of the equator is in the Southeast Atlantic Ocean. Exponential decay of heaf flow away from midocean ridges (dashed curve) is predicted by theoretical lithospheric plate models^{1,2}. Stipled region is the 'missing convective heat flow' component not measured by current observational techniques which only detect conductive heat transfer. With ageing, this convective hydrothermal circulation becomes sealed and we see a transition from predominantly convective to conductive heat flow. From then on, the observed heat flow matches the predicted values.

Roger N. Anderson is senior research associate at Lamont-Doherty Geological Observatory and adjunct associate professor in the Department of Geological Sciences of Columbia University, New York.

Rica Rift, the youngest known transition zone from convective to conductive sea floor heat flow. Two possible mechanisms could explain the transition - either a thick sedimentary blanket forms a hydraulic lid sealing convection within the oceanic crust and/or the basaltic 'plumbing system' becomes plugged by metamorphic alteration9. Only drilling would allow direct observations to be made.

Papers presented at the American Geophysical Union's spring meeting in Baltimore reported some astonishing discoveries from the integrated set of in situ geophysical and geochemical experiments carried out during these legs. M. Langseth (Lamont-Doherty Geological Observatory) et al. 10 began by presenting temperature logging results which showed that seawater was being naturally drawn down the drillstring into the oceanic crust beneath 300 m of mud and chert at site 504B. The downflow rate of 6 m3 h-1 was unchanged 54 days later when leg 70 reoccupied the site and it is anticipated that it will be continuing when the Challenger returns in November 1981.

Pore pressures 10 bars less than hydrostatic pressure as measured in situ across a hydraulically packed section of the wellbore were responsible for the downflow (M. Zoback, US Geological Survey and R. Anderson, ref. 11). Most of the downflow was disappearing into an acquifer 50 m below the sedimentbasement interface. Direct measurements showed the basalt to be several orders-ofmagnitude more permeable than the overlying sediment and chert layers11. Convection in an oceanic crust with permeability of 2-40 millidarcies (determined by the measurements) can indeed account for the observed underpressures, as shown by D. Gartling (Sandia Labs) from Finite Element Convection Modelling. It thus appears that the Challenger penetrated through a hydraulic lid into an active convection system. Interestingly, most of the real extent of such a cell is predicted to be underpressured. Thus, many, many Challenger holes drilled previously in the world's oceans may have also penetrated underpressure zones, though few measurements were ever made which might have detected such a phenomenon.

The oceanic crust does show major changes with depth, however, which must affect the form of convection. R. Von Herzen (Woods Hole Oceanographic Institution) et al. 12 measured a steady porosity decrease with depth from a long spacing electrical resistivity experiment. Anderson and Zoback¹³, using ultrasonic borehole televiewer imagery for the first time down a Challenger hole, found that a marked increase in seismic velocity downwards in the upper 200 m of the oceanic crust (layer 2A) was caused by replacement of seawater with clay and alteration products in fractures and voids at site 504B. R. Stephen (Woods Hole and see ref. 14) conducted an 'oblique' seismic experiment, shooting explosives from a surface ship to receivers latched into the wellbore. His results showed unusually high seismic velocities 1-2 km into the oceanic crust at this site. These may result from metamorphic alteration of the oceanic crust accompanying cellular convection.

J. Honnorez (University of Miami and see ref. 15) and M. Mottl (Woods Hole and see ref. 16) presented geochemical evidence for major chemical and alteration gradients both downhole and between four holes within one kilometre of each other at site 504B. These horizontal gradients probably result from heterogeneity caused by active hydrothermal convection. For example, Mottl predicts a major source of Ca-rich pore fluids somewhere just to the west of the drill sites. That source is probably the upwelling limb of a convection cell, the waters of which have travelled deep into the oceanic crust, reacted with basalt and then returned towards the surface of the basalt. Similarly, Honnorez reported marked increases in the 'grade' of alteration downhole with some of the 'hottest' metamorphic grades ever found in the drilling project (+ alc) encountered at the bottom of 504B.

It is unfortunate that 500 holes were drilled in the oceans before downhole experiments such as those using packers, televiewers, borehole seismometers and long period resistivity logs were attempted, but the spectacular success of such in situ observations, coupled with the powerful geochemical sampling and analysis techniques now available, promise to add a truly new dimension to deep-sea drilling as a scientific tool. The return to Costa Rica will very probably produce two kilometre penetration into the oceanic crust, with a full set of in situ experiments.

By then, more than 105 m3 of seawater will have been drawn into the oceanic basalt below the sea floor, never again to reappear in the ocean as long as the chert and sedimentary lid remain intact.

Such huge natural reservoirs have great potential as sites for the disposal of toxic wastes and offer several advantages over present proposals for land-based sites. The natural underpressures will draw seawater or wastes into the oceanic crust and if the seal is broken accidentally (by an unexpected earthquake, for example) oceanic bottom water will flow downwards into the crust rather than allowing toxic waste to escape into the ocean. Any natural heating which might accompany toxic waste burial should enhance the seal since it would convert more and more sediment into chert above the disposal cell and increase the seal's thickness. Once toxic waste was drawn into the immense oceanic crustal convection cell, its residence time would be far longer than the time for which it might endanger the environment.

Verification of these advantages is still awaited, of course, but the return of the Challenger to the Costa Rica Rift site should greatly help our evaluation of such deep-sea disposal sites.

Solar physics at Oxford

from I.W. Roxburgh and C. Jordan

THE growing importance of solar physics within astronomy was illustrated by a recent meeting* at Oxford which took as its theme 'Solar Activity'. Topics ranged from the underlying causes of solar activity in the interaction between the sub-photospheric convection zone and magnetic fields to the solar flares, the highest energy manifestation of the active Sun.

Weiss (University of Cambridge) described recent theoretical work on turbulent magneto-convection. Attention is now turning from the systematic largescale fields, rooted deep in the convective zone, to the small-scale feature such as ephemeral active regions. Recent results suggest that these small flux loops are examples of the intermittent turbulent magnetic fields in the upper part of the convective zone, where magnetic flux has been confined to isolated ropes winding between convective eddies.

Bruzek and Schröter (Kippenheur Institut, Freiburg) stressed the current view of solar active regions developing through the emergence and expansion of new magnetic flux. In the higher layers of the transition region and corona active region

^{1.} Sclater, J.G. and Francheteau, J. Geophys. J. RAS 20, 509 (1970).

2. Sclater, J.G., Anderson, R.N. and Bell, M.L. J. geophys.

Res. 76, 7888 (1971).

^{3.} Anderson, R.N., Langseth, M.G. and Sclater, J.G. J. Anderson, R.N., Langsein, M.G. and Sciatel, J.G. J. geophys. Res. 82, 3391 (1972).
 Anderson, R.N., Hobart, M.A. and Langseth, M.G. Science 204, 828 (1979).
 Wolery, T.J. and Sleep, N.H. J. Geol. 84, 239 (1976).

Edmond, J. Earth planet. Sci. Lett. 46, 1 (1979).
 Corliss, J.B. et al. Science 203, 1073 (1979).

Anderson, R.N., Uyeda, S. and Miyashiro, A. *Geophys. J. Res.* 44, 333 (1976).

Anderson, R.N. and Skilbeck, J.N. The Sea Vol. 7, (Interscience, 1981). 10. Langseth, M.G., Becker, K., Hobart, M. and Von Herzen,

R FOS 62, 308 (1981). Anderson, R.N. and Zoback, M.D. EOS 62, 308 (1981)

Von Herzen, R.P., Francis, T.J.G. and Becker, K. EOS 62, Anderson, R.N. and Zoback, M.D. EOS 62, 308 (1981). Stephen, R.A. EOS 62, 309 (1981).

Honnorez, J.J. EOS 62, 309 (1981).

^{16.} Mottl, M.S. and Lawrence, J.R EOS 62, 310 (1981).

^{*}The Solar Physics Section of the European Physical Society held its Triennial meeting on 13-15 April, 1981. The meeting was preceded by a two-day workshop on 'Near Future and Plans for Solar Research'.

fields show as distinct loop structures. Substantial progress has been made in determining the temperature and density structure of these loops, and in understanding their stability(Monsignori-Fossi, Arcetri Observatory). Controversy remains concerning how much information on the heafing processes can be obtained from analyses of emission line fluxes. This is an area where further highresolution observations are required, particularly time-dependent measurements of line profiles. Maltby (University of Oslo) stressed the same point with respect to the energy balance in sunspots. Gurman (Goddard Space Flight Center) reported observing, with the UVSP instrument on the Solar Maximum Mission satellite, oscillations with periods of 120 to 180 seconds in fluxes and velocities measured in the chromosphere-corona transition region over a sunspot. It has proved difficult to detect oscillations in the quiet sun with previous satellites. Thomas and Scheuer (University of Rochester) presented results of calculations which show that oscillations could be caused by resonant behaviour due to the strong vertical trapping of wave energy. Both Maltby and Stenflo (University of Zurich) drew attention to the importance of current and future measurements of the solar irradiance from spacecraft in understanding the sunspot flux deficit.

Solar flares represent the most extreme form of activity and several reviews were given of recent results from the Solar Maximum Mission satellite. This satellite has for the first time allowed the simultaneous study of active regions and flares over a broad spectral range and thus over a range of heights and temperatures in the atmosphere. Whilst spectroscopic methods are revealing the temperature and density conditions during flares, the imaging instruments are being used to show where and when flares occur in relation to the longer-term development of particular active regions. In particular, the identification by the hard X-ray imaging spectrometer team of the source of hard X rays with the foot-points of loops, rather than with the region producing softer X-ray emission, will limit the choice of suitable models for the hard X-ray emission. Measurements of Fe Ka emission caused by fluorescence and the first resolved spectra of the Fe xxvi resonance plus di-electronic satellite lines were also reported by the X-ray polychrometer team.

Before the meeting, delegates from all parts of Europe discussed their plans for the rest of the 1980s. A remarkable consensus emerged concerning important priorities including, for example, measurements of velocity fields and magnetic fields related to the development of emerging

I.W. Roxburgh is Head of the Department of Applied Mathematics at Queen Mary College, London and C. Jordan is lecturer in the Department of Theoretical Physics, University of Oxford. magnetic flux; and studies of small magnetic elements in the convective super-granulation structure.

Of the ground-based programmes the most ambitious concerns the development of a large European Solar Telescope which would offer European solar physicists a high-resolution telescope situated in the Canary Islands. This project can be seen as a logical extension of the previous sitetesting activities of the Joint Organization for a Solar Observatory.

Several countries will continue to participate in the USSR's Interkosmos and Prognoz series of rockets and satellites, with particular interest in X-ray imaging and spectroscopy. The community within the European Space Agency is awaiting the outcome of representations to NASA concerning the US part of the two-spacecraft International Solar Polar Mission. The US spacecraft, which is due to carry out experiments to study the corona near the Sun, is

threatened by recent budget cuts.

The strength of theoretical solar physics in Europe and its close involvement in analysis of new data are particularly encouraging since they should allow a good return from participation in a variety of guest investigator programmes as opportunities arise.

There is keen European interest in two potential solar payloads for the Shuttle Spacelab: in the Solar Optical Telescope, at present under study within NASA, and in the complementary Grazing Incidence Solar Telescope, currently a potential ESA project. The future of these missions depends on the success of the Shuttle; the participants abandoned the dining hall at 7.10 on Tuesday, 14th April, to crowd round the television set and witness the safe return of the first Shuttle flight. With some relief the participants returned to continue their dinner and polish up their proposals for future experiments.

Glueballs, anyone?

from T.F. Walsh

THE QUARK MODEL, invented in 1963 by George Zweig and Murray Gell-Mann, has gradually grown into a theory of elementary particles. It is now called quantum chromodynamics. An essential element of the theory was provided by Yoichiro Nambu in 1966. He suggested that the quarks are bound together in particles by the exchange of spin one vector particles, which have a new quantum number unlike the conventional isotopic spin, strangeness and so forth typical of the quarks. Nambu's exact model has been discarded by most physicists, but the idea remains.

The vector particles which bind quarks are now called gluons and their quantum number is called colour. Gluons interact strongly with quarks over distances of 10-13 cm, binding them into particles. Quarks and gluons are thought not to exist as free particles, but only inside the known 'colourless' particles, although this has yet to be proved. As well as their interaction with quarks, gluons interact strongly with each other. They should therefore bind into particles even without quarks. These particles, made entirely of gluons, have not yet been seen, but there is a strong conviction among particle physicists that they do exist. They are called bound glue states, gluonia or glueballs. They are a new type of elementary particle and it is very important to look for them.

A recent issue of *Physical Review Letters* contained two papers on glueballs, by K.

T. F. Walsh is a visiting scientist at CERN, Geneva.

Ishikawa at DESY in Hamburg and by M. Chanowitz at the Lawrence Laboratory in Berkeley^{1,2}. Chanowitz' paper is entitled "Have We Seen Our First Glueball?" Indeed, have we? And why should the first one be seen now, after hundreds of quark states have been tabulated in the celebrated Particle Data Group tables?

If glueballs do exist, it is important to know where to look for them. According to quantum chromodynamics, heavy particles like the J/Ψ and the Υ mesons can only decay first to gluons, or a photon and gluons, which then materialize into conventional mesons. The decay of the J/Ψ meson into a photon and two gluons ought to be a good place to look for glueballs. If the two gluons materialize into ordinary mesons through the intermediary of a glueball, experimenters ought to see a nearly monoenergetic photon recoiling against a resonance which decays to mesons. The chain would look like this:

It has even been suggested that the two gluons should in some way maximally generate glueballs in this chain and that the production of mesons made of quarks should be much smaller than the glueball production. According to this view, one per cent of J/Ψ decays would contain a glueball (there might, of course, be several glueballs visible at different masses)³⁻⁵. If only gluons

maximally generate glueballs, that would explain why they have not been seen until now; J/Ψ and the Υ , themselves only recently discovered, would be the first good sources of glueballs. It has also been pointed out that the glueballs produced in this reaction would mostly have spin two, with the production of spinless glueballs slightly suppressed⁶.

Experimenters have studied J/ Ψ decays since 1974, and decays to a photon and the eta, the eta prime and the f(1250) meson (the 1250 stands for the mass in MeV) have been seen. They are produced at a rather low rate and are all well known quark states (although there is a bit of doubt about the eta prime). Recently, groups working at the SPEAR e⁺e⁻ storage ring in Stanford, California found the decays

J/ Ψ→KKπ J/ Ψ→ηππ

where the $K\bar{K}\pi$ (and, less clearly, $\eta\pi\pi$) form a narrow resonance of mass 1,440± 15 MeV and width $50\pm\frac{30}{20}$ MeV. This was a great surprise, because there is a very obscure state called the E(1420) in the particle tables, and it has roughly these parameters, although there is no convincing evidence that it decays to $\eta \pi \pi$. Why should this obscure state appear together with the well known eta, eta prime and f(1250)?

One might guess that the new state seen in J/Ψ decay is the same as the known E(1420), and that this particle is one of the long sought glueballs. However, careful examination of the experimental data shows that this interpretation cannot be correct if one does not want to upset well established ideas about the production of mesons in, for example, πp collisions. Chanowitz and Ishikawa suggest that there are two mesons of mass around 1,420 MeV. One of them is listed in the data tables, and it has spin one and even parity. It decays to $KK\pi$ but not (or seldom) to $\eta\pi\pi$. The other meson is new, seen only in J/Ψ decay (and maybe in old pp annihilation experiments at low energy). Chanowitz calls this new particle G(1440), and both authors claim that it is a spin-zero glueball of negative parity. It decays to $K\bar{K}\pi$ and also to $\eta\pi\pi$. Neither author claims to prove that there are two different mesons here - what they do is to try to square the unexpected appearance of the state in J/Ψ decay with prevalent theoretical ideas.

What next? The authors suggest several steps. First, the spin and parity of the resonance seen in J/Ψdecay should be checked. If it is 0- and the spin parity assignment of the E(1420) is really 1 $^+$, then the one seen in J/\Pdecay is definitely a new particle. Evidence that the old E(1420) and the new state decay with different rates to

ηππ would supprt this. Also, if the state seen in J/Ψ decay is 0-, it will be seen in the photon-photon process

e+e- → e+e- + "G(1440)"

in the channels $KK\pi$ and $n\pi\pi$. In this process, the colliding electron and positron act as sources of photons, which then create particles. That is why the electron and positron come out of the reaction, only slightly reduced in energy having each given up a photon to make the particle. This reaction is useful because the spin-one E(1420) is forbidden to appear by a theorem of C.N. Yang which says that a vector particle cannot couple to the two photons in this reaction. According to theoretical ideas, a glueball will not appear at a large rate in this reaction, but the

estimate of Ishikawa indicates that it can be seen. Finally, Chanowitz emphasizes that one should look for the proposed new G(1440) in gluon jets, available in the threeiet events seen at the electron-positron storage ring PETRA in Hamburg, and in decays of the \(\gamma \) meson. Again, gluons should be a good source of glueballs.

If all these experimental proposals work out and there is a new particle, G(1440), made as a glueball should be, then we will indeed have seen our first glueball. And more will follow — at the very least a spin-two glueball which is expected to be produced with a significant rate in J/Ψ decay. This would provide a welcome confirmation of the ideas of quantum chromodynamics and new impulses for its further development.

New morphological transition at the Curie temperature

from Mildred S. Dresselhaus

THE increasing sophistication of the techniques of surface science now allow the quantitative study of some long-standing problems. In a recent letter, Hamilton and Jach¹ describe a new kind of structural phase transition on low-angle nickel surfaces at the Curie temperature which gives insight into an effect discovered nearly fifty years ago2. The Hedvall effect is characterized by a discontinuous change in the temperature dependence of the chemical reaction rate (Hedvall Effect I) or as a discontinuous change in activation energy (Hedvall Effect II) at the Curie temperature (T_c) , the temperature of the ferromagneticparamagnetic phase transition. The Hedvall effects are of both theoretical and practical interest as a means to vary or control chemical reactions by external means.

The work of Hamilton and Jach1 is an extension of previous studies by Thapliyal and Blakely3 who showed a reversible morphological transition on a stepped nickel surface using LEED (low energy electron diffraction) as a structural probe and AES (Auger electron spectroscopy) as a surface compositional probe. Hamilton and Jach demonstrated by permeability measurements that this morphological transition occurred at the Curie temperature of nickel. Both studies1,3 focused on vicinal surfaces, defined as single crystal surfaces in the vicinity of lowindex planes. Vicinal surfaces are composed of terraces of low Miller index planes, joined by ledges a few atoms in height. Direct information on terrace widths and step heights is obtained from structure in the LEED patterns, using a kinematic theory of electron scattering from stepped surfaces4.

Mildred S. Dresselhaus is Professor of Electrical Engineering and Director of the Center for Materials Science and Engineering at the Massachusetts Institute of Technology.

The particular vicinal surfaces that were investigated were the Ni(111)5°[110] and Ni(111)10°[110] surfaces, denoting surfaces at 5° and 10° from the (111) plane in the [110] zone, so that the resulting stepped surfaces have (111) terraces and (110) steps. For both of these stepped surfaces, a reversible structural phase transition was observed at the magnetic ordering temperature T_c of the nickel. In the paramagnetic phase, each of the samples exhibited single-atom step heights and narrow terraces, while in the ferromagnetic phase, the Ni(111)10°[110] sample showed a two-atom step height, and the Ni(111)5°[110] sample a four to five-atom average step height and wider terraces.

In addition to this reversible structural transformation, an associated chemical transformation was also reported at $T_c^{1,3}$. To study this transformation, clean nickel surfaces were prepared by argon-ion bombardment, heat treatment and annealing, to yield a surface exhibiting less than 1/20 of a monolayer impurity, as monitored by AES. With the nickel samples in the paramagnetic state, carbon, a dominant impurity in nickel, was found to be dispersed in the bulk of the nickel samples. In contrast, with the nickel in the ferromagnetic phase, carbon from the bulk segregated to the surface, forming a carbon coverage of ~ 0.2 monolayers just below T_{c} but less than 0.003 monolayers of carbon just above T_c . Assuming no entropy change at T due to carbon segregation at the surface, the measured carbon coverage implies a change of greater than 0.2 eV per carbon atom in the heat of segregation at the Curie point.

This change in binding of a surface atom at the magnetic phase transition is also observed in various manifestations of the Hedvall effect, such as studies of the rate of Co sublimation by Sales et al.5, the rate of carbony-

^{1.} Ishikawa, K. Phys. Rev. Lett. 46, 978 (1981).

Chanowitz, M. Phys. Rev. Lett. 46, 9/81 (1981). Koller, K. & Waish, T.F. Nucl. Phys. B140, 449 (1978).

Brodsky, S.J. et al. Phys. Lett. 73B, 203 (1978). Fritzsch, H. & Minkowski, P. Nuovo Cimento 30A, 393

Billoire, A. et al. Phys. Lett. 80B, 381 (1979)

Scharre, D. et al. Phys. Lett. 97B, 329 (1980).

lation of Ni by Mehta et al.6 and the rate of Ni oxidation by Sales et al.7. In all these studies it was found that more energy must be expended to remove a surface atom from a ferromagnetic substrate than from a paramagnetic one. The observations are explained by a lowering of the internal energy of the system as magnetic order is established at the onset of the ferromagnetic state. To overcome this additional binding, more energy must be supplied to remove a surface magnetic atom from a ferromagnetic substrate.

The sublimination of a magnetic species such as cobalt represents an especially attractive process for the study of the magneto-chemical Hedvall effect because of the simplicity of the reaction and because of the removal of the reaction product once the reaction is completed. On the other hand, technical difficulties associated with the high temperature experiment $(T_{c} \sim 1,400 \text{ K for Co})$ had to be overcome to make quantitative measurements. The change in the apparent activation barrier for sublimation on passing through the Curie temperature for Co is 18 kcal per mole or about 0.8 eV per atom. By analysing the temperature dependence of the sublimation rate below and above T_s , Sales et al.5 were able to show that an additional binding energy, $E_m(T)$, was introduced in the ferromagnetic state and associated with the spontaneous ordering of the magnetic moments, $E_m(T)$ $\sim [M(T)/M(0)]^2$, where M(T) is the spontaneous magnetization at temperature T. Because of the rapid change in M(T) near T_c , a large apparent change in activation energies is found near T_c for physically reasonable values of $E_m(0)$, such as 0.2 eV per atom for Co. The magneto-chemical effects associated with this sublimation process have been qualitatively explained in theoretical models by Suhl⁸ and by D'Agliano and Huberman9.

Another chemical reaction for which the reaction product leaves the surface on completion of the reaction is the nickel carbonylation reaction, whereby nickel reacts with CO gas to form gaseous nickel carbonyl. This reaction has the further attractive features of a convenient temperature range (300< T < 440 K) and a Curie temperature controllable by dilution of the nickel with copper (which does not react with CO in this temperature range).

Just as for the sublimation reaction, the nickel carbonylation reaction also shows a change in the apparent activation barrier at the Curie temperature, which in this case is about 0.4 eV per atom. This experiment provides further evidence that an additional activation barrier $E_{\rm m}$ is associated with the ferromagnetic state, insofar as E_m decreases with increasing Cu concentration such that $E_m \rightarrow 0$ for the Cu concentration where $T_c \rightarrow 0$.

Extensive studies of the Hedvall effect have also been carried out on the oxidation of nickel⁷ and of iron¹⁰, yielding general agreement with the basic conclusions of the above magneto-chemical experiments. These oxidation experiments have, however, been more difficult to interpret

quantitatively because of the accumulation of the reaction product at the free oxide surface and the concomitant presence of the additional magnetic barrier at the internal metal-oxide interface.

The identification of a morphological surface transformation at the Curie temperature T_c suggests new insights into the magneto-chemical Hedvall effect because of the enhanced chemical activity at surface steps and kinks. At this point one can only speculate that the discontinuity in the reaction rate at T_c (Hedvall Effect I) might be associated with a morphological change in step size, while a discontinuity in the activation energy or in the derivative of the reaction rate (Hedvall Effect II) corresponds to no morphological change.

Sir Isaac Newton and his madness of 1692 and 1693

from Milo Keynes

By NO MEANS had Sir Isaac Newton a perfect character. William Whiston who succeeded him in his Lucasian professorship at Cambridge said of him that "he was of the most fearful, cautious and suspicious temper"; John Flamsteed, the first Astronomer Royal, declared he was "insidious, ambitious, and excessively covetous of praise, and impatient of contradiction". Even his firm friend, John Locke, wrote that "He is a nice" (that is, difficult and over-precise) "man to deal with, and a little too apt to raise in himself suspicions where there is no ground". There are, however, many instances of his kindness and generosity to others.

Newton had an abnormal dread of controversy, and he wrote to the experimental scientist, Robert Hooke, that "There is nothing I desire to avoid in matters of philosophy more than contention, nor any kind of contention more than one in print". Newton was neurotic, and as John Maynard Keynes wrote1: "His deepest instincts were occult, esoteric, semantic with profound shrinking from the world, a paralysing fear of exposing his thoughts, his beliefs, his discoveries in all nakedness to the inspection and criticism of the world. He parted with and published nothing except under the extreme pressure of friends. Until the second phase of his life (as Master of the Mint in London, and President of the Royal Society), he was a wrapt, consecrated solitary, pursuing his studies by intense introspection with a mental endurance never equalled" Besides all this, he underwent a period of severe emotional and mental disturbance

with sleeplessness and loss of appetite during 1692 and 1693.

In 1693, Newton wrote to Samuel Pepys: "I am extremely troubled at an embroilment I am in, and have neither ate nor slept well this twelve month, nor have I my former consistency of mind, but am now sensible that I must withdraw from your acquaintance, and neither see you nor the rest of my friends any more, if I may but leave them quietly". The same year he wrote to Locke: "Being of the opinion that you endeavoured to embroil me with women and by other means I was so much affected with it as that when one told me that you were sickly and would not live I answered twere better you were dead. I desire you to forgive this incharitableness" Both Pepys and Locke appear to have recognised that Newton's mind was deranged and maintained their friendship for him, and in other letters it is clear his memory was impaired.

It was with the publication of The Correspondence of Sir Isaac Newton2 in 1961 that the period of irrationality could be more fully recognised. Newton was born in 1642 and died in London in 1727. He matriculated at Trinity College. Cambridge in 1661, became Lucasian Professor in 1669, and Fellow of the Royal Society in 1672. He left Cambridge in 1696 to become Master of the Mint in London where "with Pepys and William Lowndes (Secretary to the Treasury) he became one of the greatest and most efficient of our civil servants. He was a very successful investor of funds, surmounting the crisis of the South Sea Bubble, and died a rich man" (Keynes1). He was elected President of the Royal Society in 1703, being annually re-elected for the next 24 years, and in 1705 he was knighted by Queen

Milo Keynes is in the Department of Anatomy, University of Cambridge.

^{1.} Hamilton, J.C. and Jach, T. Phys. Rev. Lett. 46, 745

Hedvall, J.A., Hedin, R. and Persson, O. Z. physik. Chem. B27, 196, (1934).

Thapliyal, H.V. and Blakely, J.M. J. Vac. Sci. Technol. 15, 600 (1978).

Henzler, M. Surf. Sci. 36, 109 (1973).
 Sales, B.C., Turner, J.E. and Maple, M.B. Phys. Rev. Lett.

Sales, B.C., Turner, J.E. and Graphe, A.D. Phys. Rev. Lett. 44, 586 (1980).
 Mehta, R.S., Dresselhaus, M.S., Dresselhaus, G. and Zeiger, H.J. Phys. Rev. Lett. 43, 970 (1979).
 Sales, B.C. and Maple, M.B. Phys. Rev. Lett. 39, 1636. (1977); Sales, B.C., Maple, M.B. and Vernon, F.L. III *Phys. Rev.* **B18**, 486 (1978).

Suhl, H. Phys. Rev. B11, 2011 (1975).
 Galleani D'Agliano, E. and Huberman, B.A. (private communication).

Sales, B.C., Cabrera, A.L. and Maple, M.B. Solid State Commun. 30, 119 (1979).

Anne — although for political, rather than scientific reasons.

In London, "he reigned as the most famous man of his age, of Europe, and as his powers gradually waned and his affability increased — perhaps of all time, so it seemed to his contemporaries' (Keynes¹), but in the more than 30 years he lived there after leaving Cambridge, his illness of 1692 and 1693, which consisted of insomnia, loss of appetite, loss of memory, melancholia (depression), delusions of persecution, and possibly a trembling in his writing, appears to have been forgotten. After 1693, he still produced copious writings on his mathematical work, on lunar motion, atmospheric refraction and on resisted projectile motion3. He made the corrected Proposition X of the second book of Principia Mathematica in his seventieth year, and recast his theory of the propagation of light between 1705 and 1715. Besides the comment that he spoke very little in company after 1693, there was something rather languid in his look and manner in those years, as shown in a portrait, circa 1689, by Sir Godfrey Kneller in the possession of the Earl of Portsmouth.

Various explanations have been suggested for his illness, predominantly psychological, such as the shock at the death of his mother in 1679, a fire in which he lost some papers (though this occurred far earlier than 1692), his failure to gain various posts such as the Provostship of King's College, Cambridge (which he did not get for the reason he did not attend Eton College as a boy) or the Mastership of the Charterhouse, or simple exhaustion from writing the Principia which was, however, finished in 1687. Clearly, none of these is at all satisfactory, but recently it has been suggested^{4,5} that Newton's nervous breakdown might be explained by mercury poisoning arising from his experiments in alchemy and in making mirrors. Metallic mercury absorption by inhalation as mercury vapour, or by ingestion, can produce neurological, psychiatric, oral and renal signs, and these are reversible when exposure ceases. Mercury is stored in the brain, kidneys and liver, and over a period of time after the poisoning has stopped will continue to appear in hair and nails.

Newton's interest in alchemy was first shown in 1667 when he purchased the six volumes of Zetzner's *Theatrum Chemicum*, as well as chemicals and apparatus, though the first dated chemical experiment in his notebooks was in 1678, with the last in 1696 before he moved to London. He gave up his experiments for nearly two years from May 1684 till April 1686, the period in which he wrote the greatest part of his Principia, but during 18 years he carried out several hundred chemical experiments, with metals playing a large part. It is not always sure from the note books whether the experiments were actually performed, or were just thought of, and often it is not clear any longer what were the chemicals used. It is, however, well recorded that he would heat metals, or their ores, with salts in open vessels, breathing in the fumes and sometimes tasting the formed products. He used to sleep in his 'laboratory' by the fire, which was kept burning for weeks on end while the experiments were going on, and clearly exposed himself to the dangers of metal poisoning.

Newton's great niece, Catherine Conduit, married John Wallop, first Earl of Portsmouth, and as a result Newton's relics and manuscripts mainly passed to the Portsmouth archives. Spargo and Pounds⁵ obtained some of Newton's hairs from the Earl of Portsmouth as well as from Trinity College, Cambridge, and analysed them for mercury, gold, arsenic, antimony and lead. We do not know when the hairs were collected (though we do know Newton kept his hair short in older age which increase the chance that those analysed derived from a time nearer to the experiments), but

raised levels of the metals were found, especially of mercury and lead, and this would be most unlikely, even allowing for contamination from any vessels in which the hairs were stored, in ordinary hairs. It may be added that although some of Newton's symptoms could be caused by lead poisoning, all could be caused by mercury poisoning. We can presume that Newton showed erethismus mercurialis. that is, a timidity especially in the presenceof strangers, but we do not know for certain whether he showed the tremor of mercury poisoning (Hatters' shakes, a condition found among those using mercury in the felt hat industry and from which Lewis Carroll culled his 'Mad Hatter') as his handwriting did not decisively deteriorate during breakdown.

In his recent lengthy biography, Westfall⁶ suggests that Newton's symptoms were simply due to overwork in finishing the Principia in 1687, five years before their onset. This seems an unlikely explanation in view of the delusions Newton suffered and the raised levels of mercury and lead in his hair. The epitaph remains with Newton, who shortly before he died, said: "I do not know what I may appear to the world; but to myself I seem to have been only like a boy, playing on the seashore, and diverting myself, in now and then finding a smoother pebble or a prettier shell than ordinary, while the great ocean of truth lay all undiscovered before me".

Are transport proteins porous?

from N. Michael Green

In the absence of reliable methods for determining molecular size in the lipid bilayer, the state of aggregation of proteins in membranes has proved difficult to determine. A variety of indirect methods have led to conflicting interpretations. Even when the state of aggregation has been established, further experiments are required to clarify its relation, if any, to the function of the protein and even if the oligomeric structure is proved to be essential, the further interesting question of the inter- or intra-subunit location of a transport channel requires an experimental answer.

The difficulty of evaluating the evidence is emphasized by the somewhat conflicting conclusions of two recent reviews. The more wide-ranging article (Klingenberg Nature 290; 449, 1981) considers thirteen membrane proteins or complexes of proteins for which there is evidence for an

N. Michael Green is in the National Institute for Medical Research, Mill Hill, London. oligomeric structure (these include the ADP/ATP carrier, $(Na^+ + K^+)$ - and Ca^{2+} -ATPases, anion carrier (band III), glucose carrier, porin, bacterial rhodopsin, acetylcholine receptor and various cytochrome complexes). The evidence, derived mainly from sedimentation experiments in non-ionic detergents and from cross-linking experiments in membranes, shows that these proteins are all either dimeric, or trimeric, although when the dust settles around the acetylcholine receptor it is likely that each unit of the dimer will be found to be a pentamer.

Klingenberg emphasises the useful conclusions that can be drawn from consideration of symmetry. The vectorial activities of transport proteins imply the absence of symmetry axes from the plane of the membrane and this eliminates dihedral structures from consideration. The popular tetrameric structure for soluble, globular proteins is therefore unlikely to be found among the proteins of membrane transport which must possess cyclic symmetry and a transmembrane

Keynes, J.M. Newton, the Man (The Royal Society Newton Tercentenary Celebrations) (Cambridge University Press, 1947).

The Correspondence of Sir Isaac Newton 3 (Cambridge University Press, 1961).

^{3.} Whiteside, D.T. Personal communication, 1980.

Johnson, L.W. & Wolbarsht, M.L. Mercury Poisoning: a Probable Cause of Isaac Newton's Physical and Mental Ills (Notes and Records of the Royal Society of London 34; 1, 1979).

Spargo, P.E. & Pounds, C.A. Newton's 'Derangement of the Intellect': New Light on an Old Problem. (Note and Records of the Royal Society of London 34, 11: 1979)

^{6.} Westfall, R.S. Never at Rest: a Biography of Isaac Newton (Cambridge University Press, 1981).

symmetry axis. Such a structure will be stable in a lipid environment only if the intersubunit links include a strong polar component, the symmetry axis providing a plausible location for a polar channel for translocation of substrates.

The accommodation of ligand binding sites and a gating mechanism in a dimeric channel raises a number of interesting questions and leads to the conclusion that there are likely to be only half as many active sites as subunits. Klingenberg develops this argument around the ADP/ATP exchange protein mitochondria which his laboratory has clearly shown to exhibit such half-of-sites reactivity towards the mutually exclusive inhibitors bongkrekate and carboxyatractyloside. The evidence for other carriers is less clear cut and Klingenberg concludes that while the $(Na^+ + K^+)ATP$ as and the glucose carrier of erythrocytes show halfof-sites reactivity, the anion transport protein, the B-galactoside carrier and bacterial rhodopsin probably have separate channels associated with each

Kyte (see this issue of *Nature*, p.201) directs his attention mainly to the $(Na^+ + K^+)ATP$ ase, though he considers his arguments are likely to apply with equal force to the functionally homologous Ca^{2+} -ATPase and $(H^+ + K^+)$ ATPase. He takes a sceptical attitude to the evidence for dimeric structure, pointing out the problems associated with: (1) the large corrections which have to be made for bound detergent and liquid, (2) the underestimation of the molecular weights of intrinsic membrane proteins by gel electrophoresis, and (3) the interpretation of the results of cross-linking experiments in membranes, where intermolecular distances are often very small. Nevertheless he concludes that on balance the evidence does favour a dimeric $(Na^+ + K^+)$ -

The evidence for half-of-sites reactivity is much less convincing, particularly in the light of two recent papers in which the equivalent weight of the ATPase towards ouabain and towards TNPATP has been critically re-evaluated as $180,000 \pm 20,000$. This figure, consistent with one site per 2β protomer, results mainly from use of a more objective method for determining the protein concentration (amino acid analysis) in place of the uncalibrated Lowry method. He also points out that the anti-cooperative kinetics of all three transport ATPases with respect to ATP does not necessarily imply interaction between the two sites on a dimer; it is equally consistent with a monomer in which the ATP site can exist in interconvertible low- and high-affinity conformations. In view of the elusive behaviour of apparent half-of-sites reactivity of globular proteins under critical examination, stronger evidence is required to substantiate it for the $(Na^+ + K^+)ATPase.$

Doubts also surround the Ca²⁺-ATPase of sarcoplasmic reticulum. Although it possesses one ATP site per peptide chain only half of them appear to be phosphorylated in most membrane preparations. Moreover, recent evidence (Pick and Karlish *Biochim. biophys. Acta* **626**, 255) suggests that one mole of covalently bound fluorescein isothiocyanate can block the activity of two peptide chains. On the other hand, the monomer in non-ionic detergent is a fully active ATPase showing steady-state kinetics which differ little from those

of oligomeric preparations.

Of the well characterised transport proteins showing apparent half-of-sites reactivity, only the ADP/ATP exchange protein survives unchallenged; even here the intersubunit channel is no more than the most plausible hypothesis. There is firm structural evidence for the location of the channel in bacterial rhodopsin which functions as a monomer and contains a plausible intrasubunit channel. For the remainder the definitive experiments have still to be devised.

Acid precipitation — a new study from Norway

from J.N.B. Bell

IN THE 1960s the destruction of freshwater fish stocks and the impairment of forest productivity in Scandinavia were blamed on an increase in the acidity of water and soils. It was believed that the increase resulted from 'acid rain' — precipitation containing sulphuric acid derived from the oxidation of SO₂ emitted by the more industrialized European nations. Subsequently there have been similar claims elsewhere, particularly in the northeastern United States and Canada.

Public concern has nowhere been more evident than in Norway, where in the mountainous regions of the south, an accelerating decline in fish stocks has led to the extinction of fish populations in an area of 13,000 km², with severe problems over another 20,000 km². This stimulated the establishment of a major Norwegian interdisciplinary research programme into the biological effects of acid precipitation, which has recently terminated after eight years. The numerous reports published on the findings this project have now been summarized (Overrein, Seip and Tollan Acid precipitation — effects on forest and fish. Final report of the SNSF project 1972-1980).

It is clear from the final report that the SNSF project has made a major contribution towards the understanding of the relationships between the chemistry of precipitation, soils and aquatic ecosystems. The main problems in determining the ecological significance of acid precipitation lie in extrapolating the results of short investigations to the long-term effects of relatively small changes in soil and water chemistry. It has now been established beyond doubt that the precipitation in southern Scandinavia has become more acidic as a result of long-distance transport of air pollution, with

nitrogen oxides contributing an increasing proportion of the acidity. The inputs of H^+ , SO_4^{2+} and NO_5^- are readily estimated by routine analysis of precipitation samples collected at many sites in Norway. However, some uncertainty remains as to the importance of the dry deposition of gaseous and particulate sulphur and nitrogen compounds directly onto vegetation and soils, which may make a substantial contribution to total deposition in some areas.

The report admits that there are few reliable records of the acidity of lake water before 1950 but the marked shift since then towards more acid conditions in areas subject to acid precipitation is well documented. Only five to ten per cent of precipitation in southern Norway falls directly onto lakes and rivers. Thus a thorough understanding of the chemical changes occurring in water when it comes into contact with solids and vegetation is of prime importance when considering the ultimate effect on aquatic ecosystems. A key component of the SNSF project has been the assessment of annual precipitation input and ground-water output into waterways of major ions in selected catchments. It is now apparent that, as well as increased acidity, the mobilization of aluminium from soils by acid water is a major factor in the destruction of fish stocks. The uptake of salt via the gills, necessary to maintain the osmotic balance of the fish, is impaired by acid conditions and is further aggravated by aluminium stimulating the secretion of mucous.

Particularly severe problems are experienced in southern Norway, because a large proportion of the precipitation falls as snow. Pollutants accumulate within the snow-pack during the winter and are subsequently released very rapidly during the short period of snow-melt in the spring, resulting in a flush of acid water into lakes. Knowledge of the hydrology of snow-melt is limited but the SNSF project has

J.N.B. Bell is at the Centre for Environmental Technology, Imperial College of Science and Technology, London. examined the chemical content of successive fractions of melt-water: up to eighty per cent of the pollutant content of a snow-pack may be released in the first thirty per cent of the melt-water, so increasing the threat to the sensitive larval stages of salmon and trout.

The effect of acid precipitation on terrestrial vegetation is less certain. Despite an intensive research programme, the SNSF project provides little support for earlier predictions of massive losses in timber production through leaching of nutrients from soils and direct effects on trees. Most experiments demonstrating adverse effects on soils and plant growth used artifical rain with unrealistically low pH levels. Indeed, in several cases stimulation of plant growth has been observed. The situation is complicated because acid precipitation can increase the availability of plant nutrients by providing inputs of sulphur and nitrogen from the atmosphere and releasing basic cations by weathering of minerals in the soil. It is rather unfortunate that this generally well balanced report implies that acid precipitation must be assumed to reduce tree growth, in the absence of proof to the contrary.

The causal relationship between acid precipitation and the decline of fish stocks has been questioned by Rosenqvist (Sci. Total Environ, 10, 39; 1978), who drew attention to the impact of changes in catchment land use on the chemistry of aquatic systems. During the last one hundred years, many of the Norwegian upland farms, used for summer grazing, have been abandoned and in some cases replaced by conifer woods, which would be expected to accelerate natural soil acidification processes. The SNSF programme has found no consistent pattern which could explain lake acidification on the basis of land use changes. However, an investigation in central Scotland has shown a clear effect of afforestation in increasing stream acidity (Harrison and Morrison in Ecological Impact of Acid Precipitation, eds Drabl and Tollan, SNSF Project, 312; 1980). The report concedes that afforestation may enhance the acidification caused by precipitation. This remains an area of uncertainty and there is a clear need for a better understanding of the relative importance of different sources of

acidification at sites where fish stocks are under stress.

The eight-year SNSF project has. clarified many aspects of the acid precipitation problem. The claims that reduction of fish stocks has been caused by acid precipitation have generally been substantiated, although questions remain concerning the contribution from changes in land use. In contrast, the long-term effect of acid precipitation on forest productivity is far from understood and intensive studies have failed to reveal any clear trends. The SNSF project has predicted that a reduction of at least seventy per cent in H+ and SO₄2 concentrations in precipitation is necessary to restore all lakes to their previous condition: this would involve international changes in energy policy which would encounter severe political obstacles. So far as action within Scandinavia is concerned, attempts to improve the water quality by the addition of lime, followed by restocking with fish, have achieved only limited success and a profitable course may be an extension of a preliminary SNSF programme for breeding strains of trout and salmon tolerant to acid conditions.

100 years ago

"Anthropology: an Introduction to the Study of Man and Civilisation, by Edward Tylor, D.C.L., F.R.S. With Illustrations. (London: Macmillan and Co., 1881.)

To those readers whose knowledge of ethnology or anthropology has been derived from a perusal of Prichard's "Natural History of Man," or the compilations of Wood, Brown, Peschel, or Brace, the present work will present a surprising amount of freshness and originality. They will in fact find themselves introduced to a new and very captivating science.



Andaman Islanders

The first chapter contains a brief sketch of what we learn from history, archaeology, and geology, as to man's antiquity and early condition; and in the next we are shown man's relation to the lower animals both in bodily structure and mental characteristics. The numerous remains now discovered of prehistoric man, and of his works, dating back to an undoubtedly vast antiquity, show us in no case any important deviation from the existing human type, nor any indication that his mental status was lower than (if so low as) that of many living races. At the same time the increasing rudeness of his implements as we go back, undoubtedly indicates that we have

made some approach towards the period when he first emerged from the purely brute state and became "a tool-using animal."

In the next chapter we have an excellent sketch of the chief races of man copiously illustrated by portraits, mostly from photographs and very characteristic. Among the best are those of the Andaman Islanders and the Dyaks, which we here reproduce.

The four chapters on Language, are exceedingly interesting and instructive, especially the account of the gesture language and the illustrations of how connected stories may be told to the deaf-and-dumb quite independently of any knowledge of alphabetical or even verbal signs. In treating of the origin of language Mr. Tylor doubts the sufficiency of the theory that emotional, imitative, and suggestive sounds were the basis on which all languages were founded, though he gives toleraby full illustrations of how roots thus obtained became modified in an infinite variety of ways to serve the growing needs of mankind in expressing their wants or their feelings. Putting aside all mere representations of animal sounds - as the whinny of the colt, the mew of the cat, or the bleat of the sheep consider how clearly do such words as slide, glide, and wave imply slow and continuous motion, the movement of the lips while pronouncing the latter word being a perfect double undulation. How curiously do the tongue and palate seem to be pulled apart from each other while pronouncing the words glue or sticky. How marked is the contrast between the harsh consonants used to express rough, rugged, and gritty, as compared with the soft flow of sounds in smooth, oily, even, polished. Among the Malay races, for instance, in words for large we find a prevalence of broad sounds involving a wide opening of the mouth, as busar, bake, bagut, lamu, elamo, ilahé, erämei, aiyuk, mäina and for small, words that are pronounced quickly and with slight opening of the lips, as kichil, chili, kidi, köi, roit, kemi, anan, kiiti, fek, didiki, all taken from languages of the Malay Archipelago.

The five following chapters treat of the Arts of Life, a subject which Mr. Tylor has to a great extent made his own, and which he discusses in a very interesting manner. The



doctrine of development in the arts is however somewhat strained when it is implied that the modern gun is an outgrowth of the South American or Indian blow-tube; while the origin of bank notes, and the account of the rise and progress of mathematics are hardly anthropology.

The next two chapters discuss the ideas of savage man as to the spirit-world, and the origin and development of myths; while the final chapter gives an admirable sketch of man as a social being, and of the development of that complex organism, Society. This thoughtful chapter cannot be epitomised, but the reader will find in it much curious information as to the sources of many of the customs, laws, and observances of civilised life, which are shown to be often traceable among the lowest savages.

ALFRED R. WALLACE

From Nature 24, 14 July, 242, 1881.

REVIEW ARTICLE

Molecular considerations relevant to the mechanism of active transport

Jack Kyte

Department of Chemistry, D-006, University of California, San Diego, La Jolla, California 92093, USA

A small group of closely related proteins is responsible for all active transport in animal cells, and inorganic cations are the only substances transported by these enzymes. They share a common kinetic mechanism in which two fundamental conformations participate, each receiving and dispatching substrates from its unique side of the membrane. During transport, the cations must pass through their enzyme to cross the membrane and intense interest is currently focused on the possibility that the path which they follow lies within the interface between two discrete subunits in a dimeric structure. Although 'half-of-sites' behaviour, consistent with this hypothesis, has been reported, it is now known that systematic errors were responsible for this mistaken conclusion. The number of protomers which comprise a functional unit of active transport has not been determined.

THE plasma membrane of an animal cell forms the permeability boundary which separates the cytoplasm from the external environment. A diverse collection of metabolites is transported across the plasma membrane by a group of transport proteins, each of which mediates the flux of a specific substrate or group of substrates. Enzymes responsible for the active transport of sodium and potassium¹, calcium^{2,3}, and protons and potassium⁴ have been purified to homogeneity. The minimum stoichiometries for the chemical equilibria catalysed by these enzymes are, respectively:

$$2K_{0}^{+} + 3Na_{i}^{+} + MgATP_{i} \rightleftharpoons MgADP_{i} + P_{i} + 3Na_{0}^{+} + 2K_{i}^{+}$$

$$2Ca_{i}^{2+} + MgATP_{i} \rightleftharpoons MgADP_{i} + P_{i} + 2Ca_{0}^{2+}$$

$$nK_{0}^{+} + nH_{i}^{+} + MgATP_{i} \rightleftharpoons MgADP_{i} + P_{i} + nH_{0}^{+} + nK_{i}^{+}$$

where i and o indicate the cytoplasm and extracytoplasmic spaces, respectively⁴⁻⁶. In broken membrane fragments or preparations dispersed in detergents, these reactions are expressed as a sodium- and potassium-dependent ATPase ((Na⁺+ K⁺)ATPase), a calcium-dependent ATPase (Ca²⁺-ATPase) and a proton- and potassium-dependent ATPase ((H⁺+ K⁺)ATPase). The movements of the cations in each direction and the hydrolysis of the MgATP are coupled obligatorily so that the free energy from the hydrolysis of ATP is converted into electrochemical gradients of the cations. The similarity of these three chemical reactions is quite striking.

No other enzymes which reside in the extramitochondrial membranes of animal cells and which can convert metabolic energy directly into the work required to move molecules against a concentration gradient have been described. In addition, every other known example of net extrusion or accumulation of any substance against its concentration gradient across an animal cell plasma membrane involves the coupling of that movement to the electrochemical gradient of sodium created by $(Na^+ + K^+)ATP$ ase. In other words, the three chemical equilibria shown above describe all known examples of active transport in animal cells. Therefore, when active transport is used as a term here, it will refer only to the reactions catalysed by $(Na^+ + K^+)ATP$ ase, Ca^{2+} -ATPase and $(H^+ + K^+)ATP$ ase.

These enzymes, as well as a membrane-bound ATPase in fungi responsible for active proton extrusion⁷, are very closely related to each other. They each contain a large polypeptide chain whose apparent length, from SDS-polyacrylamide gel analysis, lies between 900 and 1,200 residues^{1-4,8}. In each case

this polypeptide is the phosphorylated intermediate associated with catalysis^{3,9-12}. Additional similarities have been noted between Ca2+ ATPase of the sarcoplasmic reticulum and (Na++ K⁺)ATPase of the plasma membrane. Both proteins have an identical sequence around the aspartic acid residue which is phosphorylated during turnover¹³, and both have membraneaffiliated regions distributed throughout the catalytic subunit¹⁴⁻¹⁶. Finally, the phosphorylated aspartic acid in both proteins is located in the same region of the catalytic subunit, 200–400 residues from the amino terminus^{16,17}. Although the other enzymes which catalyse active transport, $(H^+ + K^+) \overline{ATP}$ as and the fungal H⁺-ATPase, have yet to be studied as carefully, it is reasonable to assume that they will share these similarities. These correlations suggest that all the enzymes which catalyse active transport share a common ancestor. Thus, conclusions drawn from any one of these enzymes probably apply to all of them. In particular, they all must have very similar kinetic mechanisms which proceed through analogous intermediate steps and all must have equivalent tertiary and quaternary structures, positions within the bilayer and cation channels.

It is unclear how these enzymes move cations across the membrane or how they couple the movement of the cations to ATP hydrolysis. The answers to these questions are likely to involve molecular explanations of at least two conformations which these enzymes can assume on the one hand, and on the other, of the minimum unit necessary for enzymatic function.

Kinetic mechanism

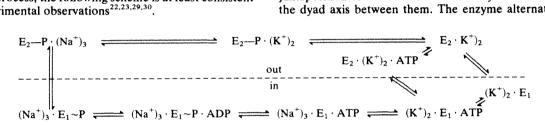
Various aspects of the sequence of kinetic steps through which (Na^++K^+) ATPase passes during its turnover have been examined both in membrane fragments and in the ATP-dependent, ouabain-inhibited, active cation fluxes of sealed systems. (Na^++K^+) ATPase can be covalently phosphorylated on an asparate residue by the γ phosphate of MgATP¹⁸; the phosphorylated intermediate is formed in the presence of sodium but hydrolysed in the presence of potassium¹⁹. This acyl phosphate has rates of formation and decay which are rapid enough for it to be an intermediate in the kinetic sequence of the reaction of the enzyme. When the rate of its forward decomposition is constant, the rate of overall turnover is directly proportional to its steady-state concentration²⁰. These experiments clearly establish that the enzyme is phosphorylated and dephosphorylated each time it turns over, and they divide active transport into two distinct steps.

Although the distinction between phosphorylated and unphosphorylated enzyme is a simple one involving the presence or absence of a covalent bond, there also seem to be at least two unique conformations of the native enzyme, the definitions of which are rather more ambiguous. Nevertheless, it is believed that the conformational changes which interconvert these states are more fundamental than phosphorylation and are responsible for the actual movement of the respective cations from one side of the membrane to the other. The two conformations, designated E_1 and E_2 , differ in their affinity for $ATP^{21,22}$, their intrinsic fluorescence spectra²³, susceptibility to trypsin^{17,24,25} and the response of their phosphorylated intermediates to ADP and K^+ (ref. 26). Transitions between E_1 and E_2 are detected by changes in any one of these properties. Both N-ethylmaleimide alkylation²⁷ and oligomycin²⁸ prevent these transitions.

Although there is still extensive disagreement about the details of the process, the following scheme is at least consistent with the experimental observations 22,23,29,30.

multimer, which has evolved under all these constraints, is a dimer whose dyad axis runs through the membrane. Furthermore, the only significant channels which pass through the centre of protein molecules lie between subunits and are often found centred on axes of symmetry. The true dyad of haemoglobin is the earliest and most striking example of this⁴². These consequences, which result when the rules that govern the symmetry of oligomeric proteins⁴¹ are applied to the two-dimensional environment of the phospholipid bilayer^{36,43}, have recently been reviewed by Klingenberg⁴⁴.

It is known that active transport of sodium and potassium through membranes does not involve the rotational or translational diffusion of $(Na^+ + K^+)ATP$ ase across the membrane⁴⁵. Thus it has been proposed that the enzyme spans the plasma membrane³⁶ and forms a channel across it through which the cations pass. In this scheme the pathway for the cations lies through the centre of the enzyme molecule, and is formed by the juxtaposition of two subunits of the enzyme and located along the dyad axis between them. The enzyme alternates between



Some of the steps in this scheme are proposed as the result of kinetic inference, but most have been directly observed and rate constants or equilibrium constants have been assigned to them. In normal circumstances the cycle operates in the clockwise direction, extruding sodium and accumulating potassium. Kinetic experiments indicate that the transition from $E_1 \sim P$ to E₂-P requires sodium to be bound to the enzyme, whereas the transition between E₂ and E₁ requires potassium. This suggests that E₁ is the inward-facing form of the enzyme which normally releases potassium as a product and receives sodium as a substrate, and E2 the outward-facing form which releases sodium as a product and receives potassium as a substrate. Therefore, it is during the transition between these two forms that the cations traverse the plasma membrane. Kinetic experiments with Ca2+-ATPase^{31,32} and (H⁺+K⁺)ATPase³³ have been interpreted in terms of a mechanism completely included within the more detailed mechanism which is currently available for (Na++ K⁺)ATPase. No counter ion, however, has been identified which would have the same role in Ca²⁺-ATPase that K⁺ does in the mechanism of $(Na^+ + K^+)ATPase$ and $(H^+ + K^+)ATPase$. It should be emphasized that this mechanism is able to explain² the negative cooperativity displayed in the MgATP kinetics of Ca^{2+} -ATPase³⁴, $(H^+ + K^+)ATPase^{33}$ and $(Na^+ + K^+)ATPase^{35}$ without any need to postulate half-of-sites behaviour or intersubunit interactions.

Molecular structure

While these kinetic observations were being accumulated, another series of experiments were being done to provide evidence for a particular structural explanation of the transition between E₁ and E₂. These experiments evolved from the following theoretical argument³⁶. In a lipid bilayer all protein molecules of the same sequence are inserted so that they point in the same direction^{37,38}. As the same area on the surface of each molecule, which is defined by a preponderance of aliphatic amino acid side chains³⁹, interacts with the boundary lipid⁴⁰, all folded polypeptide chains of the same sequence float at the same depth. With few exceptions, when two or more identical polypeptide chains combine to form a multi-subunit complex, they form a closed structure by arranging themselves about a rotational axis of symmetry⁴¹. In the membrane, this axis of symmetry is necessarily normal to the plane of the bilayer. The simplest arrangement of subunits in a membrane-bound

two conformational states, E_1 and E_2 , during turnover. These conformations differ from each other primarily in small structural alterations in the geometry of the channel; E_1 provides access to the cation compartment from the inside of the cell, E_2 from the outside. During the conformational changes which lead to net transport, each cation remains in the compartment and then departs into the medium on the side of the plasma membrane opposite to that from which it originally came. This hypothesis for the mechanism of $(Na^+ + K^+)ATPase^{36}$, and by analogy for the other enzymes catalysing active transport, was adapted from a model originally discussed by Jardetzky⁴⁶, generalized to other systems by Singer⁴⁷, and described in Klingenberg's article⁴⁴.

This proposal is only barely consistent with what is known about the structure of (Na++K+)ATPase. Purified canine renal (Na⁺+K⁺)ATPase contains two polypeptide chains, one a larger (α , molecular weight (MW) $120,000 \pm 10,000$), relatively hydrophobic protein, and the other a smaller (β , MW 55,000 \pm 5,000) sialoglycoprotein^{43,48}. It is the larger polypeptide which is the sibling of the polypeptides from Ca²⁺ ATPase and (H⁺+ K⁺)ATPase and it is generally assumed that this chain has the catalytic role. Both these polypeptides, however, are part of a specific complex⁴³ because they can be cross-linked to form a unique $\alpha\beta$ heterodimer^{48,49}. The two chains are present in the native enzyme in equimolar ratio⁴³ so that the minimum asymmetric unit is $\alpha\beta$. In certain conditions, it is possible to form a unique, covalently cross-linked α_2 dimer^{36,49,50}. Discrete complexes with apparent molecular weights of 380,000 (ref. 51) and 280,000 (ref. 52) and which are capable of regaining activity have been produced by detergent treatment. Electron micrographs have also been interpreted as indicating that the native enzyme is a multimer of $\alpha\beta$ units⁵³. The presently accepted, although far from proved, structure of native (Na+ K+)ATPase is shown in Fig. 1. If this structure is accurate, then Ca2+ ATPase and $(H^+ + K^+)ATP$ as must have the same appearance except that the β chain would be missing.

Klingenberg⁴⁴ has proposed that the present experimental evidence is sufficient to conclude that the structure shown in Fig. 1 is correct for (Na⁺+K⁺)ATPase. In addition, he assembles other experimental results which suggest that oligomeric structures are common in membrane-bound proteins. Although this may be true, it is far from from proved, and it would be useful here to review briefly the serious shortcomings of the procedures which were used to reach these conclusions.

Oligomeric associations

In the case of covalent cross-linking, the geometry of the membrane itself confuses the result. In all membranes or membrane fragments, the distances between neighbouring protein molecules are much shorter than the distances between molecules in even the most concentrated isotropic solutions of soluble proteins. As a result, collisions between unassociated molecules are much more frequent, increasing the likelihood of artefactual cross-linking. Although the overall cross-linking reactions are often pseudo-first order in their kinetics³⁶, this is consistent with either a preexisting complex or an initial ratelimiting covalent reaction followed by a very rapid collision of monomers. An additional problem caused by the two-dimensional properties of the bilayer is that on every monomer of a certain sequence, each of the specific amino acid residues which are the targets for a given cross-linking reagent will lie at the same distance from the centre of the bilayer as its twin on another monomer. When bis-functional reagents are used, this fact greatly increases the likelihood that the twins will be coupled on collision. Once a covalent, symmetrical pair is formed, however, from two previously unacquainted monomers, neither has the reactive position available for further polymerization and a unique covalent dimer will be observed with no evidence of higher oligomers. A striking example of this principle is the adventitious dimer of acetylcholine receptors which is found in preparations made in the absence of reducing reagents but which serves no biological purpose⁵⁴. Finally, cross-linking of membrane-bound proteins is rendered more ambiguous by the presence of the randomly oriented clusters of proteins often seen in electron micrographs⁵³.

The oligomeric assemblies of membrane-bound proteins which have been observed in the ultracentrifuge when these molecules are dissolved in various detergents are also cited⁴⁴ as evidence for the existence of similar, native complexes. It is not the case, however, that only one, unambiguous complex is always observed under these circumstances. Stable tetramers, dimers and monomers of Ca2+-ATPase have been separated and identified55. (Na++K+)ATPase, on the other hand, is supposed to exist as a dimer in detergent solution, compatible with the fantasy shown in Fig. 1. However the molecular weights, determined for this presumably discrete complex by ultracentrifugation in two separate laboratories^{51,52}, differ by 30% from each other. Furthermore, in neither case did the protein show (Na++K+)ATPase activity when dissolved in the concentration of detergent required to disperse it. Finally, the very substantial corrections which are necessary to account for the effects of bound detergent, carbohydrate and lipid on the hydrodynamic properties of the particle⁵¹, although theoretically pleasing, have yet to be independently verified. The cautious reader should note the errors made with aldolase⁵⁶ and aspartate transcarbamoylase⁵⁷ with the assistance of an even less complicated theoretical development in much simpler circumstances.

A clear indication of the uncertainty of the present position is that Klingenberg⁴⁴ has concluded, from experimental results of the type just discussed, that $(Na^+ + K^+)ATP$ ase is a dimer of asymmetric units, whereas $Ca^{2+}-ATP$ ase is a tetramer. If this

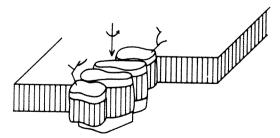


Fig. 1 Drawing of the imagined molecular structure of $(Na^+ + K^+)ATP$ ase. The subunits are drawn so that their volumes correspond to molecular weights of 120,000 and 55,000 using as a scale the width of the bilayer (4.0 nm).

were true, $(Na^+ + K^+)ATP$ ase would have a two-fold symmetry axis and Ca^{2^+} -ATPase, a four-fold one. As it is impossible to evolve a protein with a four-fold axis from one with a two-fold axis, or vice versa, the common ancestor of these proteins would have been a monomer. If this were the case, the oligomeric structures of these proteins would be irrelevant to the process of active transport. A more likely resolution of this confusion, however, is that both $(Na^+ + K^+)ATP$ ase and $Ca^{2^+}ATP$ ase have the same oligomeric structure but the true aggregation state of neither has been established.

Half-of-sites behaviour

Unfortunately, the theoretical proposals for the mechanism of active transport and the experiments which investigated the quaternary structures of membrane-bound proteins were being published at a time when enthusiasm for half-of-sites mechanisms of enzyme catalysis had reached its peak⁵⁸. It was immediately apparent that active transport was a strong candidate for half-of-sites behaviour, and many results appeared which supported the possibility. There are several ligands which bind to (Na++K+)ATPase: ouabain, vanadate, ATP and the phosphate of the covalent intermediate. Whenever comparisons are made with the same enzyme preparation, the molar concentrations of each of these sites are the same 59-63. As such, these ligands define the concentration of functional (Na*+ K+)ATPase units or active sites. There have been many estimates of the grams of protein per mol of sites in purified (Na++K+)ATPase—these usually lie between 220,000 and 280,000 g per mol of sites⁵⁹⁻⁶³. If one decides, on the basis of electrophoretic mobility, that the molecular weights of α and β are 90,000 and 35,000, respectively, it can be concluded that there is about one site for the ligands per $(\alpha \beta)_2$ or 'half-of-sites'.

This conclusion had three major weaknesses. Most of the protein concentrations were based on the Lowry method which does not yield accurate, absolute estimates of protein concentration unless extinction coefficients are independently determined⁶⁴. Second, any estimate of grams per mol of sites necessarily represents only an upper limit of the true number because some inactive protein is always present. Therefore, the lower numbers are the more important estimates. Finally, the molecular weights quoted for the chains are based only on gel electrophoresis, which gives minimum estimates of the true molecular weight of a hydrophobic protein⁶⁵. It has been pointed out that SDS-polyacrylamide gel electrophoresis underestimates the molecular weight of the subunit of Ca2+-ATPase as a determination of this quantity by sedimentation equilibrium indicates that the true molecular weight of this polypeptide chain is 120,000 (ref. 66).

New estimates of ouabain and ATP site concentrations for $(Na^+ + K^+)$ ATPase, based on protein determination by quantitative amino acid analysis, yield values of $180,000 \pm 20,000$ g per mol of sites^{22,67}. Similar values have also been obtained recently for the concentration of phosphorylation sites⁶⁸. Finally, it has been established that there are 0.8-0.9 phosphorylation sites^{10,69} and ATP-binding sites⁶⁹ present on each polypeptide chain in purified Ca^{2+} -ATPase. Clearly there is sufficient reason to doubt that active transport enzymes ever display half-of-sites behaviour.

In this context, it is illuminating to consider the present state of the half-of-sites or negative cooperativity problem in general. With the exception of hexokinase⁷⁰, which for symmetry reasons is irrelevant to the problem of membrane-bound proteins, the number of established cases of half-of-sites behaviour is rapidly dwindling. There are many reasons why mistakes were made, even with soluble proteins, and each is more likely to occur when a membrane-bound enzyme is examined. The most common problem is heterogeneity of enzyme due to the presence of contaminating, inactive protein⁷¹ of partially denatured enzyme of lower affinity^{72,73} or another protein which alters the properties of some of the enzyme⁷⁴. It is remarkable how such situations which should lead to various degrees of heterogeneous behaviour always produce half-of-sites behaviour; the concen-

Fig. 2 Hypothetical equilibrium between two monomers and a dimer of (Na++K+)ATPase asymmetric units dissolved in detergent. The detergent replaces the bilayer as a collar around the protein.

tration of sites is always half that expected. A corollary of these considerations would be the consequences which would result if some of the enzyme had been completely denatured during the detergent treatments necessary to isolate membrane-bound proteins. The partial or complete demise of an enzyme almost never alters its behaviour on polyacrylamide gels and would pass unnoticed in the ultracentrifuge. Another problem encountered with membrane-bound proteins is the presence of vesicles and multibilayers which prevent access of ligands to their otherwise normal sites. For example, the concentration of cardiac glycoside sites on (Na++K+)ATPase increases 1.6-fold when a membrane-bound, vesicular preparation is dissolved in detergent⁶⁷ even though the polyacrylamide gels of the two preparations are indistinguishable.

Finally, the shortcomings of the analytical procedures themselves must be considered. Any practising protein chemist realizes that the determination of the actual protein concentration in an experimental sample is extremely difficult²². A direct approach to the determination of the concentration of sites in a given preparation is often plagued by the problems of unstable intermediates, loss of protein, or large concentrations of unbound ligand-difficulties which can be overcome, however, by using more imaginative procedures⁷⁵. Often, the actual molecular weight of the subunits is doubtful. This quantity can only be established unambiguously from the sequence 76, but few sequences of membrane-bound proteins are available. Those

Kyte J. J. biol. Chem. 246, 4157-4165 (1971).
MacLennan, D. H. J. biol. Chem. 245, 4508-4518 (1970).
Niggli, V., Penniston, J. T. & Carafoli, E. J. biol. Chem. 254, 9955-9958 (1979).
Sachs, G. et al. J. biol. Chem. 251, 7690-7698 (1976).

Sen, A. K. & Post, R. L. J. biol. Chem. 239, 345-352 (1964). Hasselbach, W. Biochim. biophys. Acta 515, 23-53 (1978).

Nasserbach, G. A. Biochemistry 19, 2925–2931 (1980). Dufour, J. P. & Goffeau, A. J. biol. Chem. 253, 7026–7032 (1978). Uesugi, S. et al. J. biol. Chem. 246, 531–543 (1971).

10. MacLennan, D. H., Seeman, P., Iles, G. H. & Yip, C. C. J. biol. Chem. 246, 2702-2710

11. Saccomani, G., Shah, G., Spenney, J. G. & Sachs, G. J. biol. Chem. 250, 4802-4809 (1975).

Dame, J. B. & Scarborough, G. A. Biochemistry 19, 2931-2937 (1980).
 Bastide, F., Meissner, G., Fleischer, S. & Post, R. L. J. biol. Chem. 248, 8385-8391 (1973).

Karlish, S. J. D., Jørgensen, P. L. & Gitler, C. Nature 269, 715-717 (1977).
 Farley, R. A., Goldman, D. W. & Bayley, H. J. biol. Chem. 255, 860-864 (1980)

Farley, R. A., Goldman, D. W. & Bayley, H. J. tolo. Chem. 25, 800-804 (1980).
 Allen, G., Trinnamen, B. J. & Green, N. M. Biochem. J. 187, 591-616 (1980).
 Castro, J. & Farley, R. A. J. biol. Chem. 254, 2221-2228 (1979).
 Post, R. L. & Kume, S. J. biol. Chem. 248, 6993-7000 (1973).
 Albers, R. W., Fahn, S. & Koval, G. J. Proc. natn. Acad. Sci. U.S.A. 50, 474-481 (1963).
 Kanazawa, T., Saito, M. & Tonomura, Y. J. Biochem. 67, 693-711 (1970).

Karlish, S. J. D., Yates, D. W. & Glynn, I. M. Biochim. biophys. Acta 525, 252-264 (1978)

22. Moczydłowski, E. G. & Fortes, P. A. G. J. biol. Chem. 256, 2346-2356; 2357-2366 (1981).

Moczytłowski, E. G. & Potest, P. A. C., 1960. Chim. 1959, 233-233, 187-130 (1978). Karlish, S. J. D. & Yates, D. W. Biochim. biophys. Acta **527**, 115-130 (1978). Jørgensen, P. L. Biochim. biophys. Acta **401**, 399-415 (1975). Koepsell, H. J. Membrane Biol. **48**, 69-94 (1979). Fukushima, Y. & Tonomura, Y. J. Biochem. **74**, 135-142 (1973).

Fahn, S., Hurley, M. R., Koval, G. J. & Albers, R. W. J. biol. Chem. 241, 1890–1895 (1966). Fahn, S., Koval, G. J. & Albers, R. W. J. biol. Chem. 243, 1993–2002 (1968).

Fahn, S., Koval, G. J. & Albers, R. W. J. and Chem. 23, 1993-24.
 Mårdh, S. & Post, R. L. J. biol. Chem. 252, 633-638 (1977).
 Beaugé, L. A. & Glynn, I. M. J. Physiol., Lond. 289, 17-31 (1979).
 Dupont, Y. & Leigh, J. B. Nature 273, 396-398 (1978).
 Takisawa, H. & Tonomura, Y. J. Eiochem. 86, 425-441 (1979).

Wallmark, B., Stewart, H. B., Rabon, E., Saccomani, G. & Sachs, G. J. biol. Chem. 255, 5313-5319 (1980).

Neet, K. E. & Green, N. M. Archs Biochem. Biophys. 178, 588-597 (1977). Neufeld, A. H. & Levy, H. M. J. biol. Chem. 244, 6493-6497 (1969). Kyte, J. J. biol. Chem. 250, 7443-7449 (1975).

Bretscher, M. S. J. molec. Biol. 59, 351-357 (1971)

 S. J. & Nicolson, G. N. Science 175, 720-731 (1972).
 Khorana, H. G. et al. Proc. natn. Acad. Sci. U.S.A. 76, 5046-5050 (1979).
 Jost, P. C., Griffith, O. H., Capaldi, R. A. & Vanderkooi, G. Proc. natn. Acad. Sci. U.S.A. 70. 480-484 (1973).

41. Monod, J., Wyman, J. & Changeux, J. P. J. molec. Biol. 12, 88-118 (1965).

Cullis, A. F., Muirhead, H., Perutz, M. F., Rossmann, M. G. & North, A. C. T. Proc. R. Soc. A265, 161-187 (1962).

which are show that SDS-polyacrylamide gel electrophoresis significantly underestimates their true polypeptide length^{τ}

Almost all these difficulties result in an apparent decrease in the concentration of sites relative to the concentration of the polypeptide and may have contributed to the erroneous conclusion that half-of-sites behaviour is shown by active transport enzymes.

Present uncertainty

Thus it is unknown whether the boundary between two α subunits is involved in the process of active transport. The hyperbolic, non-interacting binding curves which are generally observed for the various ligands imply that the sites on each chain are well separated from each other and certainly not located within the same active site. Although dispersed forms of (Na++K+)ATPase and Ca2+-ATPase which seem to be multimeric have been identified, their existence does not necessarily imply that such arrangements are critical for function, something half-of-sites could have settled. Reports which propose that Ca2+-ATPase can function as a monomer further complicate the issue⁷⁸⁻⁸⁰. If this were true, it would eliminate the hypothesis outlined above, as well as any consideration of 'half-of-sites', in the case of active transport. There are several distinct difficulties, however, with these experiments. First, estimations of the aggregation state of the enzyme were based only on hydrodynamic parameters which are difficult to interpret in systems containing protein, lipid and detergent. Furthermore, no attempt was made to determine the quaternary structure of the enzyme at the same time that turnover was being observed. If an equilibrium such as the one depicted in Fig. 2 can occur, then the conditions of the enzyme assay may promote dimerization and reactivation even though an inactive monomer is present in the ultracentrifuge. In conclusion, it is unknown whether the functional unit of an active transport protein is monomeric or dimeric, and if a dimer is involved whether it can participate in the way described above and yet not display half-of-sites behaviour.

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43. Craig, W. S. & Kyte, J. J. biol. Chem. 255, 6262-6269 (1980).

Klingenberg, M. Nature 290, 449-454 (1981).
 Kyte, J. J. biol. Chem. 249, 3652-3660 (1974).

Kyte, J. J. biol. Chem. 239, 3652-3660 (1974).
 Jardetzky, O. Nature 211, 969-970 (1966).
 Singer, S. J. A. Rev. Biochem. 43, 805-833 (1974).
 Kyte, J. J. biol. Chem. 247, 7642-7649 (1972).
 Liang, S. M. & Winter, C. G. J. biol. Chem. 252, 8278-8284 (1977).

Giotta, G. J. J. biol. Chem. 251, 1247-1252 (1976)

Hastings, D. F. & Reynolds, J. A. Biochemistry 18, 817-821 (1979).

Esmann, M., Skou, J. C. & Christiansen, C. Biochim. biophys. Acta 567, 410-420 (1979). Deguchi, N., Jørgensen, P. L. & Maunsbach, A. B. J. Cell Biol. 75, 619-634 (1977). Anholt, R., Lindstrom, J. & Montal, M. Eur. J. Biochem. 109, 481-487 (1980). le Maire, M., Møller, J. V. & Tanford, C. Biochemistry 15, 2336-2342 (1976).

Schachman, H. K. & Edelstein, S. J. Biochemistry 5, 2681-2705 (1966). Weber, K. J. biol. Chem. 243, 543-546 (1968).

Fersht, A. R. Biochemistry 14, 5-12 (1975)

Peterson, G. L., Ewing, R. D., Hootman, S. R. & Conte, F. P. J. biol. Chem. 253, 4762-4770

60. Jørgensen, P. L. Biochim. biophys. Acta 356, 53-67 (1974)

Jørgensen, P. L. Biochim. biophys. Acta 466, 97-108 (1977).
 Perrone, J. R., Hackney, J. F., Dixon, J. F. & Hokin, L. E. J. biol. Chem. 250, 4178-4184

63. Cantley, L. C. Jr, Cantley, L. G. & Josephson, L. J. biol. Chem. 253, 7361-7368 (1978).

64. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265–275 (1951).

deJong, W. W., Zweers, A. & Cohen, L. H. Biochem. biophys. Res. Commun. 82, 532-539 (1978).

Rizzolo, L. J., LeMaire, M., Reynolds, J. A. & Tanford, C. Biochemistry 15, 3433-3437

(1976). Kyte, J. J. biol. Chem. 247, 7634-7641 (1972).

 Kyte, J. J. Biol. Chem. 241, 1034-1041 (1972).
 Askari, A., Huang, W. & Antieau, J. M. Biochemistry 19, 1132-1140 (1980).
 Meissner, G. Biochim. biophys. Acta 298, 906-926 (1973).
 Steitz, T. A., Fletterick, R. J., Anderson, W. F. & Anderson, C. M. J. molec. Biol. 104, 197-222 (1976).

Monteilhet, C. & Blow, D. M. J. molec. Biol. 122, 407-417 (1978).
Gennis, L. S. Proc. nam. Acad. Sci. U.S.A. 73, 3928-3932 (1976).
Scheek, R. M., Berden, J. A., Hooghiemstra, R. & Slater, E. C. Biochim. biophys. Acta 569, 124-134 (1979).

Maturo, J. M. & Hollenberg, M. D. Proc. natn. Acad. Sci. U.S.A. 75, 3070-3074 (1978).
 Cocivera, M., McManaman, J. & Wilson, I. B. Biochemistry 19, 2901-2907 (1980).
 Rosenbusch, J. P. & Weber, K. J. biol. Chem. 246, 1644-1657 (1971).
 Bonitz, S. G., Coruzzi, G., Thalenfeld, B. E., Tzagoloff, A. & Macino, G. J. biol. Chem. 255,

11927-11941 (1980) 78. Jørgensen, K. E., Lind, K. E., Røigaard-Petersen, H. & Møller, J. V. Biochem. J. 169, 489-498 (1978).

17. Dean, W. L. & Tanford, C. Biochemistry 17, 1683–1690 (1978).
 Møller, J. V., Lind, K. E. & Andersen, J. P. J. biol. Chem. 255, 1912–1920 (1980).

ARTICLES

Detecting CO₂-induced climatic change

T. M. L. Wigley & P. D. Jones

Climatic Research Unit, University of East Anglia, Norwich NR4 7TJ, UK

Although it is widely believed that increasing atmospheric CO_2 levels will cause noticeable global warming, the effects are not yet detectable, possibly because of the 'noise' of natural climatic variability. An examination of the spatial and seasonal distribution of signal-to-noise ratio shows that the highest values occur in summer and annual mean surface temperatures averaged over the Northern Hemisphere or over mid-latitudes. The spatial and seasonal characteristics of the early twentieth century warming were similar to those expected from increasing CO_2 based on an equilibrium response model. This similarity may hinder the early detection of CO_2 effects on climate.

OVER the past $100\,\mathrm{yr}$ there has been a steady increase of atmospheric carbon dioxide levels¹ with the concentration rising from $\sim 315\,\mathrm{p.p.m.}$ in $1958\,\mathrm{to}\,338\,\mathrm{p.p.m.}$ in $1980^{2.3}$. Most of this increase can be attributed to the ever-increasing use of fossil fuels. Carbon budget studies indicate that atmospheric CO_2 may reach $600\,\mathrm{p.p.m.}$, approximately double the pre-industrial level, some time between AD 2030 and $2080^{4.5}$. A doubling of CO_2 levels may well cause a major climatic change: however, the increase so far is probably too small for attendant climatic change to be detected above the 'noise' of natural climatic variability $^{6.7}$.

It has therefore been suggested⁸ that attention should be directed to the identification of a 'precursor signal' of the effects of CO₂ on climate. This requires a knowledge of the signal and a knowledge of the noise. The signal can be estimated only by numerical modelling experiments; the noise, a result of the considerable natural variability of climate, can be estimated using recent instrumental data. Simple radiative-convective energy balance models9 indicate that a doubling of CO2 could produce an increase in global mean annual surface temperature of 2-3 °C. More complex models 10-12 give information about the spatial and seasonal patterns of climatic change, but there is still considerable uncertainty in these model simulations. Nevertheless, for any given modelled signal we can estimate, using recent data, the corresponding noise and so calculate a signal-to-noise ratio. The best climate parameters to monitor are those with the highest signal-to-noise ratio. These are not necessarily those with the highest signal.

Signal-to-noise ratio

One approach is to look for significant changes in the mean values of particular climate parameters. Climatic noise manifests itself through uncertainties in the means, which in turn can be quantified by the statistical confidence limits for the sample mean (\bar{X}) as an estimate of the population mean. For a sample of size N(yr) with variance $\hat{s}_N^2 (\equiv \{\sum_{i=1}^N (X_i - \bar{X})^2\}/N - 1)$, the confidence limits' range, which is proportional to \hat{s}_N/\sqrt{N} , decreases roughly as $1/\sqrt{N}$. Averaging over longer time periods gives a more confident estimate of the mean and reduces he noise level. However, the reduction in noise is not strictly proportional to $1/\sqrt{N}$. Because most climate variables have a considerable amount of low-frequency variance, \hat{s}_N increases vith increasing sample size. Time-averaging reduces the noise, out not quite as rapidly as $1/\sqrt{N}$. To estimate the effect of ow-frequency variance, we suppose climate time series to ehave like first-order autoregressive processes. An additional actor

$$f(N, r) = \left\{ \frac{1+r}{1-r} - \frac{2r(1-r^N)}{N(1-r)^2} \right\}^{1/2}$$

where r is the lag-1 autocorrelation, appears, and the noise level can be defined as

$$\eta = \sqrt{2}(\hat{s}_N/\sqrt{N})f(N,r) \tag{1}$$

The signal-to-noise ratio is simply (signal)/ η . The $\sqrt{2}$ term arises because, in comparing two means, both will be subject to noise. As f(N, r) > 1, autocorrelation, or any type of enhanced low-frequency variance, effectively increases the noise level, and its presence makes the statistical detection of a signal more difficult.

Madden and Ramanathan⁷ have calculated the reduction in noise in seasonal and annual mean surface air temperatures at $60\,^{\circ}$ N due to time averaging, allowing for changes in \hat{s}_N . Their analysis suggests that, in the absence of other compensating effects, and ignoring lag effects due to the thermal inertia of the oceans, the CO_2 signal should be detectable either now or by about AD 2000, depending on the assumed character of the signal. They conclude that the effects at $60\,^{\circ}$ N should be detectable most easily in summer.

Results for 60 °N need not apply to other latitudes, and it may well be that a higher signal-to-noise ratio obtains at some other latitude band. Furthermore, because spatial averaging reduces the noise level in most climate parameters, averages over wider latitudinal ranges may increase the signal-to-noise ratio. We test these possibilities in this article.

Madden and Ramanathan used the results of Manabe and Wetherald¹⁰ and Ramanathan et al.¹³ to estimate the signal. In the latter work, the maximum signal is in high latitudes and in late spring-early summer. Here we use the general circulation model results of Manabe and Stouffer¹² in which the signal is greatest at high latitudes and in late autumn to winter. Although the signal should certainly differ from season to season, there is some doubt about the seasonal distribution⁷.

For our noise data we use gridded (5° latitude by 10° longitude) monthly surface air temperature data for the Northern Hemisphere over the period 1941–80 from ref. 14. The basic data are from World Weather Records, updated by NOAA in Monthly Climatic Data for the World, and supplemented with data from various National Meteorological Services, published records, and from the CLIMAT network. The number of individual stations varies from ~ 800 to 1,300. Data coverage becomes less complete as one moves back from 1940. Because of the sparsity of meteorological stations in ocean areas, the data grid has quite large gaps over the oceans. We have therefore used the land-area signal given by Manabe and Stouffer (their Fig. 16c) to calculate signal-to-noise ratios.

Spatial distribution of signal-to-noise ratio

In Fig. 1 we show the signal-to-noise ratio (\$N) as a function of latitude and month which is defined as

$$SN = \frac{1}{2}\Delta T_{MS}/\eta \tag{2}$$

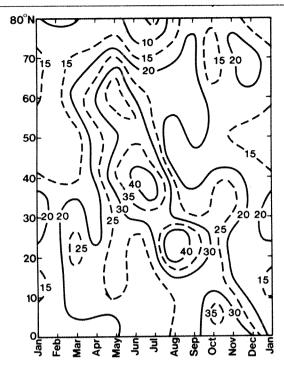


Fig. 1 Signal-to-noise ratio for predicted CO_2 -induced changes in surface air temperature as a function of latitude and month. The signal is based on the numerical modelling results of Manabe and Stouffer¹². The noise has been calculated from grid-point surface temperature data¹⁴: the value for month j at latitude L is the areally weighted average of grid points at L-5, L and L+5, and the noise level is proportional the standard deviation of month-j values over the period 1941–80, corrected for autocorrelation effects.

where η is given by equation (1), and $\Delta T_{\rm MS}$ is the temperature increase for a quadrupling of atmospheric CO₂ (the factor 1/2 arises because ΔT is approximately proportional to $\ln q$ where $q = {\rm CO_2/CO_2^0}$ (ref. 7)). We have allowed for the effect of low-frequency variance on time-averaging by including the term f(N,r). f(N,r) is generally near 1 for our data, but ranges up to a maximum near 2. Its inclusion makes little qualitative difference to our results. Although our noise levels have been calculated using the period 1941-80 (N=40), we have examined other time periods (and different values of N) and obtained very similar results. A value of SN ≥ 1.7 corresponds to a statistically significant ΔT at the 0.05 level. The temperature changes corresponding to a doubling of CO₂ are highly significant for all months and all latitudes relative to the noise level.

Figure 1 shows that the most likely season and latitude zone in which to detect the effects of CO_2 is during the summer months in middle latitudes; in spite of the fact that the assumed maximum signal is in winter in high latitudes. The importance of summer in detecting CO_2 effects is in accord with Madden and Ramanathan⁷, but our results suggest that the signal-to-noise ratio around 40 °N is higher than at 60 °N.

Monthly values of zonally averaged temperatures may not give the highest values of SN. Averaging into seasons and over the whole year reduces the variance of climate parameters, and a further variance reduction can be achieved by spatial averaging. We therefore examined seasonal and annual data for four different regions, a low-latitude zone (5° S-25° N inclusive), a mid-latitude zone (25°-55° N), a high-latitude zone (55°-85° N) and the whole Northern Hemisphere (0-85° N). SN values, defined as above, are given in Table 1. From these results, and similar analyses of different time periods (not shown here), the highest signal-to-noise ratios appear to be for summer midlatitudes, spring and summer Northern Hemisphere averages, annual mid-latitudes and annual Northern Hemisphere averages. Models with a different seasonal distribution of signal (see ref. 13) would imply even stronger signal-to-noise ratios in summer.

Recent changes in Northern Hemisphere and mid-latitude, summer and annual average surface temperatures are shown in Fig. 2. Some of the short-term variability has been smoothed out by using a 1-2-1 binomial filter, but the noisiness of the record is apparent. In neither of the summer curves is there any apparent trend (in either direction) since the mid-1960s. A slight upward trend is noticeable in the annual curves after 1972, but more pronounced and longer-lasting trends have occurred before, noticeably before 1940. We therefore examined this early warming period more closely.

Comparison with an earlier warming period

Figure 2 shows the strong warming trend in Northern Hemisphere and mid-latitude, summer and annual average temperatures between 1910 and 1938. If we compare the coldest and warmest 20-yr periods this century (1901–20 and 1934–53 respectively), then all seasons and latitude bands show a warming. Statistically, the most significant changes occurred in Northern Hemisphere averages for spring, summer, autumn and annual means, and in mid-latitude averages for summer and annual means, in striking accord with the latitude zones and seasons for which the CO₂ signal-to-noise ratio is highest (Table 1).

Although we can be reasonably sure that the early twentieth century warming was not due to increasing CO2, it is instructive to examine this possibility more closely. Two approaches may be used to test the hypothesis that CO₂ was a causal factor; the magnitude of warming can be compared with that expected from the known increase in atmospheric CO2, or the spatial and seasonal patterns of warming can be compared with those expected on the basis of CO₂ modelling experiments. The latter comparison is shown in Fig. 3 where the CO₂ signal¹² is plotted against the temperature change between 1901-20 and 1934-53 using the same seasonal and spatial grouping as before. The comparison is sufficiently good that is could be taken to support CO₂ as a cause for the early twentieth century warming. There are some interesting differences, however. Compared with the annual Northern Hemisphere temperature change, the observed low-latitude changes were too low, especially in summer. In mid-latitudes the seasonal character of the observed changes (winter minimum, summer maximum) contrasts sharply with that expected on the basis of Manabe and Stouffer's model, but

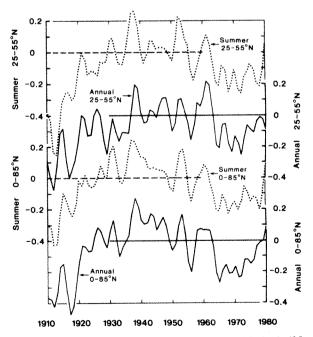


Fig. 2 Variations in Northern Hemisphere and mid-latitude (25-55° N) summer and annual average temperatures over the past 70 yr from ref. 14. The values are anomalies relative to the 1946-60 mean and have been filtered using a three-term binomial filter (except for the 1980 value).

accords better with Ramanathan et al.¹³. Given our lack of understanding of the details of both natural and CO₂-induced climatic change, these exceptions alone are probably insufficient to reject CO₂ as a possible cause of the early twentieth century warming.

However, the warming between 1901-20 and 1934-53 is, based on the most recent numerical model results, much larger than would be expected to result from the small increase in atmospheric CO₂ which occurred over this period. Additionally, between about 1940 and 1970 there was a significant cooling (Fig. 2) and this is incompatible with the CO₂ hypothesis.

The early twentieth century warming is a low-frequency component of the noise which acts to obscure any influence of increasing atmospheric CO₂ (in so far as any climatic change which is not attributable to CO₂ can be considered as noise). Because we have information about only one manifestation of this low-frequency component, we know nothing about its statistical properties. Nevertheless, this single example illustrates the similarity between natural and predicted CO₂-induced temperature changes; a similarity which might imply that analysis of spatial and/or seasonal details of future variations in surface temperature will be of little help in distinguishing natural changes from the effects of CO₂.

A second difficulty in detecting CO₂ effects arises because of lags in the response to increasing CO2 which result from thermal inertia associated with oceanic mixing¹⁵. Most models used in studying possible CO₂ effects (including that of Manabe and Stouffer¹²) have considered the equilibrium response to a stepfunction increase in CO₂. Transient response models¹⁵ indicate that oceanic influences might cause the climate response to lag 5-20 yr behind the CO₂ forcing. Such a lag, in itself, is a significant factor in determining when the effects of CO2 might first be detected. However, an equally important result is that the spatial pattern of the transient response may differ considerably from that expected on the basis of equilibrium models¹⁵. We have shown that the character of natural lowfrequency changes in surface temperature can be similar to the equilibrium CO₂ response. If transient response patterns do differ from equilibrium response patterns, this may make it easier to distinguish CO₂ effects from natural changes in climate.

Conclusions

We have examined the signal-to-noise ratio for surface temperature averaged over periods of 1 month to 1 yr and over latitude zones of widths from about 15° to 85°. The quantitative details of our results, especially those in Fig. 1, are model dependent. Our general conclusions are, however, relatively insensitive to the choice of the model used to define the CO₂ signal, because they depend strongly on the spatial and seasonal distribution of the natural variability of surface temperatures. For example, our conclusion that signal-to-noise ratio is highest for mid-latitude or Northern Hemisphere summer or annual means arises primarily because of the relatively low natural variability of summer temperatures. We can also conclude that the signal-to-noise ratio in low latitudes is of comparable magnitude to that in high latitudes (see Table 1), primarily

Table 1 Signal-to-noise ratio for different latitude bands and seasons

Latitude range	Winter	Spring	Summer	Autumn	Annual
Low (5° S-25° N)	23 (1.6)	28 (1.7)	36 (1.6)	36 (1.8)	31 (1.7)
Mid (25° N-55° N)	26 (2.7)	36 (2.4)	49 (2.2)	40 (2.4)	53 (2.4)
High (55° N-85° N)	23 (4.6)	31 (4.1)	25 (2.0)	28 (3.5)	33 (3.5)
Northern Hemisphere (0-85° N)	32 (2.6)	44 (2.4)	43 (1.9)	35 (2.4)	44 (2.3)

The signal (° C) corresponding to a doubling of CO_2 (that is $\frac{1}{2}\Delta T_{MS}$) is given in parentheses; based on Manabe and Stouffer 12.

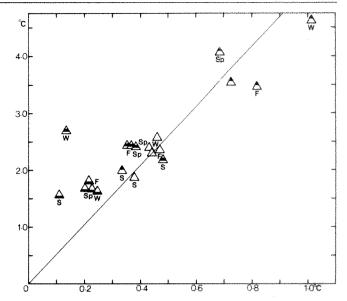


Fig. 3 Comparison of observed temperature changes between 1901–20 and 1934–53 with changes expected to result from increasing atmospheric CO_2 levels. The abscissa is the observed temperature change and the ordinate is the CO_2 signal for a doubling of CO_2 based on the land area results of Manabe and Stouffer 12. Different latitude bands are distinguished by the position of the band in the triangle. \triangle , Hemispheric averages. Seasons: W, winter; Sp, spring; S, summer; F, autumn or fall; no letter, annual average.

because of the low variability of low-latitude surface temperatures. This conclusion would only be invalidated if the ice-albedo feedback at high latitudes were considerably underestimated by currently available climate models: this is unlikely¹⁵. The uncertainty in the spatial patterns of climate response to increasing atmospheric CO₂ places considerable emphasis on monitoring large-scale averages for precursor signals of the effects of CO₂.

Because of the demonstrated similarity between observed natural changes in surface temperature and the changes predicted by Manabe and Stouffer's equilibrium model¹², it is important to investigate in more detail the differences in response between equilibrium and transient response models. If the differences are large, this would cast same doubt on the value of both equilibrium model results¹² and instrumental analogues¹⁶ in generating spatially detailed scenarios for a high-CO₂ warmer world. If the differences are small, the opposite conclusion could be drawn.

The change in surface temperature between 1901-20 and 1934-53 was similar in magnitude to that expected to result from a CO₂ increase of ~14% based on Manabe and Stouffer's model¹². Between 1970 and 1980 atmospheric CO₂ concentration increased by ~4%. A further 10% increase will almost certainly occur before the year 2000, probably by 1990. We might, therefore, anticipate changes in climate of a similar magnitude to those experienced in the first half of this century to occur over the next 10-20 yr. As most of the early twentieth century warming occurred between the late 1910s and the late 1930s (Fig. 2), the rate of future CO2 induced changes this century is unlikely to be faster than natural changes which we have recently experienced. If current model simulations are correct, then the rate of change of surface semperatures over the next 20 yr is, therefore, not likely to be useful in distinguishing CO₂ effects from natural climatic change.

Because of difficulties in using surface temperature data to detect the effects of increasing atmospheric CO₂, it is important to look at other climate parameters. There is a strong statistical and causal link between circulation changes and changes in surface temperature ¹⁷, so that surface climate variables other than temperature may not help significantly in trying to distinguish CO₂-induced changes from natural changes in climate.

Detailed spatial studies are hampered by the uncertainties in the spatial patterns of CO₂ effects. The predicted cooling of stratospheric temperatures might be useful, but our knowledge of the natural variability of stratospheric temperatures is meagre. Furthermore, other anthropogenic effects might also cause stratospheric cooling¹⁸. The same problems apply to monitoring changes in lapse rate¹⁹. An interesting possibility might be to monitor satellite radiation budget data as suggested by Madden and Ramanathan⁷.

The noise level in climate data may be reduced by eliminating variations with known causes. For example, the Southern Oscillation^{20,21} accounts for a significant part of the climate variance in some parts of the world, and this component might usefully be factored out by regression techniques. Alternatively, if there were accurate measurements of atmospheric turbidity and/or solar variability to which aspects of climatic change could be unequivocally ascribed, then the effects of these parameters

Received 23 March; accepted 27 May 1981.

- Callendar, G. S. Tellus 10, 243 (1958). Keeling, C. D. et al. Tellus 28, 538 (1976)
- Keeling, C. D. et al. in Global Energy Futures and the Carbon Dioxide Problem 2 (US Government Printing Office, Washington, DC, 1981).
- Rotty, R. in Man's Impact on Climate (eds Bach, W., Pankrath, J. & Kellogg, W. W.) 269 (Elsevier, Amsterdam, 1979).
- Zimen, K. E. in Man's Impact on Climate (eds Bach, W., Pankrath, J. & Kellogg, W. W.) 129 (Elsevier, Amsterdam, 1979).
- Mitchell, J. M. Jr in Workshop on the Global Effects of Carbon Dioxide from Fossil Fuels, (eds Elliot, W. P. & Machta, L.) (DOE/CONF-770385, 91, 1979).
- Madden, R. A. & Ramanathan, V. Science 209, 763 (1980).
 US Department of Energy Research Issues and Supporting Research of the National Program
- on Carbon Dioxide, Environment and Society (DOE/EV-1029, UC-11, 128, 1981)

could be removed, either by using numerical models or, less satisfactorily, by using empirically-based statistical relationships. Further improvements in numerical models and in our understanding of the causes of recent climatic change are required before this approach can give convincing results.

The effects of CO₂ may not be detectable until around the turn of the century. By this time, atmospheric CO₂ concentration will probably have become sufficiently high (and we will be committed to further increases) that a climatic change significantly larger than any which has occurred in the past century could be unavoidable. To avert such a change it is possible that decisions will have to be made (for example, to reduce anthropogenic CO₂ emissions) some time before unequivocal observational 'proof' of the effects of CO2 on climate is available.

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- Manabe, S. & Wetherald, R. T. J. atmos. Sci. 24, 241 (1967).
- Manabe, S. & Wetherald, R. T. J. atmos. Sci. 32, 3 (1975).
- Manabe, S. & Wetherald, R. T. J. atmos. Sci. 37, 99 (1980).

 Manabe, S. & Stouffer, R. J. J. geophys. Res. 85, 5529 (1980).

 Ramanathan, V., Lian, M. S. & Cess, R. D. J. geophys. Res. 84, 4949 (1979).
- Kamanatnan, V., Lian, M. S. & Cess, R. D. J. geophys. Res. 34, 4949 (1979).
 Jones, P. D., Wigley, T. M. L. & Kelly, P. M. Mon. Weath. Rev. (submitted).
 Schneider, S. H. & Thompson, S. L. J. geophys. Res. (in the press).
 Wigley, T. M. L., Jones, P. D. & Kelly, P. M. Nature 283, 17 (1980).
 van Loon, H. & Williams, J. Mon. Weath. Rev. 104, 365 (1976).

- Ramanathan, V. & Dickinson, R. E. J. atmos. Sci. 36, 1084 (1979) Angell, J. K. & Korshover, J. Mon. Weath. Rev. 106, 755 (1978).

- Troup, A. J. Q. Il R. met. Soc. 91, 490 (1965). Wright, P. B. The Southern Oscillation—Patterns and Mechanisms of the Teleconnection. and the Persistence (Hawaii Institute of Geophysics, University of Hawaii, HIG-77-13,

Long-period geomagnetic secular variations since 12,000 yr BP

K. M. Creer

Department of Geophysics, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, UK

A comparison of secular variation records of the geomagnetic field inclination obtained from palaeomagnetic studies of European and North American post-Glacial and late-Glacial sediments suggests that geomagnetic westward drift has persisted through the past 12,000 yr. Between 12,000 and 2,500 yr BP the overall average drift rate was between 0.14 and 0.16 deg yr^{-1} . At $\sim 2,500 \text{ yr BP}$ it is tentatively suggested that the drift rate increased and rose to a maximum of nearly 0.5 deg yr^{-1} before decreasing to historically observed values of $\sim 0.25 \text{ deg yr}^{-1}$.

OUR present knowledge of the spectrum and spacial characteristics of the geomagnetic field's longer period secular variations is derived from observatory magnetometer records which cover only the past two centuries, supplemented by the occasional magnetic compass and dip circle readings logged by ancient mariners1 thus limiting our understanding of how the field is generated. From these data, world maps contouring the various geomagnetic elements have been constructed for epochs through the past three to four centuries2-4 from spherical harmonic coefficients computed up to degree n = 4.

It is convenient to separate the observed field at any specific epoch into dipole and non-dipole components. The pattern of the non-dipole part of the field has been distributed around four positive and five negative foci throughout history. Geomagnetic secular variations are caused both by changes in intensity and orientation of the dipole and by westward drift and changes in intensity of the non-dipole part of the field. Through the past four centuries, some non-dipole foci have drifted to the west while others have stood still: some have maintained constant intensity while others have grown or decayed5.6

The geomagnetic field can be separated mathematically into drifting and standing parts⁷⁻⁹. The form of the drifting part of the vertical component of the non-dipole field is shown in Fig. 1 for the Northern and Southern Hemispheres using Yukutake and

Tachinaka's data8. Its contours exhibit an essentially quadrupolar symmetry: in each hemisphere there is one large positive and one large negative region, each of which contains its own individual structure which evolves with time. The mean rate of westward drift is ~ 0.25 deg yr⁻¹ which is similar to that determined by Barraclough² for the eccentric dipole into which the dipole and quadrupole parts of the field are sometimes combined. The direction of drift is not purely westward10; in particular, it seems that the general quadrupole has rotated clockwise at a rate of $0.25\pm \bar{0}.04$ deg yr⁻¹ during the present century¹¹ about a pole located near the Aleutian Islands rather than about the geographical pole.

The dipole component is not axial: its equatorial part has drifted to the west at an average rate of 0.29 deg yr⁻¹ since the sixteenth century10, though much slower drift rates have been obtained for epochs during the present century and have been explained by suggesting that the 'standing' part of the field actually undergoes a very slow eastward drift^{10,12}.

The lifetime of non-dipole sources was once thought to be as short as a few hundred years⁶, but recently substantially longer lifetimes of ~7,000 yr have been suggested¹⁰. For direct proof of such long lifetimes and of the persistency of westward drift we must look to archaeomagnetic and palaeomagnetic rather than observatory sources of data.

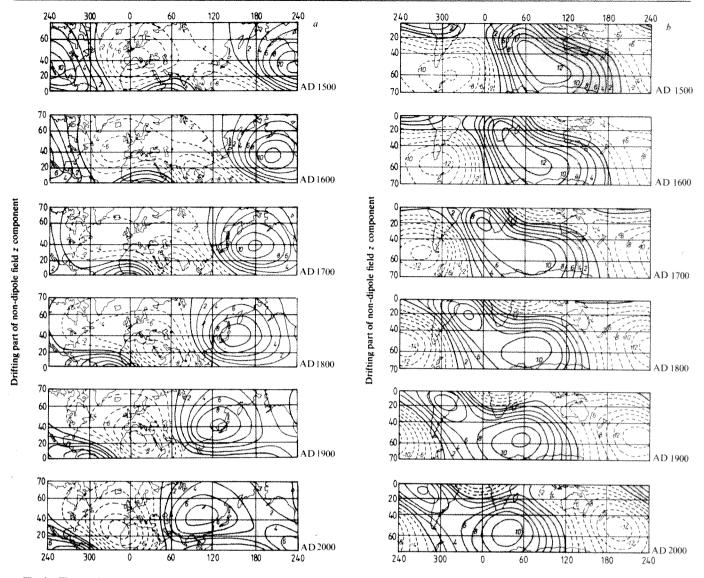


Fig. 1 The vertical component of the drifting part of the non-dipole field for six epochs from AD 1500 to AD 2000. a, Northern Hemisphere; b, Southern Hemisphere. Contour interval, 2,000 nT.

Thus, there are many gaps in our knowledge of the long term behaviour of the field during periods of settled polarity. The extended data sets becoming available from palaeomagnetic sources should help to resolve some of the outstanding problems.

Palaeomagnetic secular variation records

During the past decade significant developments have been made in techniques of taking high quality undisturbed cores of unconsolidated lake and marine sediments¹³ and it has become possible to measure their weak natural remanent magnetizations rapidly and accurately¹⁴. In favourable conditions of deposition this remanent magnetization has been found to carry a record of the secular variations of the direction of the ancient geomagnetic field at the site of deposition through the time the sediments were being accumulated. However, as might be expected, the quality of these natural records is much inferior to that we associate with instrumental records.

First, we must accept that the quality will be severely affected by any physical disturbance produced in the sediment, either naturally by slumping on the lake bottom due to, for example, to seismic activity or the action of turbidity currents, or mechanically during the coring process or during transport or during sub-sampling of cores. Another difficulty arises from the fact that corers often 'corkscrew' their way into the bottom sediments thus introducing a long wavelength trend and also discontinuities into the declination record. This is why more good quality inclination than declination records are available and why the present discussion is restricted to inclination secular variations.

Next, the natural remanent magnetization is not acquired instantaneously¹⁵: it is produced by a combination of different mechanisms such as post-depositional grain rotation, chemical changes and possibly bacterial action. Thus the recorded signal is always subjected to a variable degree of smoothing which explains why substantial variations in the amplitude of specific features identified along records from different places (see Figs 2 and 3) and even from different cores taken from the same lake are found.

Finally it is difficult to date recent sediments with the required degree of accuracy. Radiocarbon dates are often affected by the presence of recycled fossil carbon which may have been deposited into the sediment as fragments of fossil shell or plant remains ('old carbon' effect) or incorporated directly into organisms living at the time of deposition from calcium carbonate originating from pre-existing limestones dissolved in the lake water ('hard water' effect). Both these effects can produce radiocarbon ages which are too old by up to several thousand years so that we have to use indirect methods such as correlation of the 'palaeomagnetic' core with well-dated sections from the same locality using palynological or stratigraphical methods.

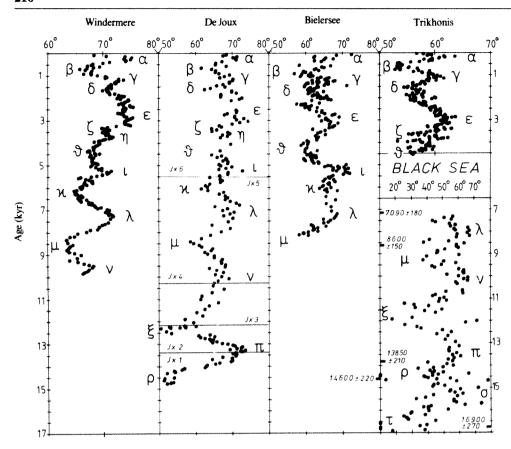


Fig. 2 Palaeomagnetic inclination logs for European sites. Time scale in uncalibrated radiocarbon years. Windermere 18 and Trikhonis 20 curves are each stacked from several individual core records. Pollen zone boundaries shown on Lac de Joux log 21. Radiocarbon ages shown along Black Sea log 22.

European post-Glacial and late-Glacial data

Limnomagnetic research started with Mackereth's study¹⁶ of post-Glacial sediments from Lake Windermere. Subsequent studies in UK (Lake Windermere, Loch Lomond, Lake Geirionydd) have produced a detailed and reproducible pattern of secular variations in inclination and declination 17,18 dated by over 30 radiocarbon age determinations. The characteristic features of the UK records, labelled $\alpha-\nu$, can also be recognized over a wide area of Europe from Poland¹⁹ to Greece²⁰. Records stacked from several cores from Lake Windermere $(54^{\circ}\text{N}\ 357^{\circ}\text{E})^{18}$ and from Lake Trikhonis $(39^{\circ}\text{N}\ 22^{\circ}\text{E})^{20}$ are shown in Fig. 2. A record from Lac de Joux $(47^{\circ}\text{N}\ 5^{\circ}\text{E})$ extending back to about 14,000 yr BP has been dated by correlation with the well established biostratigraphy for the Joux valley²¹. It exhibits features $\theta-\rho$ particularly well. The younger features $\alpha-\iota$ are exhibited more clearly for Switzerland in the Bielersee record, which as yet can be dated only by correlation of magnetic

	Table 1 Ages of inclination features						
Feature	Windermere (3°W)	St Croix (93°W)	Drift (yr)	Drift (deg yr ⁻¹)	Mean age (yr)		
α	250	0	250	0.360	125		
β	700	500	200	0.450	600		
·γ	1,150	900	250	0.360	1,025		
$\dot{\delta}$	1,650	1,250	400	0.225	1,450		
ε	3,000	2,200	800	0.112	2,600		
ζ	3,600	2,700	900	0.100	3,150		
η	4,000	3,600	400	0.225	3,800		
θ	4,500	4,000	500	0.180	4,250		
ι	5,200	4,600	600	0.150	4,900		
к	6,100	5,600	500	0.180	5,850		
λ	7,300	6,700	600	0.150	7,000		
μ	8,400	7,900	500	0.180	8,150		
ν	10,100	9,400	800	0.124	9,750		
ξ	12,400*	$10,500^{\dagger}$	1900	0.047	11,450		
π	13,400*	$12,600^{\dagger}$	1800	0.112	13,000		
ρ	14,500*						

Ages are given in radiocarbon yr BP.

declination and inclination features with the calibrated Euopean records. Another inclination record extending from $\sim\!7,000$ yr BP to $\sim\!25,000$ yr BP (Fig. 2 goes to 17,000 yr BP) is provided by Black Sea core no.1474 (42°N 38°E) which was taken using a gravity piston corer. The time scale was constructed from seven radiocarbon age determinations²². Figure 2 thus shows the extent to which inclination secular variations can now be correlated across Europe back to $\sim\!15,000$ yr BP.

North American post-Glacial and late-Glacial data

Inclination secular variation records from Lakes St Croix and Kylen, Minnesota (46°N 267°E)²³ and the Thunder Bay part of Lake Superior (48°N 271°E)²⁴ are shown in Fig. 3. Characteristic features which correlate from site to site are labelled $\alpha-\pi$, although at this stage we should not identify them with the apparently similar features observed along the European records. At Lake St Croix parallel cores of organic rich gyttja were taken in sections by making successive 1.5-m drives using a piston corer. The cores were dated with eight radiocarbon age determinations²³ as indicated along the inclination record shown in Fig. 3. Thus the dating control is quite tight. At Lake Kylen, cores were taken using the same technique and dated by four radiocarbon age determinations. Note that there is a very large error of $\pm 1,500$ yr on the oldest age of 16,500 yr (ref. 22). Four cores were taken especially for palaeomagnetic study with an Alpine piston corer from Lake Superior²⁴. The age of the base of the post-Glacial was taken as 9,500 yr BP (ref. 25). Because sedimentation occurred in the Thunder Bay part of Lake Superior back to at least 11,000 yr BP (refs 25, 26), this age was taken as the age of the base of the core. The post-Glacial sediments were dated by nine radiocarbon age determinations some of which were affected by 'hard water' and 'old carbon' effects for which it is difficult to make a precise correction. The four oldest ages shown in Fig. 3 were accepted without correction and a 'zero-error' correction of 1,700 yr (following ref. 24) was applied to the three youngest ages. Thus this time scale is likely to be in error.

Though the pattern of variations provided by St Croix/Kylen record is not very well formed, the dating control is good. If the

^{*} Lac de Joux not Windermere. † Kylen not St Croix.

Table 2 Quasi-periodicity of UK (Lake Windermere) and North American (Lake St Croix) inclination S.V.

	Age	Mean	Drift
Feature	difference	age	rate
labels	(yr)	(yr)	(deg yr ⁻¹)
Lake Windermere	•		
$\alpha - \gamma$	900	700	0.514
$oldsymbol{eta}$ δ	950	1,175	0.379
γ-ε	1,850	2,075	0.195
δ – θ	2,850	3,075	0.126
ε - ι	2,200	4,100	0.163
θ κ	1,600	5,300	0.225
ι-λ	2,100	6,250	0.171
κ-μ	2,300	7,250	0.156
$\lambda - \nu$	2,800	8,700	0.129
μ-ξ*	4,000	10,400	0.090
ν – π *	3,300	11,750	0.109
ξ-ρ*	2,100	13,450	0.171
Lake St Croix			
α - γ	900	450	0.400
β – δ	750	875	0.480
γ-ε	1,300	1,550	0.277
δ – $ heta$	2,750	2,625	0.131
ϵ - ι	2,400	3,400	0.150
θ - κ	2,000	5,000	0.180
ι-λ	1,900	5,650	0.189
κ-μ	1,900	6,950	0.189
λ-ν	2,500	8,050	0.144
μ-ξ	2,600	9,200	0.138
ν - π	2,100	10,450	0.171

All ages given in 'raw' radiocarbon years.

ages of the labelled features obtained from this record are transferred to the rather well-formed Superior record a satisfactory inclination record for the Great Lakes region is obtained, the pattern of which is confirmed by recent results from Lake Huron²⁷.

Palaeowestward drift

We now consider whether the various characteristic features of the European and North American data labelled in Figs 2 and 3 can be correlated across the North Atlantic, remembering that the Greek labels were initially attached quite independently to both sets of records. In fact a rather striking overall similarity between the patterns, especially for features $\varepsilon - \xi$ will be noted.

Considering the behaviour of the drifting part of the historic field shown in Fig. 1, it would seem reasonable to associate the observed palaeomagnetic inclination maxima and minima with the passage of the broad negative and positive regions of the non-dipole field beneath the respective sites. Thus one wavelength of the pattern of palaeomagnetic variations would be produced by each revolution of the westward drifting sources located in the outer core. Shorter wavelength variations would be caused by the more rapidly evolving structure within each broad negative and positive region and these should produce variations in the shape of the successive maxima and minima recorded at a given site and also some variation in the shape of any given labelled feature as it is recorded first in Europe and then later in North America.

In principle, we should be able to measure the rate of drift in two ways. First, the time taken for 360° of drift of the whole non-dipole field pattern is given by the time between two successive maxima or minima along the palaeomagnetic records. Figure 4 compares rates of drift through the past 12,000 yr estimated in this way, independently for European data and North American data. Dates of the labelled characteristic features have been taken from the Windermere and St Croix/Kylen records which are the best dated for Europe and North America respectively (Tables 1 and 2). Beyond 9,000 yr BP, dates from the Lac de Joux and Black Sea records have been used for Europe. Features ζ and η , originally identified along the UK records for local correlation purposes are not identified in

this discussion with the major source mechanism and have been ignored for the present analysis.

The above method does not give the sense of drift, but this can be checked as follows. Figures 2 and 3 and Table 1 show that each labelled feature is recorded later in North America than in Europe. This phase lag indicates that the sense of drift was westward and corresponds to the time taken for the centre of a positive or negative region of the non-dipole field to drift under the North Atlantic. There is a 90° longitude difference between the Lake Windermere and St Croix sites. Rates of drift estimated by the two methods are consistent with one another (Fig. 4 and Tables 1 and 2).

It is difficult to estimate quantitative errors for the plotted drift rates. These will depend mainly on the accuracy of the calibration of the palaeomagnetic records, on uniformity of deposition rates between levels dated by radiocarbon and also the accuracy with which the centre of each particular feature may be identified. The overall average drift rate before 2,500 yr BP is estimated at 0.15 deg yr $^{-1}$ (s.d. = 0.04), 0.16 deg yr $^{-1}$ (s.d. = 0.02), 0.14 deg yr $^{-1}$ (s.d. = 0.05) respectively for the European, North American and phase difference data points of Fig. 4.

Note that the characteristic times of the features $\alpha - \varepsilon$ are shorter than those of features older than ε . The sharp increase inferred for the drift rate since 2,500 yr BP (Fig. 4) results from the assumption that these shorter 'period' features originated from the same mechanism as the older features. The shorter phase differences observed across the Atlantic for features $\alpha - \delta$ (Table 1) is consistent with this assumption. Nevertheless, the possibility that higher degree terms dominated over the quadrupole term between historic times and $\sim 2,500$ BP needs to be considered. This is because, although the overall westward sense of drift is confirmed by essentially clockwise looping of Bauer plots of declination against inclination with advancing time for UK since $\sim 7,000$ yr BP (ref. 18) and for Lac de Joux since $\sim 11,000$ yr BP, a counter-clockwise loop occurs between

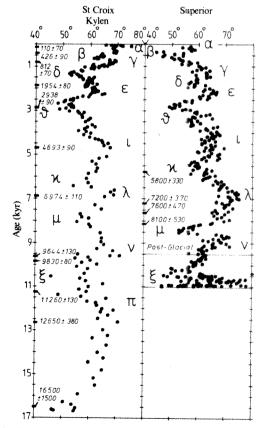


Fig. 3 Palaeomagnetic inclination logs for North American sites. Time scale in uncalibrated radiocarbon years. Radiocarbon ages defining time scale for St Croix and Kylen records²³ and also for the Lake Superior²⁴ record are shown.

^{*} Uses Lac de Joux, not Windermere, data.

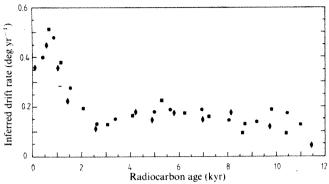


Fig. 4 Inferred westward drift rates through the past 12,000 yr. All ages are uncalibrated radiocarbon ages. Dates of labelled inclination maxima and minima as given by the Windermere and St Croix plots of Figs 2 and 3 have been used back to 9,000 yr BP, thereafter by the Lac de Joux and Kylen plots. . European data; North American data; ◆, phase difference

about 1.500 and 700 vr BP for UK data 18 and also for the Greek data²⁰. According to the Runcorn-Skiles rule⁵ this counterclockwise looping indicates eastward drifting geomagnetic sources during the time for which we have inferred fast westward drift. However, this rule breaks down under some circum-

Magnitude of the non-dipole field

In the Northern Hemisphere, the broad negative anomaly has gradually increased in strength from -8,000 to -12,000 nT while the positive anomaly has decreased from +12,000 to +8,000 nT through historic time (Fig. 1a). The magnitudes of these anomalies are too small by a factor of about 3 to account

Received 18 March; accepted 6 May 1981.

- 1. Veinberg, B. P. & Shibaev, V. P. Transl. No. 0031 (Canadian Department of the Secretary
- of State, Translation Bureau, 1970). Barraclough, D. R. Geophys. J. R. astr. Soc. 36, 497 (1974).
- Yukutake, T. J. Geomagn. Geoelect. 23, 11 (1971). Braginskii, S. I. Geomagn. Aeron. 12, 464 and 947 (1972).

- Skiles, D. D. J. Geomagn. Geoelectr. 22, 441 (1970).
 Bullard, E. C., Freedman, C., Gellman, H. & Nixon, J. Phil. Trans. R. Soc. A243, 67 (1950).
- Yukutake, T. & Tachinaka, H. Bull. Earthq. Res. Inst. Tokyo Univ. 46, 1027 (1968). Yukutake, T. & Tachinaka, H. Bull. Earthq. Res. Inst. Tokyo Univ. 47, 65 (1968).
- James, R. W. J. Geomagn. Geoelect. 22, 241 (1970). Yukutake, T. PEPI 20, 83 (1979).

- Knapp, D. G. Geol. Surv. Profess. Pap. 1118 (1980). Kawai, N. & Hirooka, K. J. Geomagn. Geoelectr. 17, 217 (1967).
- Mackereth, F. J. H. Limnol. Oceanogr. 3, 181 (1958).
 Molyneux, L. Geophys. J.R. astr. Soc. 32, 479 (1971).

for the observed amplitudes of the broad palaeomagnetic inclination variations. Thus, we infer that the historically observed non-dipole field intensity has been abnormally low relative to the dipole field intensity. Note that the intensity of the dipole has decreased by 5% per century while the quadrupole moment has increased by ~40% per century since AD 1800.

Conclusions

Overall westward drift has persisted throughout the past 12,000 yr. The average rate of 0.14-0.16 deg yr⁻¹ deduced for 12,000-2,500 yr BP is based on a reasonably secure correlation of inclination variations across the North Atlantic. Correlation of the shorter 'period' features $\alpha - \varepsilon$ is more hazardous and that adopted here must await confirmation from results of a parallel study of declination variations now being carried out. Thus the conclusion that the rate of westward drift increased to about 0.5 deg yr⁻¹ at about 800 yr BP before decreasing to the historical value of 0.25 deg yr⁻¹ should at present be regarded as

It should be possible soon to answer the question of whether the drifting part of the non-dipole field retained its historical, essentially quadrupolar, symmetry through the post-Glacial time by extending the present studies to Southern Hemisphere sites. Clearly this objective can not be achieved by observations restricted to Northern Hemisphere sites.

A full understanding of long period secular variations requires palaeomagnetic data describing the complete geomagnetic vector as a function of time. As yet, methods developed for the determination of past geomagnetic intensities from the intensity of remanent magnetization of sedimentary rocks have not been properly tested by comparison with archaeomagnetic intensity data from the same locality.

- Tucker, P. Geophys. J.R. astr. Soc. 63, 149 (1980). Mackereth, F. J. H. Earth planet. Sci. Lett. 12, 332 (1971)
- Mackeretti, F. J. Ft. Earth planet. Sci. Lett. 14, 352 (1971).

 Thompson, R. & Turner, G. M. Geophys. Res. Lett. 6, 249 (1979).

 Turner G. M. & Thompson, R. Geophys. J.R. astr. Soc. 65, 703 (1981).

 Creer, K. M. Kroll, E., Readman, P. W. & Tucholka, P. Geophys. J.R. astr. Soc. 59, 287-313 (1979).
- Creer, K. M., Readman, P. W. & Papamarinopoulos, S. Geophys. J.R. astr. Soc. 66, 193
- Creer, K. M., Hogg, T. E., Readman, P. W. & Reynaud, C. J. Geophys. 48, 139 (1980).
- Creer, K. M. Earth planet. Sci. Lett. 23, 34 (1974)
- Banerjee, S. K., Lund, S. P. & Levi, S. Geology 7, 588 (1979). Mothersill, J. S. Can. J. Earth Sci. 16, 1016.

- Mothershi, J. S. Can. J. Earth Sci. 16, 1010.
 Saarnisto, M. Can. J. Earth Sci. 12, 300 (1975).
 Prest, V. K. Geol. Surv. Can. Econ. Geol. Rep. No. 1, 676–764 (1970).)
- Mothersill, J. Can. J. Earth Sci. 18, 448 (1981). Bauer, L. A. Phys. Rev. 3, 34 (18)
- Dodson, R. E. J. geophys. Res. 84, 637 (1979)

The nus mutations affect transcription termination in Escherichia coli

Douglas F. Ward & Max E. Gottesman

Biochemical Genetics Section, National Cancer Institute, Bethesda, Maryland 20205, USA

The nusAl and nusB5 mutations result in a partial suppression of polarity and thus transcription termination in Escherichia coli. As these mutations block the transcription antitermination activity of bacteriophage \(\lambda \) N gene product, they paradoxically seem to enhance transcription termination at phage termination sites. The rho mutation HDF026 displays almost identical properties. These observations suggest that the nusA and nusB gene products may act as termination factors analgous to rho protein.

TERMINATION of transcription serves a dual function in Escherichia coli-to keep each transcription unit, or operon, separate and isolated from its neighbours and to allow fine control of expression within the operon structure. The importance of ensuring the isolation of each operon is demonstrated by the interference with expression when transcription enters an operon from without, either in the sense-direction (refs 1, 2 and S. Adhya and M.E.G., in preparation) or in the opposing direction^{3,4}. The regulation of operon expression by transcription termination within an operon ('attenuation') has been described extensively in the trp (refs 5, 6) and his (ref. 7)

Premature transcription termination can occur as a result of mutations which introduce nonsense codons into structural genes (see ref. 8) thus exposing transcription termination sites which are not normally active. Such mutations are termed

Table 1 Effect of nus mutations on polarity in the gal operon

		Galactokinase activity 32 °C			relative to gal ⁺ parent 42 °C		
Expt	gal	nus +	nusA1	nusB5	nus +	nusA1	nusB5
1	Tam76	0.15	0.20		0.20	0.33	
	TN102	0.06	0.07		0.04	0.05	
	EocB4	0.02	0.02		0.02	0.03	
2	Tam76	0.33		0.50	0.34		0.68
	TN102	0.05		0.09	0.06		0.12

Values represent the ratio of galactokinase (galK) activity in strains carrying the indicated gal mutation, expressed relative to that in the isogeneic gal^+ parent. This normalization eliminates day-to-day variations in the absolute enzyme activites. Activites in gal^+ strains varied from 12 to 29 units at 32 °C, and from 15 to 33 units at 42 °C. Results are the mean of at least two measurements and are accurate to 0.01 (or 10%). As galK is distal to both galT and galE, the level of galactokinase serves as an indication of the polarity of mutations in either of these two genes. Steady-state galactokinase activity was determined in toluenized cell extracts 12 and corrected for cell density. Growth medium was L-broth containing 5 mM D-fucose as inducer of the gal operon. All strains are derived from E. coli K12 strain N4903 (ref. 33). Expt 1 uses a set of isogeneic $nusA^+$ and nusA1 strains. Expt 2 uses isogeneic $nusB^+$ and nusB5 strains.

'polar'. DNA insertions into operons usually cause severe polarity; they can introduce transcription as well as translation termination signals.

Termination at many sites requires the action of the $E.\ coli$ rho protein⁹; rho mutants display reduced or no polarity^{10,11}. Mutations in rpoB have been isolated^{12,13} which restore polarity in some rho mutant strains. Such allele specificity suggests that interaction between the RNA polymerase β subunit and rho occurs during transcription termination.

Development of bacteriophage λ is regulated at the level of transcription termination. Rho-dependent terminators are present beyond λ genes N and cro, acting to limit transcription to these immediate-early genes. When N product is synthesized, however, these terminators are suppressed, and transcription can extend into the delayed-early gene region. As N product suppresses termination at both rho-dependent and rho-independent sites, it may act directly on the transcription termination process, rather than as a simple antagonist of rho¹⁴.

The possibility that pN might interact with RNA polymerase to prevent termination was suggested by the isolation of several mutations in the rpoB cistron which blocked pN activity 15-18 However, Greenblatt (personal communication) has failed to demonstrate binding of RNA polymerase to N protein in vitro; the putative interaction may occur via a nucleic acid or protein intermediary. A phage-specific DNA (or RNA) sequence, nut, located before the first terminator, is necessary for pN activity¹ The involvement of additional proteins in the antitermination reaction is suggested by the isolation of other host mutations which prevent N function. The nusA (ref. 20) and nusB (refs 21, 22) mutations do not correspond to any component of RNA polymerase. The nusA gene product is apparently identical to protein factor 'L', which is required for optimal expression of β galactosidase in a coupled transcription-translation system²³. nusA protein complexes with pN in vitro²³; the product of the nusB gene has not been identified.

Here we investigate the effect of the nusA1 and nusB5 mutations on host and phage physiology. We find that either mutation partially suppresses polarity in E. coli, suggesting that the nusA and nusB gene products may be transcription termination factors.

Effect of nusA1 and nusB5 on polarity in the gal operon

Suppression of transcription termination by the bacteriophage λN gene product is dependent on several host genes. The host

nusA1 (ref. 20) and nusB5 (refs 21, 22) mutations are defective for N action and restore transcription termination at sites on the phage chromosome. It seemed possible that the nus mutations might therefore promote termination in general, in the absence of N product, both on viral and bacterial DNA. To test this hypothesis, we measured the polarity of mutations in the gal operon in wild-type and nus mutant strains. As polarity produced by nonsense mutations, or DNA insertions, results from premature transcription termination, increased polarity would indicate a higher efficiency of termination.

The galTam76 mutation reduces the expression of galK to about 0.15 relative to wild-type levels (Table 1, expt 1). Introduction of the nusA1 allele elevated the relative galactokinase activity to 0.20. This relief of polarity was slightly more marked at 42 °C; galactokinase levels increased from 0.20 to 0.33 in the nusA1 strain. Recall that nusA1 totally blocks N activity and hence exerts its maximal termination effect at 42 °C. This result was contrary to expectation; the nusA1 mutation restores transcription termination in λ and it was therefore expected that nusA1 would increase polarity in E. coli.

Suppression of polarity by *nusA1* was not observed when the strongly polar mutations *galEocB4* and *galTN102*, an IS1 insertion, were assayed (Table 1).

The nusB5 mutation, like nusA1, restricts the growth of λ at high temperature by enhancing termination at sites normally suppressed by the N product. As shown in Table 1 (expt 2), the nusB5 mutation also relieves polarity in the gal operon. As was the case with nusA1, the limited polarity suppression was observed at both 32 and 42 °C.

The nusB5 mutation, unlike nusA1, reduces the steady-state rate of galactokinase synthesis in gal^+ strains (from 41 to 26 units at 32 °C; from 44 to 25 units at 42 °C). In view of the poor growth rate of strains carrying the nusB5 mutation (data not shown), the expression of other bacterial operons, including some required for cell growth, may also be adversely affected by nusB5

Interaction of nus and rho mutations

The experiments presented above suggest that the nusA and nusB gene products promote transcription termination. To determine whether the nus products act independently of rho we investigated whether nusA would influence residual polarity in a rho-deficient strain. galP3 is an IS2 insertion in the galOP region²⁴; it introduces a transcription terminator and is highly polar on the gal operon⁸. Table 2 shows that in the rho112 galP3 background, the nusA1 mutation increased galactokinase activity by more than twofold—from 0.24 to 0.61 at 32 °C, and from 0.30 to 0.85 at 42 °C. Expression of galK was similarly stimulated by nusA1 in a rho15 mutant.

The nusA1 mutation does not always enhance polarity suppression by rho mutations. Suppression of galTN102 polarity by rho112 is not markedly affected by the nusA1 mutation (Table 2).

Table 2 Suppression of polarity by rho and nusA mutations

			nase activity ! °C	relative to gal ⁺ parent 39 °C	
gal	rho	nusA+	nusA1	$nusA^+$	nusA1
P3 P3 P3	+ 112 15	0.02 0.24 0.51	0.02 0.61 0.67	0.01 0.30 0.39	0.02 0.85 0.75
TN102 TN102	+ 112	0.05 0.20	0.07 0.22	0.02 0.23	0.03 0.30
+	112	1.1	1.0	1.2	1.3

Values represent the ratio of galactokinase activity in strains carrying the indicated gal, rho and nus mutations expressed relative to the isogeneic gal⁺ rho⁺ parent. Experimental details are as indicated for Table 1.

Table 3 trp attenuation in nusA1 mutants

Tryptophan synthetase activity						
	+Attenuator	Attenuation ratio				
	(1)	(2)	(1)/(2)			
nusA+ rho+	11	64	.17			
$nusA1$ rho^+	14	56	.25			
$nusA^+$ $rho112$	10	54	.19			
nusA1 rho112	10	51	.20			

Tryptophan synthetase³⁴ activities are expressed as units per mg protein, where 1 unit is the amount of enzyme required to convert 0.1 μ mol indole in 20 min at 37 °C. Growth media was M56 minimal medium³⁵ supplemented with glucose (0.2%), casamino acids (0.05%) and thiamine (1 μ g ml⁻¹), plus 100 μ g ml⁻¹ tryptophan. All strains carry the $trpR^-$ mutation. + Attenuator strains are trpED24 (ref. 36); – attenuator strains are trpED102 (ref. 36). The attenuation ratio is accurate to 10%.

Attenuation in nusA1 mutant strains

The apparent site-specificity of polarity suppression by *nusA1* prompted us to examine transcription termination at the attenuator of the *trp* operon. Several *rho* mutations have been found to increase the expression of *trp* enzymes²⁵, suggesting a possible role of rho in *trp* attenuation.

Two sets of strains carrying combinations of *nusA1* and *rho112* were constructed. The first carried the *trpLD102* deletion which removes the attenuator; the second carried *trpED24*, a deletion similar in size and extent to *trpLD102* but which leaves the attenuator intact. *trp* expression was genetically derepressed by the introduction of a *trpR* mutation.

The effects of *rho* and *nusA1* mutations on attenuation were determined by assaying tryptophan synthetase, the product of the *trpA* and *trpB* genes (Table 3). Unlike other *rho* mutant alleles tested, *rho112* does not significantly reduce *trp* operon attenuation. By itself, the *nusA1* mutation does cause a significant, although small, suppression of *trp* attenuation. This effect was not apparent in the *rho112* background.

Interactions of *nusA* and *rho* mutations affect the growth of E. *coli* and λ

The rho15 and rho112 mutants both fail to grow in rich media at temperatures above 39 °C. In view of the pleiotropic properties of these rho mutations, it has not been possible to ascribe this conditional lethality to failure of transcription termination and, in fact, thermoresistant survivors of rho15 mutants in which polarity has not been restored have been isolated²⁶.

It was surprising to observe that a rho112 nusA1 double mutant grew well at 40 °C (Table 4). In contrast to a singly mutant strain, nusA1 or rho112, the nusA1 rho112 double mutant fails to form colonies at 32 °C. Unlike nusA1, the nusB5 mutation neither suppresses the temperature sensitivity of rho112 mutants nor renders them cryosensitive.

With respect to growth of λ the *rho112* mutation is quite clearly epistatic to *nusA1* or *nusB5*. At 37 °C the growth of λc_{17} is blocked by *nusA1* or *nusB5*; phage growth is restored by the introduction of *rho112* into the *nus* mutant strains (Table 4). In addition, the double mutant supports the growth of λN^- . Thus, the ability of the *nus* mutations to make a phage termination site resistant to N function is dependent on an active *rho* allele.

Discussion

We have presented evidence which implies that the *nus* mutations of $E.\ coli$ have opposite effects in phage λ and bacteria. In λ , the products of the *nus* genes are required for the phage N function to act and therefore λ fails to grow in *nus* mutant strains. As the role of N function is to prevent termination, the *nus* mutations can be considered to enhance transcription termination on λ DNA. However, in $E.\ coli$, the polarity of certain mutations is partially suppressed by the *nus* mutations.

As polarity is caused by premature termination of transcription, the *nus* mutations seem to reduce termination in *E. coli*.

The degree of polarity suppression by nusA1 and nusB5 depends on the polar mutation tested. Partial suppression by nusA1 occurs in the case of weakly polar gal mutations, but little or no relief of polarity is seen with more polar mutations in the gal operon such as galEocB4, IS1 or IS2 insertions. nusB5 is more effective than nusA1 in suppressing polarity and shows some relief of polarity caused by the IS1 insertion mutant, galTN102, particularly at 42 °C. The most marked effect of the nusA1 mutation on polarity is in conjunction with rho112, a rho mutation which has significant residual rho activity. Here, the nusA1 and rho112 mutations act synergistically to suppress a highly polar IS2 insertion.

The nusA1 mutation produces a partial relief of attenuation in the trp operon. An apparent stimulation by nusA protein of the $in\ vitro$ synthesis of RNA polymerase β and β' subunits has been interpreted as suggesting that nusA protein suppresses an attenuator that regulates $\beta\beta'$ expression^{23,27,28}. We have not, however, observed any significant effect of the nusA1 mutation on the synthesis of these RNA polymerase subunits $in\ vivo$ as judged by the incorporation of ¹⁴C-labelled amino acids and visualization of the proteins in SDS-polyacrylamide gels (data not presented).

We have also tested for other interactions between the *nus* mutations and *rho*. With respect to phage growth, *rho* mutations are clearly epistatic to nusA1 or nusB5; λc_{17} , which fails to form plaques on nus^- hosts, grows well on a rho^- nusA1 or a rho^- nusB5 double mutant. For bacterial growth, nusA1 is epistatic to rho^- ; a rho^- mutant which fails to form a colony at $40\,^{\circ}\mathrm{C}$ will do so when a nusA1 mutation is introduced into the strain. The double mutant is, however, cryosensitive and does not survive at $32\,^{\circ}\mathrm{C}$. Introduction of the nusB5 allele into a rho^- mutant does not alter its growth pattern; it remains thermosensitive and cryoresistant.

The thermosensitivity of rho strains is not well understood. The rho15 and rho112 mutant proteins are thermolabile in vitro. Yet the isolation of secondary mutations which suppress the thermosensitivity of the rho mutants, without restoring polarity (at least to those sites tested), suggests that failure of transcription termination may not be the lethal event (ref. 29 and A. Das, personal communication). Furthermore, the wide range defects in the rho mutants-abnormalities in DNA synthesis, in recombination and in oxidative phosphorylationpresents several potential causes for thermosensitivity. The observation that the nusA1 mutation suppresses the thermosensitivity of rho mutants in addition to enhancing suppression of certain polar mutations is not inconsistent with the above analysis. We can imagine that nusA1 affects the pleiotropic character which is responsible for cell death at 40 °C by a mechanism unrelated to transcription termination. Alternatively, the nusA gene protein may act at some bacterial terminators, vital for cell growth, as it does at λ terminators, by stimulating transcription termination.

The *rho* mutant, *rhoHDF026*, has been characterized and seems to be phenotypically very similar to nusA1 and nusB5—polarity suppression is partial and the activity of the λN function is reduced³⁰). The similarity between these mutants is consistent with the idea that nusA and nusB, like *rho*, are transcription termination factors.

Table 4 Interactions of rho, nusA1 and nusB5 mutations

		Cell g	Cell growth		Phage plating	
rho	nus	32 °C	42 °C	λnin₅	λc ₁₇	λN^{-}
+	+	+	+	+	+	
+	A1	+	+	+	****	-
+	B5	+	+	+		
112	+	+		+	+	+
112	A1	-	+	+	+	+
112	B 5	+	*****	+	+	+

In an accompanying article³¹, Greenblatt et al. present evidence that nusA protein acts in vitro as a site-specific transcription termination factor. His results are consistent with our in vivo experiments which indicate that the nusA1 mutation partially relieves polarity in the gal operon. The incomplete relief of polarity by nusA1 is best explained by assuming that nusA1 protein retains considerable termination activity. The paucity of nusA mutants suggests that nusA may have a vital function. λN protein interacts with nusA protein in vitro 23, and presumably in vivo, to antagonize nusA termination function. It is possible that the nusA1 mutation primarily affects the ability of nusA protein to interact with N rather than affecting its transcription termination activity.

The inhibition of λ growth by nusA1 is overcome by introducing a mutation in the rho gene, indicating that transcription

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- Merril, C., Gottesman, M. E., Court, D. L. & Adhya, S. J. molec. Biol. 118, 241-245 (1978).
- Hausler, B. & Somerville, R. L. J. molec. Biol. 127, 353-356 (1979). Levinthal, M. & Nikaido, H. J. molec. Biol. 42, 511-520 (1969).
- Ward, D. F. & Murray, N. E. J. molec. Biol. 133, 249-266 (1979). Bertrand, K. et al. Science 189, 22-26 (1975).

- Johnston, H. M., Barnes, W. M., Chumley, F. G., Bossi, L. & Roth, J. R. Proc. natn. Acad. Jonnston, H. M., Barnes, W. M., Chumley, F. G., Bossi, L. & Roth, J. R. Proc. natn. Acad. Sci. U.S.A. 77, 508-512 (1980).
 Adhya, S. & Gottesman, M. E. A. Rev. Biochem. 47, 967-996 (1978).
 Roberts, J. Nature 224, 1168-1174 (1969).
 Richardson, J. P., Grimley, C. & Lowery, C. Proc. natn. Acad. Sci. U.S.A. 72, 1725-1728.

- 11. Das, A., Court, D. & Adhya, S. Proc. nam. Acad. Sci. U.S.A. 73, 1959-1963 (1976).
- Das, A., Merril, C. & Adhya, S. Proc. natn. Acad. Sci. U.S.A. 75, 4828-4832 (1978).
 Guarente, L., Mitchell, D. & Beckwith, J. J. molec. Biol. 112, 423-436 (1977).
- , Gottesman, M. & deCrombrugghe, B. Proc. natn. Acad. Sci. U.S.A. 71,
- Baumann, M. F. & Friedman, D. I. Virology 73, 128-138 (1976).
 Georgopoulos, C. P. Proc. natn. Acad. Sci. U.S.A. 68, 2977-2981 (1971).
- 17. Ghysen, A. & Pironio, M. J. molec. Biol. 65, 259-272 (1972).

termination caused by nusA1 must be occurring at rho-dependent sites. We cannot eliminate the possibility that other terminators may exist which are dependent solely on nusA protein.

We will be presenting elsewhere evidence that the nusA and nusB gene products interact. It is not unreasonable, therefore, that the nusA1 and nusB5 mutants should have a similar phenotype.

Further investigation of the role of nusA and nusB proteins in bacterial and phage metabolism requires the isolation of additional nus mutations, of secondary mutations which suppress the Nus phenotype and of reproduction of mus protein action in vitro. This work is in progress.

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- Sternberg, N. Virology 73, 139-154 (1976).
 Salstrom, J. S. & Szybalski, W. J. molec. Biol. 124, 195-221 (1978).
- 20. Friedman, D. I. The Bacteriophage Lambda (ed. Hershey, A. D.) 733-738 (Cold Spring Harbor, New York, 1971).
- Friedman, D. I., Baumann, M. & Baron, L. S. Virology 73, 119-127 (1976). Georgopoulos, C. P. et al. Molec. gen. Genet. 179, 55-61 (1980).
- Greenblatt, J. et al. Proc. natn. Acad. Sci. U.S.A. 77, 1991-1994 (1980). Adhya, S. & Shapiro, J. A. Genetics 62, 231-247 (1969).

- Korn, L. & Yanofsky, C. J. molec. Biol. 103, 395-409 (1976).
 Das, A., Court, D. & Adhya, S. Molecular Basis of Host Virus Interactions (ed. Chakravarty, M.) 459-467 (Science Press, Marrikville, 1978)

- Zaruchi-Schulz, T. et al. Proc. natn. Acad. Sci. U.S.A. 76, §115-6119 (1979).
 Barry, G., Squires, C. L. & Squires, C. Proc. natn. Acad. Sci. U.S.A. 76, 4922-4926 (1979).
 Guidi-Rontani, C., Danchin, A. & Ullmann, A. Proc. natn. Acad. Sci. U.S.A. 77, 5799-5801 (1980).
 30. Simon, L. D., Gottesman, M., Tomczak, K. & Gottesman, S. Proc. natn. Acad. Sci. U.S.A.
- **76,** 1623–1627 (1979).
- Greenblatt, J., McLimont, M. & Hanly, S. Nature 292, 215-220 (1981).
- Sherman, J. R. & Adler, J. J. biol. Chem. 238, 873-878 (1963). Gottesman, M. E., Adhya, S. & Das, A. J. molec. Biol. 140: 57-75 (1980).
- Smith, O. H. & Yanofsky, C. Meth. Enzym. 5, 801-802 (1962)
- Gottesman, M. E. & Yarmolinsky, M. B. J. molec. Biol. 31, 487~505 (1968)
- Jackson, E. & Yanofsky, C. J. molec. Biol. 71, 149-161 (1972)

Termination of transcription by nusA gene protein of Escherichia coli

Jack Greenblatt, Marjorie McLimont & Sarah Hanly

Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6

The nus A gene protein of Escherichia coli and N gene protein of bacteriophage λ interact in vitro and cooperate in vivo to prevent transcription termination. In vitro the nusA gene protein causes RNA polymerase to pause in the t_{R2} terminator region of λ DNA. A completed termination event at t_{R2} requires both the nusA gene protein and the previously described E. coli termination factor rho. The nusA gene protein is therefore both a transcription termination factor and a protein which couples antitermination factors to the elongating transcription complex.

BEYOND the ends of the first genes N and cro of the P_L and P_R early operons of bacteriophage λ are the transcription termination sites t_{L1} and t_{R1} (see Fig. 1). Termination of transcription occurs at these sites in vitro or in vivo only in the presence of the transcription termination factor rho¹⁻⁵. In an infection of rapidly growing Escherichia coli bacteria by phage λ the E. coli RNA polymerase is able to transcribe λ genes distal to the N and cro genes because the product of the N gene of the phage prevents termination of transcription at t_{L1} and t_{R1} (refs 2-11). The N gene protein also prevents termination of transcription at other more distal termination sites in both of the early operons^{4,12–14} It is thought that N protein recognizes particular sites, called nut sites, in the nucleic acid of the λ early operons^{5,15} and somehow modifies RNA polymerase molecules at these sites so that they will resist termination of transcription at most termination sites¹⁶⁻²⁰

Friedman and Georgopoulos and their co-workers (and others) have isolated E. coli mutants in which phage λ cannot grow because its N gene protein cannot prevent transcription

termination²¹⁻²⁴. One such mutation is the nus Al mutation at 68 min on the standard E. coli map, which blocks λ growth at 42 °C, but not at 30 °C (refs 21, 25). The nusA1 mutant protein does not inactivate N protein because the nusA* allele is dominant to the nusA1 allele25. This suggests that the nusA protein is involved in preventing termination of the transcription of λ DNA. That a phage λ N gene mutant, λN punA, grows on nusA1 bacteria at 42 °C (ref. 26) suggests that the nusA gene protein might interact directly with the N gene protein. Recently, the nusA gene protein has been identified as a 69,000dalton acidic protein which binds directly to N protein²²

The nusA gene protein is identical to L factor²⁸, a protein which was purified as an activity necessary for DNA-dependent β -galactosidase synthesis in vitro²⁹. L factor also stimulates the DNA-dependent synthesis in vitro of the β and β' subunits of E. coli RNA polymerase30. These observations can perhaps be rationalized by noting that there is a rho-sensitive transcription termination site in the middle of the lacZ gene³¹ and a probable transcriptional attenuator proximal to the rpoB gene³². These

results suggest that the *nusA* gene protein may have a role in overcoming transcription termination at bacterial terminators.

We have now examined the effects of wild-type and mutant nusA gene proteins on the transcription $in\ vitro$ of λ DNA by E. coli RNA polymerase. Our results show that the nusA gene protein can act to terminate the transcription of the P_R operon of λ . Other experiments, to be published elsewhere 33 , demonstrate that the nusA protein binds specifically, directly and with equimolar stoichiometry to the transcribing form (core enzyme) of RNA polymerase. The nusA gene protein is therefore both a termination factor for RNA synthesis and a protein which can couple transcription antitermination factors, such as the λ N protein, to the transcription complex.

Inhibition of transcription in vitro by the nusA gene protein

In our first preparations of the nusA gene protein we followed the L factor purification scheme of Kung et al.29 and pooled those fractions containing a protein with the same SDS-polyacrylamide gel mobility and isoelectric point as a radiolabelled nusA gene protein standard²⁷. We then noted that preparations of the nusA gene protein at least 90% pure contain an activity that inhibits the transcription in vitro of λ DNA by pure E. coli RNA polymerase (Fig. 2). The dose-response curve is similar to that produced by rho factor¹ in that the inhibition never becomes greater than about 50% even at very high concentrations of nusA protein (Fig. 2). Moreover, as the nusA gene protein does not inhibit transcription in reactions carried out in the presence of rho factor (Fig. 2), our results suggested that the nusA gene protein might be acting to terminate the transcription of λ DNA at a site or sites located either at or distal to the rho-sensitive termimnators.

A consideration of the genetic evidence dealing with the *nusA* gene²¹ and of the biochemical data on L factor^{29,30} suggested that the *nusA* gene protein was involved in preventing the termination of transcription (see above). The idea that it might be causing the termination of transcription was therefore sufficiently unexpected for us to seek convincing evidence that the apparent termination activity present in our purified *nusA* protein was indeed a property of the *nusA* protein itself and not of a contaminant present in the preparation.

Purification of wild-type and mutant nusA gene proteins

Figure 3a,c,e demonstrates that an activity which inhibits λ transcription in vitro has the same chromatographic behaviour as the nusA protein on DEAE-cellulose (Fig. 3a) and hydroxylapatite (Fig. 3c) and the same sedimentation velocity as the nusA protein during centrifugation in a glycerol density gradient (Fig. 3e). In the purification scheme illustrated in Fig. 3 we modified the L factor purification procedure of Kung et al. 29 by using a buffer of higher ionic strength during the high speed centrifugation step and by replacing their gel filtration column

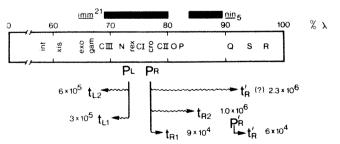


Fig. 1 Partial genetic and transcriptional map of bacteriophage λ . The sizes of the P_R - t_{R1} and P_R' - t_R' transcripts have been determined by sequencing⁴⁵⁻⁴⁷ and the indicated sizes, in daltons, of the other transcripts estimated by electrophoresis^{5,34}. For simplicity, the known λ transcripts beginning at prm, pre, P_O and P_I have been omitted from the diagram. nin_5 is a deletion and imm^{21} is a deletion-substitution.

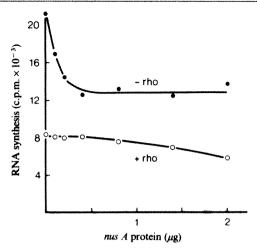


Fig. 2 Effect of the nusA protein on total λ transcription in the absence and in the presence of rho factor. Each reaction contained, in a total volume of 50 μ l, 20 mM Tris-HCl, pH 7.9, 80 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol, 100 μ M EDTA, 200 μ M each of ATP, CTP and GTP, 50 μ M 3 H-UTP at 16,000 c.p.m. per nmol 2 μ g λ CI₈₅₇S₇ DNA, 1 μ g E. coli RNA polymerase holoenzyme 48 , the indicated amount of nusA protein, and either no rho factor (\bullet) or 50 ng rho factor (\bigcirc). Incubation was for 30 min at 37 $^{\circ}$ C and 3 H-UTP incorporated into RNA was determined using DE81 filters 49 .

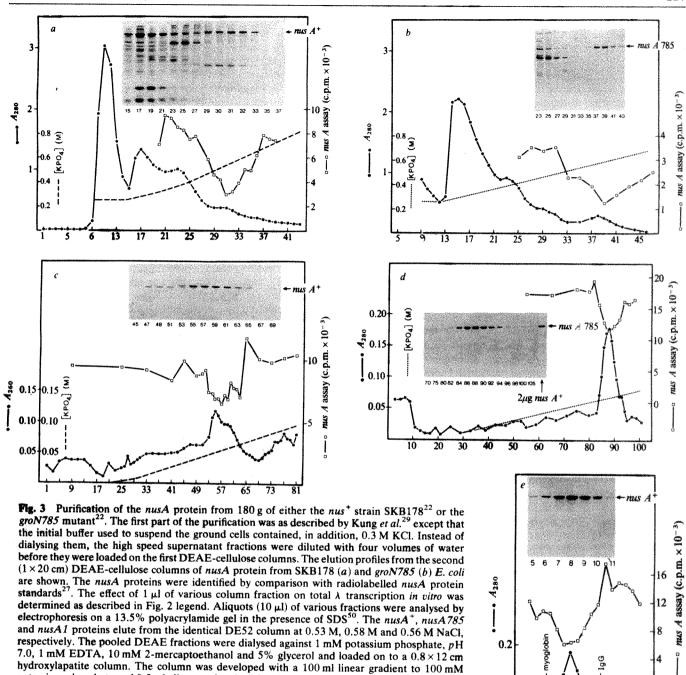
with a hydroxylapatite column. In our hands, at least, the result is a higher yield of nusA protein with a greater degree of purity. The fact that an activity that inhibits transcription of λ DNA in vitro co-purifies with the nusA protein is consistent with the idea that such an activity is indeed a property of the nusA protein.

To establish this point more securely we purified the nusA protein from two strains, nusA1 (ref. 21) and groN785 (ref. 22), that each has a nusA protein with a modified isoelectric point²⁷. We were able to do so because the nusA mutation in groN785 seems to be a silent mutation²⁷ and because the nusA1 mutation is a conditional defect that is very weak at low temperature²¹. Presumably as a result of the charge change or a configurational change introduced by the mutation, each of the mutant nusA proteins behaves somewhat differently from the wild-type protein during chromatography on DEAE-cellulose and hydroxylapatite. The entire purification of the nusA protein from the groN785 strain is illustrated in Fig. 3b,d. Both mutant proteins elute later than the wild-type protein from columns of both DEAE-cellulose and hydroxylapatite.

Our purification scheme leads to a high degree of purity (>90%) for the wild-type nusA protein (Fig. 3e) and for both mutant nusA proteins (see Fig. 3d for nusA785). All three proteins preserve their isoelectric homogeneity throughout the purification (not shown). Most importantly, both the mutant nusA785 protein (Fig. 3b,d) and the mutant nusA1 protein (not shown) co-purify with an activity that inhibits λ transcription in vitro, just as does the wild-type protein (Fig. 3a,c,e). As the behaviours during purification of the three allelic proteins are non-identical, the ability to inhibit transcription of λ DNA in vitro is therefore a genuine property of the nusA gene protein.

Termination of λ transcription in vitro at t_{R2} by the nusA gene protein

We have used gel electrophoresis to examine the effect of the nusA gene protein on λ transcripts made in vitro in the presence of $[\alpha^{-32}P]UTP$. We chose an electrophoresis system used by Roberts³⁴ to analyse λ transcripts made in reactions containing RNA polymerase and no additional factors. Roberts noted that most RNA polymerase molecules transcribing the P_R operon synthesize a large 2.3×10^6 -dalton transcript (see Figs 1, 4a). His mapping data suggested that this long transcript was probably co-terminal, at t_R' , with the short 6×10^4 dalton RNA transcribed in vitro from the late promoter P_R' (Figs 1, 4a).



tively. The wild-type nusA protein was precipitated with ammonium sulphate at 50% saturation and then further purified by centrifugation for 42 h at 40,000 r.p.m. in a Beckman SW 40 rotor through a 10-30% (W/V) glycerol gradient containing 10 mM potassium phosphate, pH 7.0, 100 mM NaCl, 1 mM EDTA and 10 mM 2-mercaptoethanol (e). 1-µl Aliquots of gradient fractions were tested in transcription reactions and 5-µl aliquots were analysed by electrophoresis. The nusA gene protein sediments at 4.2S. The yields of the wild-type and groN785 nusA proteins were, respectively, about 2 mg and 4 mg per 200 g wet weight of E. coli. Each was more than 90% pure, as was the nusA1 protein, purified in a similar manner (not shown).

Some of the RNA polymerase molecules transcribing the P_R operon seem to stop at a site t_{R2} located about midway between t_R' and the rho-dependent terminator t_{R1} (ref. 34 and Fig. 1). The size of the resulting transcript is 1.0×10^6 daltons (Fig. 4a). The t_{R2}-terminated transcript is easily identified as the one which is eliminated by the nin_5 deletion (Fig. 4h), which deletes at least one P_R operon terminator in $vivo^{12,35}$. The P_R operon transcript made from the λnin_5 template is the P_R -t'_R transcript shortened to 1.6×106 daltons by the length of the nin5 deletion. Experiments in which rifampicin was used to synchronize initiation of transcription and then gel electrophoresis to monitor the kinetics of RNA chain elongation (not shown) have indicated that the RNA polymerase molecules pause for an average of

potassium phosphate and 0.5-µl aliquots of various fractions were tested for their effect on RNA

synthesis. The $nusA^+$ protein (c) and the nusA785 protein (d) were located by electrophoresis of 10 μ l aliquots of the various fractions. The $nusA^+$, nusA785 and nusA1 proteins elute from the identical hydroxylapatite column at 50 mM, 66 mM and 62 mM potassium phosphate, respec-

> 1-2 min at t_{R2}; ultimately, all of them synthesize a large 2.3× 106-dalton RNA.

11

15

0.1

The effect of the *nusA* gene protein on λ transcription in vitro is shown in Fig. 4b. It eliminates the long P_R-t'_R transcript and leaves the shorter P_R-t_{R2} transcript. As shown in Fig. 4i, it does not eliminate the P_R - t_R' transcript when λnin_5 , a template which lacks t_{R2}, is transcribed. Thus, the major effect of the nusA gene protein that can be observed by gel electrophoresis is to enhance the efficiency to apparent transcription termination at t_{R2}.

The molar yield of the P_R-t_{R2} transcript made in the presence of the nusA protein is somewhat less than expected based entirely on a termination model (that is, $[P_R-t_{R2}]$ in the presence of $nusA < [P_R-t_{R2}] + [P_R-t_R']$ in the absence of nusA). This is

because RNA polymerase molecules arrive nonsynchronously at t_{R2} , because the nusA protein increases the transit time for RNA polymerase from P_R to t_{R2} by about 50%, and because the nusA protein decreases the rate of RNA chain initiation by about 20% (J. G., unpublished data). These other effects of the nusA protein on λ transcription in vitro can be accounted for by the tight association of the nusA protein with core RNA polymerase at sites other than terminator sites³³ and by competition between the nusA protein and the RNA polymerase σ subunit for binding to core RNA polymerase³³. The experiment of Fig. 2 suggests that the effect of the nusA protein on transcription initiation is, in any case, only minor.

Figure 4e demonstrates that the nusA gene protein is a termination factor that, to be effective, must be present during the transcription reaction. When transcription is carried out in the absence of the nusA gene protein, subsequent incubation in the presence of the nusA gene protein and the transcriptional inhibitor streptolydigin does not result in the conversion of the P_R-t'_R transcript to the shorter P_R-t_{R2} transcript. Therefore, the nusA gene protein is not a ribonuclease that processes the P_R-t'_R transcript to produce a shortened derivative. Kinetic experiments (not shown) have indicated that the nusA protein does not cause irreversible chain termination at t_{R2}, but instead causes RNA polymerase to pause for 10-15 min at several closely linked sites in the t_{R2} region. Irreversible termination at t_{R2} requires both the nusA protein and a low concentration of rho factor (3 µg ml⁻¹, a concentration which does not cause termination at t_{R2} in the absence of the nusA protein).

In the absence of the nusA protein and rho factor, RNA polymerase synthesizes a 6×10^5 dalton transcript from the P_L operon, terminating at t_{L2} (ref. 34 and Figs 1, 4a). Kinetic experiments have indicated that the event at t_{L2} is a pause of very long, but not indefinite, duration (about 10 min) which is not affected by the nusA protein. Hybridization of λ RNA synthesized in vitro to various fragments of λ DNA (J. G., unpublished data) confirmed that the nusA protein inhibits the synthesis of distal RNA from the P_R operon, but has little effect on the synthesis of distal RNA from the P_L operon (unlike rho, which inhibits both). This suggests that, at least in vitro, the nusA protein acts as a strong termination factor for the P_R operon, but not for the P_L operon.

Other large λ RNAs are made in the absence of the *nusA* gene protein and not in its presence (see Fig. 4), and are particularly evident when the nin_5 deletion moves the long t_R' -terminated transcript lower in the gel (Fig. 4h, i). Experiments with λimm^{21} DNA (Fig. 4f, g) suggest that some of them come from the immunity region, but they have not been studied in any greater detail.

Termination of transcription in vitro at t_{R2} by mutant nusA gene proteins

To show that termination at t_{R2} is caused by the *nusA* gene protein and not by a contaminant present in our preparation, we carried out similar experiments with *nusA* protein derived from the *groN785* and *nusA1* strains^{21,22,27}. Thus, *nusA785* protein (Fig. 4l) is just as effective as the *nusA*⁺ protein (Fig. 4k) in terminating transcription at t_{R2} , as is consistent with the lack of phenotype conferred on λ or *E. coli* growth by the *nusA785* mutation²⁷. In fact, the *nusA785* protein is just as effective as the *nusA*⁺ protein at t_{R2} even when the mutant RNA polymerase of the *groN785* strain^{22,36} is used in the experiment (Fig. 4m-o). As the *nusA785* protein behaves differently during purification from the *nusA*⁺ protein (it elutes later from both DEAE cellulose and hydroxylapatite; see Fig. 3), it must be the *nusA* gene protein itself that causes termination at t_{R2} .

The mutant nusA1 protein does behave differently from the wild-type protein (Fig. 5). It is able to cause termination at t_{R2} , but does so with a specific activity several-fold lower than normal. This is true even in reactions carried out at 30 °C and the effect is only slightly accentuated in reactions carried out at higher temperatures. The lack of a strong temperature effect on nusA1 termination activity in vitro is not unexpected: in vivo

experiments have suggested that the nusA1 mutant protein is only temperature sensitive for antitermination²¹, not for termination. The low specific activity of the nusA1 protein is not a consequence of its having been almost totally denatured during purification because the pure nusA1 protein is fully capable of binding to λN gene protein and to RNA polymerase (J. G. and Joyce Li, unpublished data). It seems likely that the reduced activity of the nusA1 mutant protein is adequate or almost adequate in vivo for terminating transcription (see accompanying article³⁷), but inadequate for coupling the N gene antitermination protein to the transcription complex. The fact that the nusA1 protein has an altered t_{R2} termination activity provides additional evidence that it is really the nusA gene protein that causes termination at t_{R2} .

Termination of transcription in vitro at t_{R2} by a mutant RNA polymerase

The experiment shown in Fig. 4p-s suggests that the termination effect of the nusA protein $in\ vitro$ represents a real physiological phenomenon, and is not simply an artefact of our conditions for transcription $in\ vitro$. We purified RNA polymerase from an $E.\ coli$ strain isolated by Lecocq and Dambly³⁸ which has a mutation, rif501, conferring resistance to rifampicin in the β subunit of its RNA polymerase. One of the properties of this strain is its ability to support λ growth at high temperature in the absence of a functional N gene protein³⁸. The experiments of Lecocq and Dambly³⁸ suggest that the rif501 RNA polymerase is resistant in

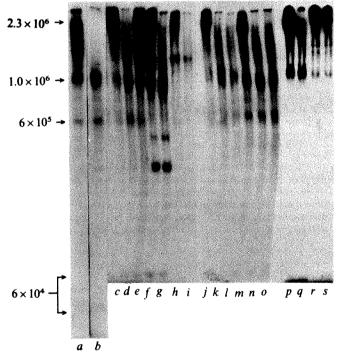


Fig. 4 Electrophoretic analysis of λ transcripts made in vitro. Transcription reactions were carried out as described in Fig. 2 legend except that they contained [α-32P]UTP at 200 μCi per μmol instead of ³H-UTP. Reactions contained the indicated DNAs and, where indicated, 0.5 μg nusA protein. Reactions were stopped with 100 μl of 9 M urea, 1% SDS and aliquots were electrophoresed on a slab gel containing 0.5% agarose, 2.5% polyacrylamide and 0.1% SDS³⁴. a, λ DNA; b, λ DNA, nusA⁺ protein; c, λ DNA; d, λ DNA, nusA⁺ protein; e, λ DNA, reaction stopped with 10 μg ml⁻¹ streptolydigin and incubated for another 30 min in the presence of nusA⁺ protein; f, λ imm²¹ DNA; g, λ imm²¹ DNA, nusA⁺ protein; h, λ nin₅ DNA; i, λ nin₅ DNA, nusA⁺ protein; j, λ DNA; k, λ DNA, nusA⁺ protein; l, λ DNA, nusA⁺ protein; l, λ DNA, nusA⁺ protein; m, n, o, same as j, k, l except RNA polymerase; p, λ DNA, q, λ DNA, nusA⁺ protein; r, s, same as p, q except that the core component⁵¹ of the RNA polymerase was from the nif501 mutant³⁸ instead of from wild-type E. coli.

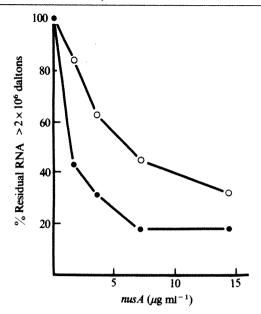


Fig. 5 Comparison of the effects of wild-type nusA protein and the nusA1 mutant protein on λ transcription in vitro. Reactions containing the indicated concentrations of the $nusA^+$ protein (\odot) or the nusA1 protein (\odot) were carried out and the products analysed as in Fig. 4. The high molecular weight regions of the gel tracks were cut out and counted and the results expressed as a percentage of the counts obtained in tracks of reactions containing no added nusA protein.

vivo to termination at terminators of the P_R operon of λ , but terminates normally at the terminators of the P_L operon of λ . As the nusA gene protein is active in vitro on the transcription of the P_R operon, but not on the P_L operon, it seemed likely that the rif501 RNA polymerase was resistant to the nusA protein. As shown in Fig. 4r, s the rif501 RNA polymerase does not respond in vitro at t_{R2} to the presence of the nusA protein. It also has a shorter pause at t_{R2} than wild-type RNA polymerase in the absence of the nusA protein (Fig. 4p, r), but does respond normally at t_{R1} to rho factor (not shown). As the in vitro binding of the nusA protein to the rif501 RNA polymerase seems to be normal, the polymerase may be unable to recognize nusAdependent terminators. These results imply that, at least at high temperatures, the nusA gene protein is an important physiological block to P_R operon expression and λ growth; they also imply that transcription termination in vitro by the nusA gene protein is an in vitro reflection of a phenomenon that occurs in vivo. Additional support for the concept that the nusA protein causes transcription termination in vivo is found in the accompanying article³⁷.

Discussion

We have shown that the nusA protein of E. coli causes RNA polymerase to pause at a site t_{R2} in the P_R operon of phage λ . Final termination and release of the RNA chain at t_{R2} requires a low concentration of rho factor (a rho factor concentration that is ineffective in the absence of the nusA protein) in addition to the nusA protein.

How, then, can we reconcile the action of the nusA gene protein as a termination factor for RNA synthesis with the demonstration by Friedman²¹ that nusA is required to prevent the termination of λ early operon transcription and with the observations of Weissbach and his collaborators that nusA is required for the $in\ vitro$ expression of the genes for β -galactosidase²⁹ and the β and β' subunits of RNA polymerase³⁰? We think that there are two major possible explanations.

The first relies on our observation, to be published elsewhere, that the *nusA* gene protein behaves as an RNA polymerase subunit in causing the termination of transcription. It could, then, alter the entire termination specificity of the enzyme so

that, although it enables the enzyme to recognize a new set of termination sites, it also enables the enzyme to ignore another set of termination sites that it would otherwise recognize. In accordance with this principal, it might cause RNA polymerase to read through the rho-dependent terminator in lacZ (ref. 31) and the attenuator in front of rpoB (ref. 32) even though it causes RNA polymerase to stop at t_{R2} in the P_R operon of λ .

If this idea is correct, it is clearly necessary to repeat, in the presence of the nusA gene protein, all the studies done in vitro on the effects of rho factor on the transcription of various operons. For example, an interesting anomaly is posed by studies on the attenuator of the trp operon of E. coli. Termination at trpA is efficient in vitro in the absence of added factors³⁵ even though genetic studies have suggested that rho factor is involved in termination at trpA in vivo 40. It is also particularly interesting to speculate that the insertion element IS1 may possess nusA-dependent terminators because it has no rhodependent or factor-independent terminators in vitro 41 and yet is polar in both orientations in vivo 42. In fact, it is already known that the nusA protein weakens rho-dependent termination at a terminator in the early region of bacteriophage T7 DNA (M. Chamberlin, personal communication) and that the nusA protein strengthens rho-dependent termination at one site and weakens it at other sites at the end of the trp operon of E. coli (T. Platt, personal communication).

A second intriguing possibility, by no means incompatible with the above, is suggested by our observation that the N gene transcription antitermination protein of bacteriophage λ binds directly to the *nusA* gene protein²⁷. This fact suggests how the nusA gene protein can be necessary for preventing transcription termination at many sites, including t_{R2}, even though it is a termination factor. We imagine that the λ N protein interacts with the RNA polymerase at a nut site by binding to the nusA gene RNA polymerase subunit. A subtle modification of the nusA protein, such as that introduced by the nusA1 mutation, might block proper binding of the N gene protein without significantly interfering with the ability of the modified nusA protein to cause transcription termination. The nusA1 mutant protein, which blocks λ growth in vivo, does indeed have termination factor activity in vitro. We expect that an inactive nusA gene protein would allow λN^- phage to grow and might very well be lethal for the host: these are the properties of a strain carrying the rif501 RNA polymerase, an RNA polymerase which is resistant to the nusA protein in vitro.

We can also imagine that E. coli might possess operon-specific regulatory proteins, analogous to the N gene protein of λ , that bind to the nusA gene protein and alter the termination site recognition properties of the RNA polymerase when it is transcribing particular operons. In the case of the lac operon, such a regulator might enable RNA polymerase to read through the rho-dependent terminator in the lacZ gene and still terminate transcription at the end of the lac operon. Such a regulator, if it exists, would probably have been present in the crude E. coli extract used in the original L factor assay of the nusA protein29. There is no strong genetic evidence for the existence of such a regulator, but some experiments 43,44 suggest that CAP, the 3', 5'-cyclic AMP-binding protein of E. coli, could have such a role. Of course, the existence of such a regulator would not be necessary if the nusA gene protein can by itself prevent transcription termination at the rho site in the lazZ gene.

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- 1. Roberts, J. W. Nature 224, 1158-1174 (1969).
- Heinemann, S. F. & Spiegelman, W. G. Cold Spring Harb. Symp. quant. Biol. 35, 315–318 (1971).
- Kourilsky, P., Bourguignon, M.-F. & Gros, F. in The Bacteriophage Lambda (ed. Hershey, A. D.) 647-666 (Cold Spring Harbor Laboratory, New York, 1971).
- Lozeron, H. A., Dahlberg, J. E. & Szybalski, W. Virology 71, 252-277 (1976).
 Rosenberg, M., Court, D., Shimatake, H. & Brady, C. Nature 272, 414-423 (1978)
- Skalka, A., Butler, B. & Echols, H. Proc. natn. Acad. Sci. U.S.A. 58, 576-583 (1967).
 Kumar, S., Bovre, K., Guha, A., Hradecna, Z., Maher, V. M. & Szybalski, W. Nature 221, 823-825 (1969).
- 8. Luzzati, D. J. molec. Biol. 49, 515-519 (1970).
- 9. Nijkamp, H. J. J., Bovre, K. & Szybalski, W. J. molec. Biol. 54, 599-604 (1970).

- 10. Portier, M.-M., Marcaud, L., Cohen, A. & Gros, F. Molec. gen. Genet. 117, 72-81 (1972).
- Lozeron, H. A., Anevski, P. J. & Apirion, D. J. molec. Biol. 109, 359-365 (1977).
 Court, D. & Sato, K. Virology 39, 348-352 (1969).
 Hopkins, N. Virology 40, 223-229 (1970).

- Butler, B. & Echols, H. Virology 40, 212–222 (1970).
 Salstrom, J. S. & Szybalski, W. J. molec. Biol. 124, 195–221 (1978).
- Friedman, D. I., Wilgus, G. S. & Mural, R. J. J. molec. Biol. 81, 505-516 (1973) 17. Adhya, S., Gottesman, M. & de Crombrugghe, B. Proc. natn. Acad. Sci. U.S.A. 71, 2534-2538 (1974).
- 18 Franklin N. C. I. malec. Riol. 89, 33-48 (1974)
- Segawa, A. & Imamoto, F. J. molec. Biol. 87, 741-754 (1974).
- Adhya, S. & Gottesman, M. A. Rev. Biochem. 47, 967-996 (1978)
- 21. Friedman, D. in The Bacteriophage Lambda (ed. Hershey, A. D.) 733-738 (Cold Spring Harbor Laboratory, New York, 1971).
- 22. Georgopoulos, C. in The Bacteriophage Lambda (ed. Hershey, A. D.) 639-645 (Cold Spring Harbor Laboratory, New York, 1971).

 23. Keppel, F., Geogopoulos, C. & Eisen, H. *Biochimie* 56, 1503–1509 (1974).

 24. Friedman, D., Baumann, M. & Baron, L. S. *Virology* 73, 119–127 (1976).

 25. Friedman, D. I. & Baron, L. S. *Virology* 58, 141–148 (1974).

 26. Friedman, D. I. & Ponce-Campos, R. *J. molec. Biol.* 98, 537–549 (1975).

- Greenblatt, J. & Li, J. J. molec. Biol. 147, 11–23 (1981).
 Greenblatt, J. et al. Proc. natn. Acad. Sci. U.S.A. 77, 1991–1994 (1980).
 Kung, H., Spears, C. & Weissbach, H. J. biol. Chem. 250, 1556–1562 (1975).
- Zarucki-Schulz, T. et al. Proc. nam. Acad. Sci. U.S.A. 76, 6115-6119 (1979)
- 31. de Crombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. Nature new Biol. 241, 260-264 (1971)

- 32. Barry, G., Squires, C. L. & Squires, C. Proc. natn. Acad. Sci. U.S.A. 76, 4922-4926 (1979).
- 33. Greenblatt, J. & Li. J. Cell 24, 421-428 (1981)
- Roberts, J. W. Proc. natn. Acad. Sci. U.S.A. 72, 3300-3304 (1975).
- 35. Fiandt, M., Hradecna, Z., Lozeron, H. A. & Szybalski, W. in The Bacteriophage Lambda (ed. Hershey, A. D.) 329–354 (Cold Spring Harbor Laboratory, New York, 1971).
 36. Georgopoulos, C. P. Proc. natn. Acad. Sci. U.S.A. 68, 2977–2981 (1971).

- Ward, D. F. & Gottesman, M. E. Nature 292, 212–215 (1981).
 Lecocq, J.-P. & Dambly, C. Molec. gen. Genet. 145, 53–64 (1976)
- Lee, F., Squires, C. L., Squires, C. & Yanofsky, C. J. molec. Biol. 103, 383-393 (1976). Korn, L. J. & Yanofsky, C. J. molec. Biol. 103, 395-409 (1976).
- 41. de Crombrugghe, B., Adhya, S., Gottesman, M. & Pastan, I. Nature new Biol. 241, 260-264 (1973).
- Das, A., Court, M., Gottesman, M. & Adhya, S. in DNA Insertion Elements, Plasmids, and Episomes (eds Bukhari, A. I., Shapiro, J. A. & Adhya, S. L.) 93–97 (Cold Spring Harbor Laboratory, New York, 1977)
- 43. Ullmann, A., Joseph, E. & Danchin, A. Proc. natn. Acad. Sci. U.S.A. 76, 3194-3197
- 44. Guidi-Rontani, C., Danchin, A. & Ullmann, A. Proc. natn. Acad. Sci. U.S.A. 77, 5799-5801 (1980)
- 45. Roberts, T., Shimatake, H., Brady, C. & Rosenberg, M. Nature 270, 274-275 (1977).
- Schwarz, E., Scherer, G., Hobom, G. & Kössel, H. Nature 272, 410-414 (1978). Lebowitz, P., Weissman, S. & Radding, C. J. biol. Chem. 246, 5120-5139 (1971).
- Burgess, R. R. & Jendrisak, J. J. Biochemistry 14, 4634-4638 (1975). Weil, P. A. & Blatti, S. P. Biochemistry 14, 1636-1642 (1975).
- Laemmli, U. K. Nature 227, 680-685 (1970)
- 51. Burgess, R. R. J. biol. Chem. 244, 6168-6176 (1969)

Microwave emission from flare star AT Mic

O. B. Slee.* I. R. Tuohv.† G. J. Nelson* & C. J. Rennie†

* Division of Radiophysics, CSIRO, PO Box 76, Epping, New South Wales 2121. Australia

† Mount Stromlo and Siding Spring Observatories, Australian National University, Canberra, ACT 2600

There have been several comprehensive optical and lowfrequency radio surveys of flare stars^{1,2} but much less attention has been directed to the microwave emission from such stars. Slee and Page³ claimed to have detected one short 5-GHz flare from Proxima Centauri, and several weak, short 5-GHz bursts were reported from YZ CMi (ref. 4) and Proxima Centauri (refs 5, 6) during simultaneous X-ray, optical and microwave observations. We describe here the most convincing detection (7σ) vet made of a microwave flare during simultaneous optical and 5-GHz observations of the dMe 4.5 flare star AT Mic on 25 October 1980. This event was notable for its long duration and the good temporal correlation between the optical and microwave intensities. Unless the dimensions of the source are very much larger than those observed for solar flares, the 5-GHz source has too great a brightness temperature to be produced by the gyrosynchrotron mechanism usually invoked for solar microwave bursts. Much higher energy particles or a coherent emission mechanism must be involved in the stellar microwave flare. The observed correlation between the optical and microwave fluxes from this flare support the theories in which non-thermal electrons are responsible for both emissions.

The optical monitoring of AT Mic was conducted using the 26-inch Yale Columbia refractor at the Mount Stromlo Observatory. A series of 1-min exposures on blue sensitive IIaO plates were taken at intervals of ~5 min. No filter was used, but the response of the emulsion coupled with the UV cutoff of the telescope gives a good approximation to a photoelectric B magnitude. Images of AT Mic on the plates were measured with an iris photometer, and the resulting intensity data were normalized relative to a set of comparison stars.

The observing method used in 5-GHz flare observations at Parkes has been described in detail elsewhere3. In the 5-GHz observations high stability against receiver gain fluctuations and thermal emission from passing clouds was achieved by using the equipment in the 'beam wagging' mode. A dual feed at the focus of the 64-m reflector produces two beams which are alternately placed on and off the flare star, a beam switch and synchronous detector being used to extract the difference signal at 39-Hz between the two beams. A complete integration cycle lasts for 1.7 min, with the driving of the telescope, sampling of the data and analysis of the difference signal being controlled by a computer. The r.m.s. fluctuation level for the integrations is

Figure 1 shows the 5-GHz and optical results for the flare on AT Mic on 25 October 1980. Clearly the microwave emission rose rapidly in less than one integration cycle of 1.7-min duration and thereafter declined slowly over the next 60 min. The base level shown dashed in Fig. 1 was obtained by fitting a straight line to the data taken during an identical observation on the previous evening. The slope evident on this base line is caused by the slow rotation of the aerial pattern on the sky, resulting in small changes in the contributions of confusing sources and ground radiation to the on-source and off-source beam configurations. Some secondary intensity changes may be present on the declining side of the microwave burst but their significance is difficult to assess in view of the relatively high noise level.

The optical exposures give a light curve of similar shape with a peak flare increase corresponding to 0.5-1.0 mag; in view of the significantly higher integration cycle time of \sim 5 min (as opposed to 1.7 min for the 5-GHz observation), it is difficult to decide whether or not the microwave and optical flares started simultaneously. The peak optical flux is probably considerably

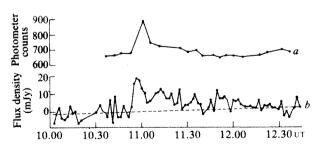


Fig. 1 The optical and 5-GHz light curves for the flare on AT Mic on 25 October 1980. Curve a shows a measure of the density of the photographic images, which were exposed for durations of 60 s at intervals of ~ 5 min. The peak of the optical curve corresponds to a flare luminosity of $\sim 5 \times 10^{30}$ erg s⁻¹. Curve b shows the 5-GHz flux densities, which are averaged over 60 s and taken at intervals of 1.7 min. The dashed baseline was obtained by averaging data taken during an identical observation on the previous evening. The r.m.s. fluctuation in the base line is 2.8 mJy.

brighter than shown in Fig. 1 and occurred near the time of the peak 5-GHz flux density.

The photographic measurements enabled the two stellar components of the AT Mic system to be resolved and thus simultaneously monitored. The two stars, separated by ~4 arc s. are both believed to be flare-active4. Our data unambiguously show that the 25 October flare originated from the southern component of the pair.

There have been previous attempts to observe stellar flares simultaneously at various combinations of X-ray, optical and radio wavelengths^{1-3,5-7}. In fact, only a few flares have been detected simultaneously in more than one of these wavelength ranges. The most striking features of the flare reported here, as compared with the earlier results, are the very long duration and the close correlation between the microwave and optical time

The 5-GHz flare exhibits a temporal variation similar to that observed in very large solar microwave bursts; for these comparatively rare events the well-known, short-lived impulsive phase is followed by a very much extended second phase which typically lasts for ~30 min (ref. 8). Both the impulsive and extended phase of solar microwave bursts are closely associated with hard X-ray emissions^{9,10} and the same population of electrons probably produces both the hard X rays and microwaves^{11,12}. The electrons responsible for the extended phase are generally harder and probably higher in the corona than those generating the impulsive bursts^{8,13}.

It is interesting to consider whether the observed stellar burst can be produced by the synchrotron mechanism which is believed responsible for the solar microwave emissions. In the latter case the synchrotron emission can account for the observed brightness temperatures of up to $\sim 10^9$ K. In the case of AT Mic (assuming a distance of 8.2 pc and a stellar radius of $0.32~R_{\odot}$) the brightness temperature $T_{\rm b}$ of the 20 mJy flare is $8.5 \times 10^9/\beta^2$ K, where β is the diameter of the radio source in stellar diameters. Thus if the emission is synchrotron radiation from ~ 100 -keV electrons $(10^8 < T_b < 10^9 \text{ K})$ the source must have a diameter at least three times larger than the star. If, as is more likely, the source is much smaller than the star, then very much higher brightness temperatures are implied. In this case, particles with energies greater than 100 keV or coherent emission mechanisms need to be considered 1,2,5

Assuming that there are enough high-energy particles in the source region to produce the microwave burst, the same particles (by analogy with the solar flare) may be expected to generate hard X-ray emission with intensity dependent on the ambient (thermal) particle density in the source. Hard X rays have not yet been detected from stellar flares during the several bright optical flares that have occurred during satellite observations in the energy range up to 60 keV (refs 4, 5, 14). On the other hand, soft X rays (0.1-10 keV) have been detected from several flare stars^{6,14,15}, but these are probably of thermal origin and therefore not directly related to the microwave burst.

The observed temporal correlation between the optical and microwave intensities for the flare on AT Mic may only be coincidental. Indeed, if the theory due to Mullan 16 is correct, the optical flare energy is conducted downwards from the coronal plasma which emits the soft X rays. In this case a correlation between the thermal X-ray and optical fluxes would result; any correlation with the non-thermal microwave emission would be much more indirect. On the other hand, if there is a direct relationship between the optical and microwave fluxes, as suggested by the observations in Fig. 1, then theories which are consistent with such a common origin should be investigated. One such theory 17 ascribes the increase in optical brightness to a wavelength shift of IR photons to visible wavelengths by inverse Compton scattering from MeV electrons. The mechanism may be very efficient in the cool flare stars, which have most of their quiescent emission in the IR region of the spectrum. This theory has recently been supported by Bruevich et al. 18, who observed decreases in the IR fluxes of several flare stars immediately before flares in the visible spectrum; the magnitudes and durations of the negative IR flares were sufficient to account for the excess flare energy radiated subsequently in the visible spectrum. The significance of this mechanism for the current observations is that the same MeV electrons responsible for the optical emission could also produce the microwave flare, thus accounting for the observed correlation.

The fact that the present 5-GHz flare on AT Mic lasts much longer than the microwave bursts generally observed either from the Sun or from the flare stars Proxima Centauri^{5,6} and YZ CMi (ref. 4) suggests that the extended part, at least, may result from a previously unobserved mechanism. On the other hand, long-duration optical flares¹⁷ and metre-wave radio bursts² have been previously observed on flare stars. It may simply be that the extended parts of previous stellar microwave bursts have been below the threshold for detection.

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- Spangler, S. R. & Moffett, T. J. Astrophys. J. 203, 497 (1976). Nelson, G. J. et al. Mon. Not. R. astr. Soc. 187, 405 (1979).
- Slee, O. B. & Page, A. A. *Proc. IAU Colloq.* **46**, 150 (1979). Kunkel, W. E. *Proc. IAU Symp.* **67**, 15 (1975).
- Karpen, J. T. et al. Astrophys. J. 216, 479 (1977). Haisch, B. M. Astrophys. J. Lett. 225, L35 (1978)

- Haisch, B. M. et al. Astrophys. J. 245, 1009 (1981).

 Frost, K. J. & Dennis, B. R. Astrophys. J. 165, 655 (1971).

 Crannell, C. J., Frost, K. J., Mätzler, C., Ohki, K. & Saba, J. L. Astrophys. J. 223, 620 (1978).

 Stewart, R. S. & Nelson, G. J. Proc. astr. Soc. Aust. 3, 390 (1979).

- Stewart, R. S. & Nelson, G. J. Proc. astr. Soc. Aust. 3, 393 (1979). Nelson, G. J. & Stewart, R. T. Proc. astr. Soc. Aust. 3, 392 (1979). Holt, S. S. & Ramaty, R. Solar Phys. 8, 119 (1969). Hoyng, P., Brown, J. C. & van Beek, H. F. Solar Phys. 48, 197 (1976). Kahn, S. M. et al. Astrophys. J. Lett. 234, L107 (1979).
- Heise, J. et al. Astrophys. J. Lett. 202, L73 (1975). Mullan, D. J. Astrophys. J. 207, 289 (1976).
- Gurzadyan, G. A. Flare Stars (Pergamon, Oxford, 1980).
 Bruevich, V. V. et al. Proc. Crimean Astrophys. Obs. 61, 90 (1980).

Fine structure in the ionospheric D-region

E. V. Thrane & B. Grandal

Norwegian Defence Research Establishment, PO Box 25, N-2007 Kjeller, Norway

T. Flå & A. Brekke

Institute for Mathematical and Physical Sciences, University of Tromsø, N-9001 Tromsø, Norway

Assuming that their observations of the weak echoes of high frequency radio waves scattered from the lower ionosphere were caused by Fresnel reflections from discontinuities in the refractive index, Gardner and Pawsey¹ developed a method for determining the height distribution $N_{\rm e}(h)$ of the D-region electron densities from observations of the ratio A_x/A_0 (h) of the back-scattered amplitudes of the extraordinary and ordinary magneto-ionic components. This 'partial reflection' method turned out to be a useful tool for studies of the ionospheric D-region²⁻⁵. We compare here observations of weak radio echoes from the ionospheric D-region, with model computations of radio wave scattering, based on in situ measurements of ionospheric irregularities. The results show excellent agreement, indicating that an important source of the weak partial reflections has been identified.

The nature of the irregularities and the scattering mechanism has been extensively discussed and the theory has been gradually developed to describe different types of scattering 6-13. Part of this discussion dealt with the possibility that the irregularities in refractive index could be due to changes in electron-neutral collision frequency⁷ as well as changes in electron density, as originally assumed by Gardner and Pawsey. Belrose *et al.*¹⁰, however, presented data supporting the original hypothesis, and in the following we assume that only changes in electron density cause the partial reflections. The remaining important problem is to map the structure of these electron density irregularities. Because the air in the D-region is only weakly ionized, the degree of ionization being in general smaller than 0.1 p.p.m., we may expect a close coupling between changes in the ionized and neutral constituents. Studies of irregularities in ionization density may therefore give information about the dynamical state of the mesosphere.

The simple theory of Fresnel reflection implicity assumes that sharp vertical gradients in refractive index exist extending horizontally over at least a Fresnel zone (≈5 km for HF-waves near 70-km altitude). The vertical change in electron density $\Delta N_e/N_e$ must be of the order of 1-8% within a fraction of a radio wavelength (30-100 m) to produce the observed scattered amplitudes¹⁴. A revised theory by Flood⁸ assumes volume scattering from a medium in which irregularities with a spectrum of sizes are present. Constructive interference of backscattered signals will occur where the spatial variations have scales near half the radio wavelength. The radar will therefore preferentially be sensitive to the intensity of fluctuations with such scales. Ground based experiments to determine the nature of the scattering mechanism have not yielded conclusive results, but indicate that both Fresnel reflection from extended irregularities and volume scattering may occur. There seems to be a tendency for the larger irregularities to occur at low heights $(below 70 \text{ km})^{14,15}$.

We now compare observations of partial reflections from the D-region with computed intensities of radio echoes. The model computations are based on volume scattering from irregular ionospheric structures measured in situ in a sounding rocket. The experimental data were obtained from a rocket-borne electrostatic ion-current probe combined with simultaneous recordings of partial reflections. The experiments were made in North Norway, at the Andøya Rocket Range (69°17′N, 16°01′E) and at Ramfjord Research Station (69°58N, 19.22°E) where the University of Tromsø operates a partial reflection facility. The latter station is situated near Tromsø, 120 km to the north-east of the Andøya Rocket Range.

On 1 March 1978 at 01.13UT a research rocket code F-47, was launched from Andøya Rocket Range into a moderately disturbed auroral ionosphere. Before and during launch the ionospheric conditions were monitored by the partial reflection experiment at Ramfjord and by riometers and magnetometers at both sites. The launch criteria were: the presence of strong, stable partial reflections in the height range 65–95 km, and that both riometer absorption and magnetic activity were of similar magnitudes at the two sites. At the time of launch a moderate disturbance with riometer absorption between 0.5 and 1 dB at both sites had lasted for several hours with only small changes in absorption and partial echo structure. Magnetic activity was low during this period.

The F-47 payload was carried by a Nike Apache rocket and reached an apogee of 127.5 km. The electrostatic probe was designed to measure positive ion current with high time resolution and accuracy. The sampling rate was 1,136 Hz, providing one measurement of ion current per metre along the rocket trajectory through the D-region. The rocket experiment and the results have been described elsewhere 16. Some important assumptions made in the analysis of the rocket data should be mentioned. First, the observed ion current I is assumed to be proportional to ambient ionospheric ion number density N_i over small height intervals ($\Delta h \approx 1$ km). As the probe moves through the plasma with a speed about three times larger than the thermal velocity of the ions, the observed time variation in ion current is assumed to reflect the spatial structure of irregularities in the ambient ion density, that is $\delta I/\langle I \rangle = \delta N_i/\langle N_i \rangle$, where δI and δN_i are deviations from the respective running means $\langle I \rangle$

and $\langle N_i \rangle$ taken along a suitable length of the rocket trajectory. Furthermore, local charge neutrality is assumed so that $\delta N_i/N_i = \delta N_e/N_e$ where N_e is the electron number density. The instrument provides power spectra in the scale range 3-100 m. In the D-region the spectral slope is near -5/3, indicating that the irregularities are caused by homogeneous, isotropic turbulence, whereas the spectra observed above 95 km have a 'white noise' character. Of particular interest is the intensity in the D-region of irregularities with scales equal to $1/2 \lambda$ where λ is the radio wavelength of the partial reflection radar. The height variation of this intensity is shown in Fig. 1. The accuracy of the rocket results shown in Fig. 1 depend on several factors. The determination of the relative fluctuations in positive ion density $\sqrt{\langle \delta N_i^2 \rangle / \langle N_i \rangle}$ at the length scale $\lambda/2$ is accurate to better than 5%. The absolute magnitude of the electron density fluctuations is found from the equation

$$\sqrt{\left\langle \delta N_{\rm e}^2 \right\rangle} = \frac{\sqrt{\left\langle \delta N_{\rm i}^2 \right\rangle}}{\left\langle N_{\rm i} \right\rangle} \left\langle N_{\rm e} \right\rangle$$

where the electron density profile $\langle N_e \rangle(h)$ is determined from a radio wave propagation experiment in the rocket (Faraday rotation and differential absorption measurements, M. Friedrich personal communication). The structure in Fig. 1 represents a real variation in the fluctuation intensity, it is not associated with any large-scale structure in the electron density profile $\langle N_e \rangle(h)$, which is a smooth monotonic function of height¹⁶. The absolute accuracy of this profile changes with height from ~30% near 65 km to better than 10% at 95 km. Note that the observed fluctuations in ion density could not have been caused by fast time variations in the flux of the ionizing source (precipitating energetic electrons), because the time constant of the ions in the lower ionosphere is of the order of 100–1,000 s.

lower ionosphere is of the order of 100-1,000 s. The partial reflection experiment (PRE)^{17,18} facility near Tromsø operates on 2.75 MHz ($\lambda = 109$ m) and using a 17 dB gain antenna, transmits circularly polarized, 20 μ s wide, pulses vertically with a pulse repetition rate of 50 Hz. The equivalent

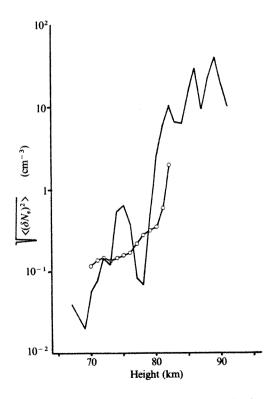


Fig. 1 Root mean square fluctuation of ion density for scales of $\frac{1}{2}\lambda$ ($\lambda = 109$ m, the radio wavelength for the PRE radar).

—, Fluctuations derived from the rocket measurements; one of the amplitude of the ordinary magneto-ionic component.

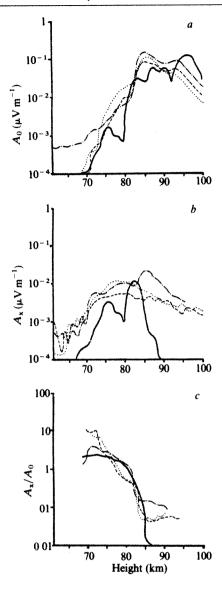


Fig. 2 Comparison between partial reflection measurements and results from calculations of partial reflection amplitudes based on rocket measurements of ionization density fluctuations. Computed values; ----, measurements 01.12.23-01.13.09UT (1 March 1978); ---, measurements 01.13.54-01.14.34UT; measurements 01.15.21UT-01.16.02UT. a, r.m.s. amplitude of ordinary magneto-ionic component versus height; b, r.m.s. amplitude of extraordinary magneto-ionic component versus height; c, r.m.s. amplitude ratio A_x/A_0 versus height.

isotropically radiated peak power is about 125 kW. The polarization is switched between pulses, so that alternate pulses correspond to the ordinary and extraordinary magneto-ionic modes. The transmitter and receiver systems are computer controlled, and the weak partial echoes are recorded on magnetic tape for every 1-km height interval, typically in the height region 50-100 km. Three examples of PRE observations of ordinary echo amplitude $A_0(h)$ and extraordinary amplitude $A_x(h)$, averaged over 40 s intervals during the rocket flight are shown in Fig. 2. The electron density profile $N_e(h)$ may be derived from the ratio $A_x/A_0(h)$, and this ratio is also shown. Observations from the three different time intervals are quite consistent, indicating that no significant ionospheric changes occurred during the flight. The height distribution of electron number density was also determined from Faraday rotation measurements in the rocket (M. Friedrich, personal communication). These number densities agreed well with those determined from the PRE results. Thus the D-region showed no significant changes over a distance of 120 km.

The ratio of received to transmitted powers for the partial reflections may be expressed as 8 (T. F. unpublished data):

$$\frac{P_{R}(h)}{P_{T}} = \operatorname{constant} \frac{1}{h^{2}} \int_{0}^{\infty} dz_{1} g^{2} \left(\frac{z_{1} - h}{2}\right) |C(z_{1})|^{2}$$

$$\times \exp\left(-4 \int_{0}^{z_{1}} k_{i}(z') dz'\right) \tilde{R}(z_{1}) \tag{1}$$

Here h is the height from which the power $P_{\rm R}(h)$ is received, $g((z_1-h)/2)$ represents the pulse shape, $C(z_1)$ is a known function based on an assumed height variation of electronneutral collision frequency 19 , and k_i is the imaginary part of the wavenumber. The constant is determined by calibration of the radar system, and $\tilde{R}(z_1)$ is the intensity of ionospheric fluctuations with scales corresponding to $\lambda/2$ or $2k_r$, where k_r is the real part of the wavenumber.

The relation between the rocket and partial reflection observations was tested in two ways. First, the amplitudes $A_x(h)$, $A_0(h)$ and ratio $A_x/A_0(h)$ were computed directly from equation (1), using values of $\tilde{R}(z_1)$ determined from the rocket probe for 1-km intervals. In Fig. 2 the results of the computations are compared with the measurements. The agreement, both regarding absolute magnitudes and height variation is very good. The main sources of uncertainty are the possible ionospheric changes over the distance of 120 km between the radar and the rocket, and possible inaccuracies in the absolute calibration of the radar system. The discrepancy between calculated and measured amplitudes for the extraordinary wave above 85 km, could be due to contamination of the measurements by oblique echoes, or to the effects of not correcting for the change in the extraordinary mode group velocity above 85 km.

In the second test equation (1) was solved for the term $\hat{R}(z_1)$, using the measured ratio $P_{\rm R}(h)/P_{\rm T}$. To achieve this, the pulse $g((z_1-h)/2)$ was assumed to be a square wave of width 3 km centred at a height h, and all other terms inside the integral were assumed constant within this height range. In Fig. 1 the computed term $R(z_1)$ representing the intensity of the fluctuations is compared with the rocket measurements. Again the agreement is good, although the computations do not reproduce the detailed structure observed in the rocket. At least part of this discrepancy could be due to the fact that the computed fluctuation intensity is averaged over a scattering volume of width

We conclude that the intensity and height variation of partial reflections measured during the rocket flight are consistent with volume scattering from the ionospheric irregularities measured by the electrostatic probe in the rocket. The power spectra of ion density fluctuations derived from the rocket data, indicate that these fluctuations are caused by turbulence in the neutral air¹⁶. We may therefore conclude that such turbulence is an important source of partial reflections.

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- Gardner, F. F. & Pawsey, J. L. J. atmos terr. Phys. 3, 321-344 (1953), Fejer, J. A. & Vice, R. W. J. atmos terr. Phys. 16, 291 (1959). Gregory, J. B. J. geophys. Res. 66, 429-435 (1961).

- Thrane, E. V., Haug, A., Bjelland, B., Anastassiades, M. & Tsagakis, E. J. atmos. terr. Phys. 30, 135-150 (1968)
- Gallett, R. M. Proc. IRE 43, 1240-1252 (1955).
- Piggott, W. R. & Thrane, E. V. J. atmost terr. Phys. 28, 311-314 (1966).
 Flood, W. A. J. geophys. Res. 73, 5585-5598 (1968).
 Austin, G. L. & Manson, A. H. Radio Sci. 4, 35-40 (1969).
 Belrose, J. S., Burke, M. J., Coyne, T. N. R. & Reed, J. E. J. geophys. Res. 77, 4829-4838.

- Briggs, B. H. & Vincent, R. A. Aust. J. Phys. 26, 805 (1973)
- Rastogi, P. K. & Bowhill, S. A. J. atmos. terr. Phys. 38, 399(1976) Hocking, W. K. J. geophys. Res. 84, 845-851 (1979).
- Vincent, R. A. & Belrose, J. S. J. atmos. terr. Phys. 40, 35-47 (1978). Schlegel, K., Brekke, A. & Haug, A. J. atmos. terr. Phys. 40, 205-213 (1978).
- Thrane, E. V. & Grandal, B. J. atmos. terr. Phys. 43, 179-189 (1981).
- Haug, A., Thrane, E. V., Bjørna, K., Brekke, A. & Holt, O. J. atmos. terr. Phys. 39, 1333-1340 (1977)
- 18. Holt, O., Brekke, A. & Hansen, T. Proc. 5th ESA Symp., ESA SP-152, 387-392 (1980).
- 19. Thrane, E. V. & Piggott, W. R. J. atmos. terr. Phys. 28, 721-737 (1966)

Photovoltaic conversion by macromolecular thionine films

R. Tamilarasan & P. Natarajan*

Department of Chemistry, University of Madras, Tiruchirapalli 620 020, Tamil Nadu, India

On excitation by light, thionine dye oxidizes Fe^{2+} ions in solution and the photoproducts undergo dark reactions to restore the starting materials. The photogalvanic (photovoltaic) potentials generated by this cyclic process can be demonstrated by means of a cell with two platinum electrodes, one illuminated and one in the dark. Light is converted more effectively into electricity in the totally illuminated thin-layer (TITL) cell with platinum and tin oxide electrodes, but the efficiency of the conversion is limited by energy-wasting back reactions in solution and by restriction on the concentration of the dye¹. We now show that these limitations can be overcome if thick films (~10 μ m) of thionine condensed with macromolecules are coated on to inert electrodes.

Recent investigations of photoredox reactions in heterogeneous media and of charge-transfer reactions in micellar systems², organized monolayers³ and chemically modified electrode systems4 are aimed at understanding the reactions involving biological membranes including photosynthetic apparatus. Microheterogeneous environments drastically change the features of chemical reactions occurring in homogeneous media. In a photoinduced electron-transfer reaction, the products readily recombine in a homogeneous medium to regenerate the starting materials whereas charge separation is favoured in the micellar surface or in organized monolayer assemblies⁵. Such systems could be used to store and utilize light energy. Here we describe the photocatalysed redox reactions induced by the thionine covalently bound to poly(N-methylolacrylamide) and related copolymers. Films of polymer-bound dye were coated on to inert electrodes. The electrode reactions are totally different from those of the excited dye in homogeneous solution.

Purified thionine on condensation⁶ with poly(N-methylolacrylamide) produces the polymer-dye complex, poly(acrylamidomethylthionine-co-methylolacrylamide). Uncondensed dve is removed by dialysing the reaction mixture for several days with water. The ratio of thionine units present to the hydroxymethylacrylamide units in the macromolecular chain could be varied depending on the reaction conditions; the monomer/dye (M/D) ratio in the polymer-dye complex is 120 ± 10 . In solution the dye centres present in the polymeric chain do not form aggregates at this M/D ratio. However, with increasing amounts of dye present in the chain, the dye centres aggregate and blueshift the absorption maximum by 15 nm with respect to the free dye7. Evaporation of the solvent in a flash evaporator at 40 °C leaves behind an insoluble dye-polymer complex: presumably some cross-linking occurs. However, the polymer-dye complex remains in solution without forming a precipitate when the solvent is not evaporated. A drop of the polymer-dye solution placed on a glass or metal surface produces a film on evaporation in vacuum at 90 °C. The absorption spectra of the polymer-dye in solution and of the film coated on to an electrode plate are shown in Fig. 1. In the film some of the dye has aggregated as indicated by the absorption spectrum. In all the present experiments a 1-cm² platinum electrode was coated with the polymer-dye. The film was 12-15 µm thick.

The photoelectrochemical cell consisted of a platinum electrode covered with a film of the polymer-thionine complex, and a plain platinum electrode. The electrodes were immersed in a de-aerated solution containing 10^{-2} M ferrous sulphate in 0.5×10^{-2} M sulphuric acid. The dye-coated electrode was irradiated

Table 1 Polymer-dye coated electrodic behaviour on irradiation

Substrate	$rac{\Delta E_{ m oc}}{({ m mV})}$	$I_{\rm sc} \ (\mu { m A})$	$P_{ ext{max}} \ (\mu ext{W})$
\boldsymbol{A}	+37.4*	2.84	0.027
	+31.4†	3.70	0.029
В	+32.0*	1.80	0.014
	+29.6†	2.76	0.020
C	+ 4*	Nepadopte	
	+ 3†		

A, Poly(N-acrylamidomethylthionine-co-methylolacrylamide); B, poly(N-acrylamidomethylthionine-co-methylolacrylamide-co-acrylic acid); C, poly(N-acrylamidomethylthionine-co-methylolacrylamide-co-yinyl pyridine).

by a 300 W tungsten projection lamp. The open-circuit photopotential ($\Delta E_{\rm oc}$), short circuit current ($I_{\rm sc}$) and maximum power output ($P_{\rm max}$) were measured from the plots of photopotential, photocurrent and power output as a function of applied resistance. The results are shown in Table 1 for the thionine bound to poly(N-methylolacrylamide) and its copolymers. For comparison, the power output and the potential generated on irradiation in identical conditions in the TITL⁸ cell with the distance of 1 mm between the SnO₂ and platinum electrode were measured for unbound thionine. The open-circuit potential measured was -74.5 mV and the power output $0.038~\mu W$ for unstirred solutions. The direction of the current is reversed with respect to the plain platinum electrode in the TITL cell compared with that with polymer-dye film coated electrode.

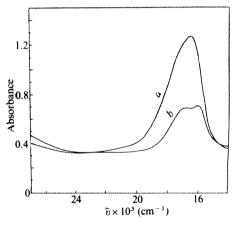


Fig. 1 Absorption spectra of poly(N-acrylamidomethylthionine-co-methylolacrylamide) in aqueous solution (a) and as a film (b).

For the electrode reactions occurring at the dye-coated electrode current-voltage curves were determined in a PAR cyclic voltammeter. The results (Fig. 2) indicate that the thionine-leucothionine couple is more reversible at the dye coated electrode than the Fe²⁺/Fe³⁺ couple. The peak potentials of the polymer-bound thionine/polymer-bound leucothionine are not affected by coating it on to a platinum electrode. However, the cathodic peak potentials of Fe²⁺/Fe³⁺ system is shifted cathodically by 110 mV at the polymer coated electrode compared with the uncoated platinum electrode. Albery et al. have reported that electrolytically coated thionine on a platinum electrode shows a similar behaviour.

That the polymer-dye coated electrode functions as a cathode on irradiation suggests that the electrode reaction reduces Fe³⁺ or some other species. However, the reduction of free Fe³⁺ ions is not a favourable process at this electrode. We propose that the electroactive species is a complex of semithionine with Fe³⁺ ions surrounded by the polymer network along with solvent. As thionine is immobilized in a polymer film the photoproduced semithionine does not undergo the disproportionation reaction

^{*} To whom correspondence should be addressed.

^{*} Stationary condition; † stirring condition.

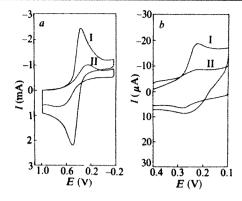


Fig. 2 Cyclic voltammograms versus SCE. Scan rate, 100 mV s⁻¹ electrode area, 1 cm², in 0.25 M [H₂SO₄]. a, Fe²⁺/Fe³⁺ couple at (I) plain platinum and (II) polymer-thionine coated electrodes ² M of Fe²⁺). b, Polymer-bound thionine/leucothionine couple at (I) plain platinum electrode with polymer-dye in solution $(5\times10^{-5}\,\mathrm{M}$ dye); and (II) for the polymer-dye coated electrode without the polymer-dye in solution.

observed in homogeneous medium. The electrode reactions are

Cathode reaction

$$P-TH^{+} + Fe^{2+} + H^{+} \xrightarrow{h\nu} [P-TH_{2}-Fe]^{4+}$$

 $[P-TH_{2}-Fe]^{4+} + e^{-} \longrightarrow P-TH_{2}^{+} + Fe^{2+}$

Anode reaction

$$Fe^{2+} \longrightarrow Fe^{3+} + e^{-}$$

Fe³⁺ ions produced at the anode combine with the semithionine at the cathode to generate the starting materials. The proposed mechanism implies that the oxidation of the semithionine in the complex [P-TH₂-Fe]⁴⁺ present near the electrode seems to be a much slower process than the reduction of the complex at the electrode. Oxidation of P-TH₂⁺ by the Fe³⁺ ions produced at the anode probably occurs by charge migration through interacting thionine-semithionine centres in the polymer network. Charge can be transferred in thin films of dyes and polymers coated on to electrodes and electrons can tunnel through stacks of monolayers 10. However, in quite thick films of polymer a high ohmic resistance is expected to result in negligible amount of current flow. In the present case with the ~15-µm thick polymer film, charge transfer to electrode must be explained either by the migration of charge through the dye centres or the penetration of ferrous ion through the porous polymer network or a combination of both. It has been suggested 11 that the polymer films of 10 µm thickness in suitable solvents exhibit considerable flexibility, which facilitates charge transfer through bound chromophores.

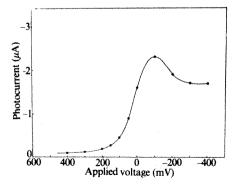


Fig. 3 Photocurrent plotted against applied voltage (versus SCE) across the working electrode-reference electrode for the polymerdye coated electrode.

Decrease in the open-circuit photopotential and the practically negligible amount of current flow observed in the case of poly(acrylamidomethylthionine-co-methylolacrylamide-covinylpyridine) indicates the inhibition of free movement of Fe² ions through the polymer network due to the strong binding between the ferrous ion and pyridine ligand. With the corresponding polyacrylic acid copolymer at pH 2.0, as much of the carboxylic acid is in the protonated form, lack of coordination of acrylate with Fe²⁺ ions does not affect the mobility of these ions through the polymer network.

The polymer-dye coated electrode in the electrochemical cell was illuminated at various applied potentials and the resulting photocurrents are shown in Fig. 3.

The present study has shown that high concentrations of the light absorbers in contact with electrodes facilitate absorption of more light per unit thickness and bring about specific electrode reaction at the electrode. The catalyst, which brings about the light induced electrode reaction, is mounted on the electrode, eliminating unwanted waste-ridden bulk reactions. Comparison of the TITL cells, which show the maximum efficiency for the Fe2+-thionine photogalvanic cell, with the polymer-dye coated electrode system shows that nearly 75% of the power output of the former cell is attained by absorbing light in less than onehundredth of the thickness of the thin layer. As the bulk solution contains no dye, operation of the system becomes simpler. The polymer-dye film is stable for days in the dark and under illumination shows no observable change in its electrochemical behaviour.

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- 1. Albery, W. J., Bowen, W. R., Archer, M. D. & Ferreira, M. I. J. Photochem. 11, 27-37
- Gratzel, M. Isr. J. Chem. 18, 364-368 (1979)
- Kuhn, H. Pure appl. Chem. 61, 341-352 (1979). Wrighton, M. S. Chem. Engng News 29-47 (1979)
- Whitten, D. G. Accts Chem. Res. 13, 83-90 (1980).
- Kamogawa, H., Kato, M. & Sugiyama, H. J. polym. Sci. At 6, 2967-2991 (1968).
- Tamilarasan, R. & Natarajan, P. Ind. J. Chem. 20A, 213-215 (1981).
 Hall, D. E., Wildes, P. D. & Lichtin, N. N. J. electrochem. Soc. 125, 1365-1371 (1978).
- Albery, W. J. et al. Nature 282, 793-797 (1979). Oyama, N. & Anson, F. C. J. electrochem. Soc. 127, 640-647 (1980).
- 11. Kaufmann, F. B. & Engler, E. M. J. Am. chem. Soc. 101, 547-549 (1979).

Viscosity of high-pressure ice VI and evolution and dynamics of Ganymede

J. P. Poirier, C. Sotin & J. Pevronneau

Institut de Physique du Globe, Université Paris VI, 4, Place Jussieu. 75320 Paris Cedex 05, France

Current models1-5 of the evolution of the jovian satellite, Ganymede, assume that it was formed by homogeneous accretion of water ice and silicate particles (about 50 mass%) with chondritic abundance of the radioactive elements U. Th and K. As the satellite reached its final size it would probably have been composed of a mixture of silicate particles and high-pressure phases of ice, from the centre outwards: ice VIII, ice VI, ice II and finally, near the surface, ice I4. Due to radioactive decay, the temperature would rise in the interior. presumably transforming ice VIII into ice VII, and bringing ice VII and VI close to their melting point. We have measured the viscosity of the high-pressure ice VI at room temperature and pressures of 1.1-1.2 GPa in a sapphire anvil cell; fine particles were used to visualize the flow of ice down the radial pressure gradient. The low value we found for the viscosity $(\eta = 10^{14} \text{ P})$ suggests that solid state convection might have taken place during the early evolution of Ganymede, thus preventing melting and differentiation.

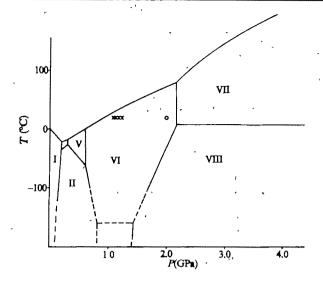


Fig. 1 Phase diagram of ice⁶ showing our experimental conditions (×); and conditions for which the value of viscosity was extrapolated (O).

Parmentier and Head³ have shown that if the viscosity of high-pressure ices near their melting point is lower than 10^{17} P, solid state convection can take place, effectively removing heat and preventing melting and differentiation of the planet. However, if the viscosity is higher than 10^{17} P, melting could eventually occur leading to differentiation and silicates could sink forming a rocky core surrounded by a liquid water mantle and a lithosphere of ice I and II i; if the viscosity of ice I is low enough ($\sim 10^{14}$ P) the lithosphere itself can convect², extract heat from the liquid layer which will freeze in a time of the order of 10^8 yr, and form a mantle composed mostly of ice VI 4 which, in turn, can convect if its viscosity is low enough. More generally, if the viscosity of ice in the interior were as low as 10^{14} P, a steady state body, either differentiated or undifferentiated, would be primarily solid⁵.

Ice VI is easily formed in a diamond anvil cell and observed by optical microscopy⁶. The phase diagram of ice⁷ (Fig. 1) shows that liquid water transforms to ice VI at 0.9 GPa, at 20 °C. For such relatively low pressures, the anvils can be made of sapphire instead of diamond with the result that a gasket with a larger hole can be used, allowing a wider field of observation. The viscosity of the ice can be measured by following the motion of marker particles down the radial pressure gradient in the cell.

A cell of the usual Block-Piermarini type was fitted with anvils machined from a 6-mm diameter single crystalline rod of Al_2O_3 with the c-axis along its length. The anvils were 5 mm

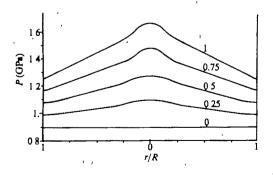


Fig. 2 Hydrostatic pressure in the sapphire anval cell plotted against r/R, where r is the distance from the centre and R the radius of the hole in the gasket. Each curve corresponds to a different load and is labelled in fractions of one turn of the screw.

high and were tapered to an end diameter of 1.3 mm. The gaskets were made of annealed copper foil 0.35 mm thick with a 0.6-mm diameter hole. The hole is filled with tap water containing particles of 2 µm alumina grit and the load is applied, through a lever, by turning a screw (by capillarity, most of the particles stay close to the anvil surfaces); the cell is installed under an optical microscope. At first the gasket is extruded a little inwards, reducing the diameter of the hole and the hydrostatic pressure increases until 0.9 GPa, when ice VI crystals begin to form, and eventually fill the hole. From then on, further increases in the load cause the ice to be extruded outwards and the diameter of the hole to increase slightly. There is a radial pressure gradient from the maximum pressure at the centre to the edge and it can be reasonably assumed that the ice flows fully plastically, having reached its shear strength. In such cases, one can assume that the radial pressure gradient is equilibrated by the shear stresses along the anvil surfaces and that

$$\frac{\delta P(r)}{\delta r} = \frac{2\sigma_{rs}}{h} \tag{1}$$

where P(r) is the hydrostatic pressure at a distance r from the centre, σ_{rz} is the shear stress along the anvil surfaces (in cylindrical coordinates); h is the thickness of the ice disk which can be measured for a given load by focusing the microscope on the

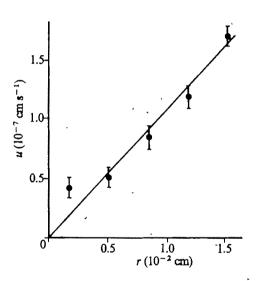


Fig. 3 Plot of the radial velocity (u) of the markers against radius (r).

upper and lower surfaces. P(r) is determined for several values of the load (expressed by N, number of turns of the screw after the ice has formed) by using fixed transition points for alkali halides at room temperature: 0.36 and 1.16 GPa for NH₄F (ref. 9), 0.52 GPa for RbCl (ref. 10) and 1.8 GPa for KBr (ref. 11). Holes in gaskets 150-um thick (the thickness of the gasket when the ice starts to form) are filled with alkali halide powder. As the load is increased the powder first sinters and the transformation appears at the centre of the hole; the transformed phases can be seen as dark circular patches propagating outwards as the load is increased. The pressure at the value of r corresponding to the rim of the dark patch is equal to the transformation pressure. For each of several values of r, it is possible to determine the number of turns of the screw for which the transitions appear; these data are converted into smoothed curves giving P(r) for $\frac{1}{4}, \frac{1}{2}, \frac{3}{4}, 1$ turn of the screw. We assume that the same curves will be approximatively correct for ice VI if we take P(r) to be the pressure over 9 kbar for the same number of turns of the screw after formation of the ice. The pressure distribution in ice VI is shown in Fig. 2.

Table 1 Experimental results at various distances from the centre r

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r/R	r (cm)	$(\operatorname{cm s}^{-1})$	(s^{-1})	P (GPa)	$\frac{\delta P}{\delta r}$ (cgs)	η (P):	$T_{\mathbf{M}} - T^{(r)}$ (K)
0.25 0.5 0.75 1	$4.2 \times 10^{-3} 8.4 \times 10^{-3} 1.3 \times 10^{-2} 1.7 \times 10^{-2}$	4.2×10^{-8} 8.4×10^{-8} 1.3×10^{-7} 1.7×10^{-7}	$3.2 \times 10^{-6} \\ 6.5 \times 10^{-6} \\ 10^{-5} \\ 1.3 \times 10^{-5}$	1.22 1.17 1.12 1.08	$1.4 \times 10^{11} 1.2 \times 10^{11} 1.2 \times 10^{11} 9.5 \times 10^{10}$	$ \begin{array}{c} 1.4 \times 10^{14} \\ 6.1 \times 10^{13} \\ 4 \times 10^{13} \\ 2.4 \times 10^{13} \end{array} $	16 14 12 10

u is the radial velocity of the particles; $\dot{\gamma}$, the shear strain rate; P, the hydrostatic pressure; $\delta P/\delta r$, the pressure gradient; η , the viscosity; $T_{\rm M}-T$ is the distance to the melting point.

The viscosity has been determined for the load corresponding to $\frac{1}{2}$ turn of the screw after formation of the ice. Microphotographs of the field of ice with particles of alumina close to the anvil surfaces were taken 2 and 17 min after the load was applied. Comparison of enlarged photographs shows that the particles at a distance r from the centre of the cell have moved radially by Δr during the time Δt between the two photographs. Their average velocity $u(r) = \Delta r(r)/\Delta t$ is found to be proportional to $r: u(r)/r = 10^{-5} \,\mathrm{s}^{-1}$ (Fig. 3). The markers are passively carried by the outwards creeping ice. As a first approximation, we take $\dot{\gamma} = u(r)/h$ for the viscous shear rate of ice.

 $\dot{\gamma}$ is related to the shear stress by

$$\sigma_{rz}(r) = 2\eta \dot{\gamma}_{rz}(r) \tag{2}$$

Hence, from equations (1) and (2)

$$\eta(r) = \frac{\delta P(r)}{\delta r} \frac{h}{4\dot{\gamma}_{rz}(r)} \tag{3}$$

For $N = \frac{1}{2}$, $h = 1.3 \times 10^{-2}$ cm. The value of $\eta(r)$ can be computed from the data in Table 1 for values of r corresponding to $\frac{1}{4}R$, $\frac{1}{2}R$, $\frac{3}{4}R$, R (R being the radius of the hole). The viscosity of ice VI at temperatures between 10 and 16 °C below the melting point and pressures between 1.08 and 1.22 GPa varies from 2.4×10^{13} to 1.4×10^{14} P. The accuracy of our determinations is thought to be no better than $\pm 20\%$, $(\Delta P/P \approx 5\%, \Delta h/h \approx 1\%, \Delta u/u \approx 10\%,$ $\Delta r/r = 0$ hence $\Delta \eta/\eta = 20\%$; the viscosity found in a repeated experiment differred by 18%) yet the difference is probably significant and the trend is certainly correct indicating an

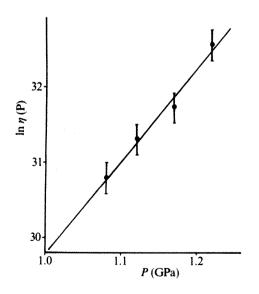


Fig. 4 Plot of the logarithm of the viscosity (η) against pressure (P). The slope of the line gives an activation volume $V^* \simeq$ 30 cm³ mol⁻

increase in viscosity with pressure. The activation volume $V^* =$ $RT(\partial \ln \eta)/\partial P$ can be estimated from plotting $\ln \eta$ against P (Fig. 4), it is found to be approximately $V^* = 30 \text{ cm}^3 \text{ mol}^{-1}$. If we use this value to extrapolate the viscosity at 2 GPa near the ice VI-VII boundary (and 52 °C below the melting point) we find $\eta \simeq 1.7 \times 10^{18} \, \mathrm{P}.$

The assumptions used to determine the viscosity probably lead to overestimating rather than underestimating it; the assumption of fully plastic strain regime (compatible with the fact that the volume variation in the deforming regions has experimentally been found close to zero) leads to an upper limit for the shear stress as does the equilibrium condition (1) for a given load. The annealed copper gasket has been found to be just of the right stiffness to allow formation of ice VI without preventing its creeping outwards (platinum was too soft and Inconel too hard). Finally, we assume in equation (2) that viscous flow takes place under the constant plastic shear stress, which is obviously not correct: some stress relaxation must occur.

Finally, in taking $\dot{\gamma} = u/h$, we made the hidden assumption that the velocity profile across the sample is constant up to the vicinity of the anvils where the particles lie. If we make the more reasonable assumption that the profile u(z) is parabolic with u = 0 at z = h/2 (anvils) and that the particles with the measured velocity u lie at a distance of the anvil surfaces equal to 0.05 h (that is z = 0.45 h), the strain rate is then $\dot{y} = du/dz \approx 20 u/h$, reducing the viscosity by a factor 20.

In these conditions, it is reasonably safe to state that the viscosity of ice VI in most of its domain of existence and within 20 °C of its melting point, is certainly lower than 10¹⁷ P, and may even be lower than 10¹⁴ P. Two important consequences follow:

First, if the accretion of Ganymede was homogeneous, subsolidus convection was possible in the ice VI and most of the planet could have remained undifferentiated.

Second, for reasons not taken into account in the current models (such as rapid accretion rate, tidal dissipation and high initial luminosity of Jupiter), melting and differentiation might occur; in that case the liquid mantle could probably freeze again by lithospheric instability or ice diapirism⁵ and transfer heat by solid state convection.

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- Consolmagno, G. J. & Lewis, J. S. Icarus 34, 280-293 (1978).

- Consolmagno, G. J. & Lewis, J. S. Icarus 34, 280-293 (1978).

 Reynolds, R. T. & Cassen, P. M. Geophys. Res. Lett. 6, 121-124 (1979).

 Parmentier, E. M. & Head, J. W. J. geophys. Res. 84, 6263-6276 (1979).

 Squyres, S. W. Geophys. Res. Lett. 7, 593-596 (1980).

 Parmentier, E. M. & Head, J. W. Proc. 10th Lunar planet. Sci. Conf. 2403-2419 (1979).

 Whatley, L. S. & Van Valkenburg, A. Advances in High Phessure Research Vol. 1 (ed. Bradley, R. S.) Ch. 6 (Academic, New York, 1966).

 Mishima, O. & Endo, S. J. chem. Phys. 73, 2454-2456 (1980).

 Sung, C. M., Goetze, C. & Mao, H. K. Rev. scient. Instrum. 48, 1386-1391 (1977).

 Kuriakose, A. K. & Whelley, E. Lehem. Phys. 49, 2023 (2021) (1969).

- Kuriakose, A. K. & Whalley, E. J. chem. Phys. 40, 2025-2034 (1968)
 Peyronneau, J. & Lacam, A. Rev. Phys. Appl. 13, 107-113 (1978).
 Pistorius, C. W. J. Phys. Solids 26, 1003-1011 (1965).

Ordering of aluminium and silicon in synthetic faujasites

S. Ramdas, J. M. Thomas* & J. Klinowski

Department of Physical Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EP, UK

C. A. Fyfe & J. S. Hartman

Guelph-Waterloo Centre for Graduate Work in Chemistry, University of Guelph, Ontario, Canada N1G 2W1

The distribution of aluminium and silicon atoms on the tetrahedral sites in synthetic faujasites (zeolites X and Y) has been debated for many years. Although the framework structure of the zeolite is known¹, the detailed distribution of Si and Al in the lattice cannot be established by conventional methods, partly because of the very similar scattering powers of silicon and aluminium for X rays and partly because of the difficulties in obtaining crystals of adequate size and perfection. Some inferences have, however, been made on the basis of four indirect approaches: variation of the (cubic) lattice parameter as a function of aluminium content^{2,3}; electrostatic calculations⁴; change of zeolitic acidity on successive extraction of aluminium from the framework⁵⁻⁷; and the kinetics of crystallization⁸. All these approaches have assumed the validity of 'Loewenstein's rule' which forbids Al atoms from occupying neighbouring tetrahedral sites. High-resolution solid-state ²⁹Si NMR (with magic angle spinning) can determine the detailed Si, Al ordering in zeolites⁹⁻¹⁶. NMR coupled with electron diffraction studies has revealed that Loewenstein's rule, generally treated as axiomatic, is broken in Linde $A^{10,13,14}$ and in at least three other zeolites where Si/Al is nominally unity15. In view of its importance as an archetypal zeolite, particularly commercially, we have re-examined the question of framework ordering in faujasite. We propose new ordering schemes and explain discontinuities in the unit cell constant as a function of composition.

The basis of the solid-state NMR method is the fact that ²⁹Si chemical shifts fall into five distinct ranges, depending on whether a given SiO₄⁴⁻ tetrahedron is linked by oxygen bridges to four, three, two, one or no AlO₅⁴⁻ tetrahedra⁷, these ordering modes are denoted Si(4Al), Si(3Al), Si(2Al), Si(1Al) and Si(0Al), respectively (following ref. 7).

We have carried out a detailed NMR examination of synthetic faujasites with Si/Al ratios in the range 1.18-2.42. Spectra were obtained at 20 °C using a Bruker CXP-100 solid-state highresolution spectrometer with a home-made magic-angle-spinning attachment. Cross-polarization and proton decoupling were not used. The conical rotors, ~0.5 ml internal volume, were made from Delrin, an acetal resin and spun at a rate of ~3 kHz. The ²⁹Si spectral line widths at half-height were close to 2 p.p.m. All chemical shifts are given from tetramethylsilane, with high field shifts being negative. Time intervals of 1 s between the radio frequency pulses were allowed, and 2,000-10,000 free induction decays were accumulated per sample. Relative intensities of peaks corresponding to the different Si, Al ordering modes have been measured from the spectra and compared with many speculative distributions of Si and Al generated for each crystal composition. Relative populations of the five types of ordering modes were calculated for each such configuration and compared with experiment. We have given special attention to those configurations which do not involve the creation of a net dipole moment, and those associated with the lowest electrostatic energy. The latter has been approximately calculated for a lattice model with fixed point charges on Al sites.

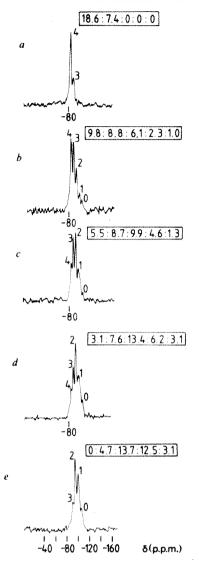


Fig. 1 High-resolution solid-state ²⁹Si NMR spectra of synthetic faujasites. Chemical shifts are given in p.p.m. from tetramethylsilane. Relative populations of the five possible ordering modes, Si(4Al): Si(3Al): Si(2Al): Si(1Al): Si(0Al), estimated from the spectra, are given in the right-hand corner of each box. Peaks corresponding to the respective ordering modes are marked as 4, 3, 2, 1 and 0. The Si/Al ratio for each crystal composition studied, which sometimes slightly deviates from the ideal, is given in the left-hand corner of each box. a, Si/Al = 1.19; b, Si/Al = 1.35; c, Si/Al = 1.67; d, Si/Al = 2.00; e, Si/Al = 2.45.

Unless restrictions are imposed on the size and symmetry of the unit cell, an infinite number of ordered structures is possible. We have considered a basic unit composed of two sodalite cages linked through a hexagonal prism with a centre of symmetry in the middle. Such basic units are, for heuristic purposes, placed at the points of a face-centred cubic faujasite lattice. Each unit contains 24 pairs of tetrahedral sites. The four-fold multiplicity of the F-lattice allows only certain numbers of Al atoms per unit cell of 192 tetrahedral atoms: 96, 88, 80, 72, 64, 56, and so on, equivalent to Si/Al ratios of 1.00, 1.18, 1.40, 1.67, 2.00, 2.42, and so on, respectively. In faujasite there are two distinct types of six-membered rings in each sodalite cage: four 'type I' rings involved in linkages with neighbouring cages, and four 'type II' (non-linking) rings which face the supercage.

The NMR spectra, given in Fig. 1, show that as the Si/Al ratio approaches unity, the ordering converges towards Si(4Al), that is, towards strict alternation of Si and Al in the lattice. This is in contrast to the situation which prevails in Linde A, where the ordering is Si(3Al) (refs 9, 12 and 13), although the zeolite is also built of sodalite cages. The fact that Loewenstein's rule is broken

^{*} To whom correspondence should be addressed.

in Linde A but obeyed in faujasite will be discussed elsewhere. It would seem reasonable to expect that Al-O-Al linkages would be less likely in less aluminous faujasites, that is, those with higher Si/Al ratios. The perfect ordering in a hypothetical faujasite with Si/Al = 1.00 is given as C1 in Fig.2. All sixmembered rings are of the meta type with respect to both Si and Al. Following Dempsey et al.², by now replacing any pair of Al atoms which are related through the centre of symmetry, by Si atoms, thus increasing Si/Al ratio to 1.18, we arrive at configuration C2 with the relative population of the ordering modes of 16:8:0:0:2 which, although the smallest peak cannot be identified, is in good agreement with the intensities in the NMR spectrum (Fig. 1a). Detailed examination revealed that no other speculative configuration can explain NMR results for this composition. The NMR spectra rule out complete disorder.

The transition from Si/Al = 1.18 to Si/Al = 1.40, which is equivalent to altering the Si: Al content per sodalite cage from 13:11 to 14:10, leads to four possible population ratios of ordering modes: 12:8:4:0:4; 10:12:2:0:4; 14:4:6:0:4 (neither shown in Fig. 2) and 8:16:0:0:4 (C3 in Fig. 2), none of which is compatible with the NMR results. However, if we abandon the centre of symmetry requirement and thus the condition of zero dipole moment, we arrive at configurations C4 and C5. C4 agrees very well with experiment (G. Engelhardt, E. Lippmaa and M. Mägi have independently reached the same conclusion) however, C5 (which breaks Loewenstein's rule) does not agree so well with experiment. Approximate calculations of electrostatic energy indicate that either C4 or C5 are acceptable from that point of view. However, the Si (4Al) peak in measured spectra may be higher than Si(3Al) peak, only because the zeolite composition deviates from the ideal Si/Al = 1.40 (see the proportion of these peaks in C2). This means that C5 cannot be unequivocally eliminated.

Closer examination of Fig. 2 reveals that para occupancy of type II rings begins at Si/Al = 1.40 and not at Si/Al = 2.00, as postulated by Dempsey et al.². We suggest that this change in mode of occupancy is responsible for the discontinuity in the plot of lattice parameter, occurring at the composition equivalent to 80 Al atoms per unit cell (that is at Si/Al = 1.40). Note that for any composition para occupancy of a type I ring must lead to the violation of Loewenstein's rule if the centre of symmetry is preserved. There is no violation, however, if para occupancy occurs only in type II rings. For Si/Al = 1.40 it is not possible to limit the para occupancy only to type II sites; some para rings of type I have to be admitted if agreement with NMR data is to be achieved. The resultant loss of centrosymmetry gives rise to a net dipole moment. This may, however, be an acceptable consequence, especially if one considers that small adjustments of cationic positions can compensate that dipole moment and hence retain overall non-polarity.

At Si/Al = 1.67 (a 13:9 ratio, that is two Al atoms are eliminated from C5, one of which was involved in an Al-O-Al linkage) configuration C7 is produced with para occupancy in some type I and II rings. C7 is in good agreement with NMR results. However, an alternative configuration, C6, in which para occupancy is restricted to type II rings, also fits the experimental data. Only very accurate measurements, not feasible at present, could distinguish between the two possibilities

For Si/Al = 2.00 NMR indicates that all type II rings must be of para occupancy (configuration C8). This conclusion agrees with the distribution of Si and Al postulated by Dempsey et al.² and evidently accounts for another discontinuity in the cell parameter plot, observed at Si/Al = 200. Intensities calculated from C8 agree well with experiment. So do those derived from C9 for Si/Al = 2.42, although good agreement with NMR could be achieved in both cases also by abandoning the μ = 0 condition. In other words, for high Si/Al ratios (from 2.00 upwards), ²⁹Si NMR is a progressively less sensitive tool for the choice of Si, Al ordering. It is, however, invaluable in highly aluminous zeolites, where the correct distribution of tetrahedral atoms can be easily identified.

One further discontinuity in the lattice parameter versus composition plot has been observed², at $Si/Al = \sim 2.80$. Although we have no experimental proof, we can speculate, using analogies with more aluminous phases, that the break in the curve is due to the onset of single occupancy of six-membered rings. A configuration in which all six-membered rings are occupied by only one Al atom corresponds to Si/Al = 5.00 and is given in C12. Introduction of partial double occupancy, to reach Si/Al = 3.00 (C10) and Si/Al = 3.80 (C11) involving the necessary existence of meta and para rings, as well as singly-occupied rings, is most likely.

The most reasonable configurations from the many possible are marked with circles in Fig. 2. Unlike any other known and readily applicable technique, ²⁹Si NMR directly probes the immediate environment of the Si atom.

While some of our conclusions may have to be modified when even higher-resolution, higher-sensitivity, solid-state ²⁹Si NMR becomes available, we have already arrived at the progression of

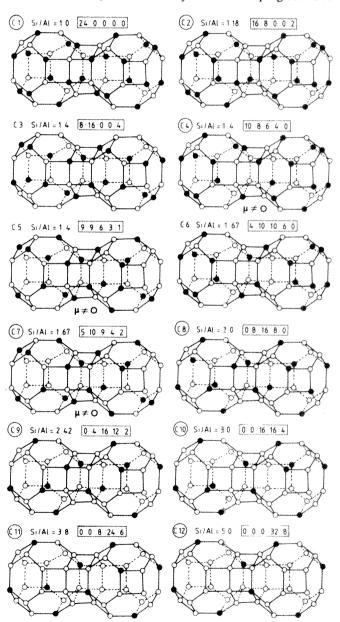
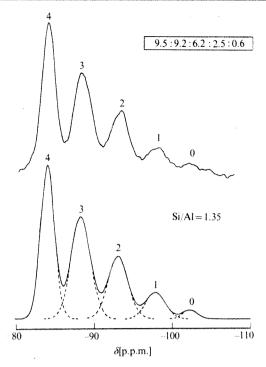


Fig. 2 Possible distributions of Al (●) and Si (○) in the basic structural unit of faujasites with different compositions (given in the left-hand corner of each box). Calculated relative populations of the five possible ordering modes (see Fig. 1) for each diagram are given in the right-hand corner of each box. $\mu \neq 0$ denotes a net dipole moment of the basic structural unit. The most probable configuration for each composition is circled. Configuration C5 violates Loewenstein's rule.



Observed (at 79.6 MHz) a, and simulated b high-resolution ²⁹Si NMR spectra for a Si/Al ratio of 1.35.

ordering schemes that give rise to the following unique configurations of Si/Al ratios of 1.0, 1.18, 2.0, 2.42 and 5.0: (1) at Si/Al = 1.0 and 1.18, all meta configurations for sixmembered rings of both type I and type II; (2) at Si/Al = 2.0and 2.42, para occupancies are limited to only the type II rings; and (3) at Si/Al = 5.0, both type I and type II rings have only single occupancies.

In the intermediate configuration, Si/Al = 1.4, para occupancy occurs in some of the type I and type II rings, with consequential generation of a dipole moment. For Si/AI = 1.67, an extension of the ordering schemes applicable for both 1.4 and 2.0 is possible.

Since this work was completed, we have recorded spectra at much higher resolution, and the conclusions drawn from this recent development vindicate our earlier ones. More precise values of the relative populations are now available, and one of the newly recorded spectra, together with its computer-stimulated analogue (based on Gaussian peak shapes) is shown in Fig. 3.

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Note added in proof: Since this work was submitted we have heard from Engelhardt and Lippmaa that they have obtained essentially the same results and reached the same conclusions¹

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- Olson, D. H. J. phys. Chem. 74, 2758 (1970).
 Dempsey, E., Kühl, G. H. & Olson, D. H. J. phys. Chem. 73, 387 (1969).
 Breck, D. W. & Flanigen, E. M. in Molecular Sieves, 47 (Society of Chemical Industry. London, 1968).
- Dempsey, E. in Molecular Sieves, 293 (Society of Chemical Industry, London, 1968). Dempsey, E. J. Catal. 39, 155 (1975); 33, 497 (1974).
- Beaumont, R. & Barthomeuf, D. J. Catal. 27, 45 (1972). Olson, D. H. & Dempsey, E. J. Catal. 13, 221 (1969).
- Cason, D. H. & Lechert, H. J. phys. Chem. 80, 1291 (1976). Kacirek, H. & Lechert, H. J. phys. Chem. 80, 1291 (1976). Lippmaa, E., Mägi, M., Samoson, A., Englehardt, G. & Grimmer, A.-R. J. Am. chem. Soc.
- 102, 4889 (1980). 10. Engelhardt, G., Kunath, D., Samoson, A., Tarmak, M. & Mägi, M. Workshop on Adsorption
- of Hydrocarbons in Zeolites, Berlin-Adlershof (1979).

 11. Lodge, E. A., Bursill, L. A. & Thomas, J. M. JCS Chem. Commun. 856 (1980)
- 12. Thomas, J. M., Bursill, L. A., Lodge, E. A., Cheetham, A. K. & Fyfe, C. A. JCS Chem. Commun. 276 (1981).
- Bursill, L. A., Lodge, E. A., Thomas, J. M. & Cheetham, A. K. J. phys. Chem. (in the press).
 Bursill, L. A., Lodge, E. A. & Thomas, J. M. Nature 286, 111 (1980).
 Klinowski, J., Thomas, J. M., Fyfe, C. A. & Hartman, J. S. (submitted).

- Klinowski, I. et al. JCS Chem. Commun. (in the press)
- 17. Engelhardt, G. & Lippmaa, E. Z. anorg. allg. Chem. (in the press).

Geophysical evidence for non-newtonian gravity

F. D. Stacey & G. J. Tuck

Physics Department, University of Queensland, Brisbane 4067, Australia

Measurements of the variation of gravity with depth in mines and boreholes permit the densities of intervening rock strata to be inferred. In the few cases in which reliable absolute values of density have been independently determined, the calculations can be used to check the value of the newtonian gravitational constant, G. Such large-scale measurements of G are important because the validity of the inverse square law of gravity at short range is being questioned. We have made such a series of measurements and have found four other data sets in the literature that suffice for the estimation of G. We also report here a statistical analysis of 1,100 km² of overlapping sea floor and sea surface gravity data from the Gulf of Mexico (made available by Exxon). All these estimates of G give values that are higher than the conventional, laboratory-determined one. While the possibilities of systematic errors in these data sets preclude a definite conclusion that Newton's law of gravity fails at short range, the strong circumstantial evidence suggests that well controlled large-scale experiments on the inverse square law are urgently required.

There are theoretical reasons for postulating a non-newtonian gravitational effect^{1,2}, and Long's³ claim that, contrary to earlier, less precise measurements⁴, a departure from the inverse square law is observable on a laboratory scale has stimulated novel experiments on gravity⁵⁻⁷. Essentially evidence is sought that, in terms of Newton's law for the attractive force between masses m_1 , m_2 at separation r

$$F = Gm_1m_2/r^2$$

the factor G is not strictly a constant but varies with r. If it exists, the variation is almost certainly small and at astronomical distances it vanishes altogether, but because we have no way of determining the astronomical scale value of G, it is important to extend laboratory type observations to as large a scale as possible.

The earliest estimates of G were geophysical, relying on the attractive effects of mountains of assumed known masses8. With the acceptance of the superiority of Cavendish-type laboratory experiments the geophysical attempts became of historical interest only, but now that a scale dependence of G is being sought, geophysical measurements acquire a new significance. They permit the measurement of the gravitational constant over distances of 10-5,000 m, greatly extending the normal laboratory range of 0.05-1 m. Of particular interest are measurements of the vertical gravity gradient in mines, boreholes and at depth within the sea. Airy first used this method in a coal mine in Durham and it was later pursued more successfully in Czechoslovakia by Von Sterneck¹⁰. The basic idea is that at depth within the Earth the attracting mass is less than that of the whole Earth by the amount of the shell outside the point of measurement. Domzalski¹¹ emphasized instead the potential usefulness of underground gravity surveying as an exploration tool and in the past 30 yr there have been numerous reports of gravity measurements in mines and boreholes, pursuing this idea, but not referring to the possibility of estimating G. The values obtained from those that enable G to be calculated $^{12-15}$ are compared in Table 1 with our own observations in a mine in North-west Queensland, undertaken specifically to determine G, and a new analysis of marine gravity surveys. These seem to be the only reports that provide sufficient information on independent measurements of rock densities, as well as the

Table 1 Values of G from modern geophysical data

	Type of		Depth range		
Data source	measurement	Gravity data	(m)	Density data	$(G \pm \sigma)(10^{-11} \text{ m}^3 \text{ kg}^{-1} \text{ s}^{-2}$
Ref. 12	Mine	21	96-587	~400	6.795 ± 0.021
Ref. 13	Mine	31	57.3-684.8	47	6.7390 ± 0.0025
		11	57.3-208.5		6.724 ± 0.014
		10	223.1-388.9		6.726 ± 0.012
		10	418.2-684.8		6.746 ± 0.013
		31	57.3-684.8		$*6.7427 \pm 0.0024$
		35	0-684.8		6.7334 ± 0.0037
Ref. 7	Mine	8	0-948	565	6.712 ± 0.037
Ref. 14	Borehole	3	3,712-3,962	16	6.81 ± 0.07
Ref. 15	Mine	7	251-590	53	6.705 ± 0.060
Exxon Exploration Department	Marine surveys	703	113-687		6.797 ± 0.016

^{*}Result obtained with an assumed deep mass anomaly biasing the gravity profile.

variation of gravity with depth, to permit G to be calculated with formal standard deviations that indicate the scatter of the data. The method of analysis follows closely that used in ref. 7. The values in Table 1 should be compared with the conventional laboratory-determined value of $G = (6.672 \pm 0.004) \times$ $10^{-11} \,\mathrm{m}^3 \,\mathrm{kg}^{-1} \,\mathrm{s}^{-2}$ (ref. 16).

The suspicion that the in situ densities of rock strata are systematically underestimated in core and hand samples makes measurements at sea most important. We have been given access to an extensive set of overlapping sea floor and sea surface gravity data from the Gulf of Mexico (Exxon). The surface and sea floor data were obtained independently at different times. but tied to common sea shore reference stations, and both had been free air and Bouguer-corrected to sea level with the assumption that the sea was filled in with sediment. We have tabulated 703 pairs of sea floor values, with corresponding sea surface values and depth, uniformly spaced over an approximately square area of 1,100 km². We are interested in the difference between the surface and sea floor values g(0) and g(z)as a function of water depth, z. The combined effect of the numerical values used in the adjustments is to subtract 0.068223 mGal ft⁻¹ from [g(z)-g(0)]. Values of $\Delta g =$ [g(z)-g(0)-0.068223 z(ft)] are plotted as a function of z in Fig. 1. If the adjustments were perfect, Δg would average to zero for all values of z, so we are interested in the trend apparent in these rather scattered values. The statistical properties are: n = 703; $\sum_{1}^{n} z = 857782.1$ ft; $\sum_{1}^{n} \Delta g = -802.5$ mGal; $\sum_{1}^{n} z^{2} = 1203853183$ ft²; $\sum_{1}^{n} (\Delta g)^{2} = 2744.7754$ mGal²; $\sum_{1}^{n} (z\Delta g) = -1266254.728$ mGal ft; $\sum_{1}^{n} (\Delta g/z) = -554.61526$ mGal ft⁻¹; $\sum_{1}^{n} (\Delta g/z)^{2} = 2303.2955$ mGal² ft⁻².

We initially having the detection of the linear regression through

these data to obtain the departure of dg/dz from the reference

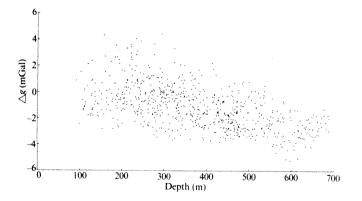


Fig. 1 Gravity differences between overlapping surveys in the Gulf of Mexico. Surface values are subtracted from bottom values after both have been adjusted to correspond to the surface of a sea filled with sediment. If these adjustments were perfect the plotted gravity differences would be zero at all depths, apart from effects of sub-surface features. The scatter of data is due at least partly to sub-surface gravity anomalies.

value (0.068223 mGal ft⁻¹), but a correlation with sea floor topography was noticed. Data from local high points appear systematically above the general trend, although the tendency for data from hollows to give below-average Δg is not as noticeable. The effect may be due to imperfection of the correction for sea floor topography, which was carried out as part of the Bouguer adjustment of the data, but could also arise from a correlation with topography of gravity anomalies from subbottom features; in either case it probably gives an appreciable contribution to the gradient of Δg against z. However, the mean value of $\Delta g/z$ is unaffected by topography; positive and negative contributions to $\Delta g/z$ from underlying inhomogeneities average to zero if a large enough area is properly sampled. Using this average, adding the reference gradient and converting to SI units we obtain the result

$$\frac{dg}{dz} = (2.2124 \pm 0.0020) \times 10^{-6} \quad s^{-2}$$

Using equation (6) in ref. 7, the theoretical gradient is, with numerical values of the geometrical parameters of the Earth

$$\frac{dg}{dz} = 3.089539 \times 10^{-6} - 12.56616G\bar{\rho} \quad s^{-2}$$

and taking the mean value of the seawater density to be $\bar{\rho}$ = $1,027 \text{ kg m}^{-3}$, the observed gradient gives the value of G listed as the last item in Table 1. Alternatively, if we attempt to reduce the error resulting from the greater uncertainties of the $\Delta g/z$ values for shallow data by taking the linear regression constrained through the origin, then the $d(\Delta g)/dz =$ $-(0.526 \pm 0.038) \times 10^{-3}$ mGal ft⁻¹ and the resulting estimate of G is $(6.730 \pm 0.010) \times 10^{-11}$ m³ kg⁻¹ s⁻².

There are possibilities of systematic errors, not represented by the quoted standard deviations, in all of these estimates. In the case of the marine data we rely on the accuracy of reference baseline ties of the two surveys and there is now no effective way of checking these. In the mine and borehole measurements there is a possibility of a systematic discrepancy between in situ and laboratory densities, although this is certainly slight in the case of our own data⁷. We have also considered the possibility of a bias arising from deep anomalous masses. Neither direct measurements⁷ of the free air gradient nor the evidence of a surface gravity survey give evidence of a significant bias. There are sufficient data points in McCulloh's tabulations to provide an internal check and these data are of particular interest because of the small standard deviation that they give. Of the 35 gravity values, we ignored the top 4 which were in the weathered surface layer where densities are unreliable. In Table 1 we have also treated three subsets of 11, 10, 10 of the remaining values. We have also considered the bias of a deep mass anomaly by least square fitting the data with the postulate of an anomalous mass of arbitrary size and depth, without materially affecting the estimate of G.

The evidence of a high value of G is not conclusive but is strongly circumstantial-better controlled experimental data are needed and two new experiments are planned. One is to

measure gravity profiles to a depth of about 4 km in topographically and gravitationally flat areas of the ocean 17 other involves a balance comparison of the weights of 10-kg masses suspended in evacuated tubes at different depths in a lake which will undergo rapid level changes during operation of a hydroelectric pumped storage facility and will offer the possibility of the most precise measurement of G so far.

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- 1. Fuiii. Y. Nature phys. Sci. 234, 5 (1971).
- Long, D. Nuovo Cimen. **55B**, 252 (1980). Long, D. R. Nature **260**, 417 (1976).

- Mackenzie, A. S. *Phys. Rev.* 2, 321 (1895). Hirakawa, H., Tsubono, K. & Oide, K. *Nature* 283, 184 (1980).
- Spero, R., Hoskins, J. K., Newman, R., Pellam, J. & Schultz, J. Phys. Rev. Lett. 44, 1645
- 7. Stacey, F. D., Tuck, G. J., Holding, S. C., Maher, A. R. & Morris, D. Phys. Rev. D23, 1683
- Bullen, K. E. *The Earth's Density* (Chapman and Hall, London, 1975). Airy, G. B. *Phil. Trans. R. Soc.* 146, 297, 343 (1856).
- Von Sterneck, R. in The Mean Density of the Earth (ed. Poynting, J. H.) (Charles Griffin, London, 1894).
- Domzalski, W. Geophys. Prospect. 3, 212 (1955).
 Whetton, J. T., Myers, J. O. & Smith R. Geophys. Prospect. 5, 20 (1957).
- 13. McCulloh, T. H. Geophysics 30, 1108 (1965).
 14. Hinze, W. J., Bradley, J. W. & Brown, A. R. J. geophys. Res. 83, 5864 (1978).
- Hussain, A., Walach, G. & Weber, F. Geophys. Prospect. (in the press) Cohen, E. R. & Taylor, B. N. J. phys. Chem. Ref. Data 2, 663 (1973).
- Stacey, F. D. Geophys. Res. Lett. 5, 377 (1978)

Structure of the Earth's inner core

David R. Fearn* & David E. Loper

Geophysical Fluid Dynamics Institute, Florida State University, Tallahassee, Florida 32306, USA

Paul H. Roberts

School of Mathematics, The University, Newcastle upon Tyne NE1 7RU, UK

Jacobs proposed that the Earth's inner core is growing through the freezing of outer-core material as the Earth gradually cools². Recent studies have shown that compositional effects associated with this freezing process can release energy at a rate sufficient to power the geodynamo^{3,4} and may be crucial in determining the dynamic state of the outer core⁵. Here we investigate the effects of composition on the freezing process itself, drawing on metallurgical experience, and speculate on the structure and state of the inner core. We propose that the interface separating the inner and outer core is dendritic and argue that the region in which freezing takes place may extend throughout the entire inner core. Consequently the compositionally driven convective motions which stir the outer core and sustain the geodynamo also occur within the interdendritic spaces of the inner core. The seismic evidence which corroborates this proposal is briefly reviewed.

The material composing the Earth's core has often been treated as being pure iron but it is in fact an "uncertain mixture of all the elements"6. The freezing of a mixture can be very different in nature from the freezing of a pure substance so it is most important that the effects of composition be included in any study of the evolution of the Earth's core. In particular there are two properties which must be modelled. First the temperature (the liquidus temperature $T_{\rm L}$) below which material freezes out of a fluid with a certain composition is a function of that composition. Second, in general, the composition of the solid which freezes is different from that of the fluid from which it has frozen. As a first step towards investigating the effects of composition we shall study the simplest possible system which incorporates these two properties; a binary alloy. We shall assume that as freezing proceeds, one component of the binary mixture goes to form the solid while the other is expelled into the remaining fluid. We shall use ξ to denote the mass fraction of the latter (so by definition $\xi = 0$ in the solid). As already mentioned, the liquidus temperature is a function of composition and we shall use ξ_e to denote that composition ξ for which T_1 takes its minimum T_e . In applying the results of studies of binary alloys to the Earth's core, it is very likely that ξ represents the less dense constituents and the solid which freezes is richer in the denser constituents.

A binary mixture in a single liquid phase has, by Gibbs' phase rule, three independent thermodynamic variables. When a solid phase is present in equilibrium with the adjacent liquid, a constraint is placed on the variables reducing the number of independent variables to two. Usually the constraint is expressed as the liquidus condition $T = T_L(p, \xi)$ where T is the temperature of the liquid and p is the pressure. If

$$T < T_1(p, \xi) \tag{1}$$

the liquid is said to be supercooled and is unstable to the growth of solid, usually in the form of dendrites extending from a boundary. In particular if this condition is caused by a spatial gradient of composition normal to a freezing interface, the liquid is said to be constitutionally supercooled. To determine whether equation (1) is satisfied near the inner-core boundary of the Earth, let us assume the boundary to be spherical and T = $T_1(p, \xi)$ on the boundary. Expanding equation (1) as a Taylor series in powers of $p-p_0$, where p_0 is the pressure at the boundary, and keeping only the linear terms allows equation (1) to be rewritten as

$$(T/L)(\delta - \bar{\mu}\xi \, d\xi/dp) < dT/dp \tag{2}$$

evaluated at the boundary, where L is the latent heat, δ is the specific-volume change on melting, $\bar{\mu}$ is the energy of mixing⁹, and

$$\partial T_{\rm L}/\partial p \equiv T\delta/L, \qquad \partial T_{\rm L}/\partial \xi \equiv -T\bar{\mu}\xi/L$$

Because fluid motions are inhibited at the rigid freezing boundary, the fluxes $\xi \dot{m}$ of light material and $L\dot{m}$ of heat, generated by the freezing of material at a mass rate m per unit area, must be removed from the interface by diffusion. In addition to the usual flux down the gradient, there is an upward barodiffusive flux of light material, giving 9.10

$$\xi \dot{m} = \rho^2 Dg[(\bar{\delta}/\bar{\mu}) + d\xi/dp]$$
 (3)

$$L\dot{m} = k\rho g \, dT/dp \tag{4}$$

where ρ is the fluid density, D the material diffusion coefficient, g the acceleration due to gravity, δ the specific-volume change with composition and k the thermal conductivity. In writing equations (3) and (4) we have assumed a hydrostatic balance to replace radial derivatives by derivatives with respect to pressure. Using equations (3) and (4) to eliminate $d\xi/dp$ and dT/dp from equation (2) yields

$$\dot{m} > \dot{m}_c = (\delta + \xi \bar{\delta}) \rho^2 g D / \bar{\mu} \xi^2$$
 (5)

In writing equation (5) a thermal term, which can be shown to be negligible, has been neglected. This is the condition for constitutional supercooling to occur in the liquid outer core immediately above a spherical inner-core boundary.

Using parameter estimates from ref. 11 ($\rho = 1.2 \times 10^4 \text{ kg m}^{-3}$, $D = 3 \times 10^{-9} \,\mathrm{m}^2 \,\mathrm{s}^{-1}$ $\bar{\mu} = 4.4 \times 10^7 \, \bar{\mathrm{J}} \, \mathrm{kg}^{-1}$ $g = 4.3 \text{ m s}^{-2}$ $\rho \bar{\delta} = 1.1$, $\rho \delta = 0.016$, $\xi = 0.05$), the critical growth rate is $\dot{m}_c = 10^{-10} \,\mathrm{kg} \,\mathrm{m}^{-2} \,\mathrm{s}^{-1}$. On the other hand, assuming the solid inner core formed 3,000 Myr ago and grew at a constant rate since then, we have $\dot{m} = 5 \times 10^{-8} \text{ kg m}^{-2} \text{ s}^{-1}$. By these estimates, the inner core is growing at a rate roughly 500 times faster than the critical value for the occurrence of constitutional supercooling. Even allowing for a considerable variation in the parameter estimates, it appears that if the inner-core boundary were

^{*} Present address: Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Silver Street, Cambridge CB3 9EW, UK

spherical, the fluid immediately above it would be constitutionally supercooled. With the above parameter estimates, the supercooling increases with distance from the boundary at a rate of 0.8 K m⁻¹. It is extremely doubtful that such a strongly supercooled fluid could persist for long in the vicinity of a solid crystalline interface. Studies by metallurgists⁷ of the freezing of binary alloys have shown that for growth rates satisfying equation (5), the system reduces the local rate of freezing \dot{m} by increasing the surface area over which freezing occurs, most commonly by forming dendrites extending into the fluid.

The process of freezing and dendritic growth has been described by Copley, et al. 12 who studied the evolution of a solution of ammonium chloride in water as it was cooled from below. This solution was studied because it is transparent, allowing the freezing process to be observed directly, and because it has a low entropy of fusion, thus solidifying, as would a metallic solution¹³⁻¹⁵. The cooling causes crystals of ammonium chloride to freeze onto a dendritic interface at the bottom of the solution. The interdendritic liquid is enriched in water and hence is lighter than the overlying solution. This unstable layering drives convective motions despite the stabilizing temperature gradient. The buoyant plumes of lighter fluid erode the dendrites and create isolated chimneys free of solid.

In a quasi-steady state, the cooling system studied by Copley et al.12 may be divided into three regions: a lower region completely solid, an upper region completely liquid and a middle region, the 'mushy zone', in which both solid (dendrites) and liquid are present. The compositionally driven convective motions circulate throughout the upper two regions. The top of the mushy zone is the level at which $T = T_L(p, \xi_0)$ where ξ_0 is the mass fraction of the less dense constituent (water) in the wellmixed upper region, while the bottom is the level at which $T = T_{\rm L}(p, \xi_{\rm e}).$

The mushy zone may be thought of as a layer which matches the composition ξ_0 of the upper region to the lowest-meltingpoint composition ξ_e . The thickness of the zone thus depends on the difference $\xi_e - \xi_o$. In the laboratory the dependence of the liquidus temperature on pressure is unimportant and the imposed temperature gradient is sufficiently large that the mushy zone is only a few centimetres deep. In the Earth, pressure effects are important and the temperature gradient is much smaller, causing the mushy zone to be much thicker. To estimate its thickness, the liquidus relation may be rewritten as

$$g\rho\delta dr = -\bar{\mu}\xi d\xi - (L/T) dT$$
 (6)

Within the mushy zone, dT/dr < 0 and a lower bound on its thickness can be found by setting dT = 0 in equation (6). Integration yields

$$g_{o}\rho\delta(r_{o}^{2}-r^{2})/r_{o} > \bar{\mu}(\xi^{2}-\xi_{o}^{2})$$
 (7)

where $g = g_o r/r_o$, subscript o denotes conditions at the innercore boundary (at the top of the mushy zone) and we have assumed $\bar{\mu}$, ρ , δ , r_o and g_o are constant. We may use this inequality to calculate the greatest compositional difference which can be accommodated by a mushy inner core. Setting r = 0, $\xi = \xi_c$, $r_o = 1.2 \times 10^6$ m and using the parameter estimates given previously, equation (7) predicts that $\xi_e - \xi_o < 0.016$. This figure is, of course, very uncertain. If we had chosen a larger value of ξ_0 , say 0.15, then the allowable difference would have been smaller: $\xi_e - \xi_o < 0.006$. An independent bound on the compositional difference sustained by a mushy inner core can be found using Fig. 5 of ref. 16. With the pressure difference across the outer core known¹⁷ to be 1.93×10^{11} N m⁻², the liquidus gradient may be estimated as $(d\xi/dp)_T \le 10^{-13}$ m² N⁻¹. With the pressure difference across the inner core being17 $10^{10} \,\mathrm{N} \,\mathrm{m}^{-2}$, it follows that $\xi_e - \xi_o \le 0.003$.

Whether the composition of the core satisfies such an inequality is not known. The point of this calculation is to show that the thickness of the mushy zone is strongly dependent on the difference in composition between the outer core and the corresponding liquid with the lowest melting point. In fact, unless the two compositions are very nearly equal, the mushy

zone will be very thick, possibly extending to the centre of the

When the idea of compositionally driven convection in the core was first introduced, it was assumed that the resulting flow patterns would be quite similar to those found in thermally driven convection. With the model of a dendritic inner-core boundary, that assumption is not valid. As the core gradually cools, compositionally buoyant fluid is produced within the inner core. This fluid rises upwards into the outer core as thin plumes and is replaced by a general downward motion of heavier fluid. An important feature of this flow pattern, resulting from the fact that diffusion of material is very slow, is that all streamlines must pass through the inner core. Models of fluid flow within the outer core, such as those constructed for kinematic dynamo problems, should have this property.

There may be some seismic evidence which corroborates the view that the inner core is in a partially molten state. Specifically, several authors 18-20 have suggested that the attentuation of seismic waves in the inner core may be due to the presence of fluid inclusions. It has also been demonstrated experimentally²¹ that the anelasticity of a simple binary system (NaCl-H₂O) is much larger if partially molten than if completely solid. Several attempts 19,20 have been made to demonstrate that thermal diffusive effects in a partially molten inner core can account for the observed anelasticity, but preliminary calculations show that compositional effects are likely to dominate thermal effects; whether these can account for the observed anelasticity remains an open question.

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- Jacobs, J. A. Nature 172, 297 (1953).
 Stacey, F. D. Phys. Earth planet. Inter. 22, 89-96 (1980).
 Gubbins, D. J. Geophys. 43, 453-464 (1977).
 Loper, D. E. Geophys. J. R. astr. Soc., 54, 389-404 (1978).
 Fearn, D. R. & Loper, D. E. Nature 289, 393-394 (1981).
 Birch, F. J. geophys. Res. 57, 227-286 (1952).
 Chalmers B. Principles of Solidification (Wiley, New York).
- Chalmers, B. Principles of Solidification (Wiley, New York, 1964)
- Fearn, D. R. & Loper, D. E. in The Fluid Mechanics of Astrophysics and Geophysics Vol. 2
- (ed. Soward, A. M.) (Gordon and Breach, London, in the press). Loper, D. E. & Roberts, P. H. Geophys. Astrophys. Fluid Dyn. 9, 289-321 (1978)
- Loper, D. E. & Roberts, P. H. Geophys. Astrophys. Fluid Dyn. 16, 83-127 (1980).
 Loper, D. E. & Roberts, P. H. Phys. Earth planet. Inter. 24, 302-307 (1981).
- 12. Copley, S. M., Giamei, A. F., Johnson, S. M. & Hornbecker, M. F. Met. Trans. 1, 2193-2204 Jackson, K. A. & Hunt, J. D. Acta. met. 13, 1212-1215 (1965).
 Jackson, K. A., Hunt, J. D., Uhlmann, D. R. & Seward, T. P. Trans. met. Soc. AIME 236,
- 149-158 (1966).
- Streat, N. & Weinberg, F. Met. Trans. 3, 3181-3184 (1972). Usselman, T. M. Am. J. Sci. 275, 291-303 (1975).
- Jacobs, J. A. The Earth's Core (Academic, London, 1975)
- Doornbos, D. J. Geophys. J. R. astr. Soc. 38, 397-415 (1974).
 Stiller, H., Franck, S. & Schmit, U. Phys. Earth planet. Inter. 22, 221-225 (1980)
- Cormier, V. F. Phys. Earth planet Inter. 24, 291-301. Spetzler, H. & Anderson, D. L. J. geophys. Res. 73, 6051-6060 (1968).

Iron-manganese banding in Oneida Lake ferromanganese nodules

Willard S. Moore

Department of Geology, University of South Carolina, Columbia, South Carolina 29208, USA

Ferromanganese nodules in the deep-sea and in freshwater lakes usually accrete layers rich in manganese oxides alternating with layers rich in iron oxides1. The mechanism producing these alternating layers is unknown; indeed, the mechanism producing the nodules themselves is unknown. In Oneida Lake, a large freshwater lake near Syracuse, New York, precipitants from the lake water and the surfaces of nodules at the sediment-water interface are enriched in Mn, whereas nodules buried in lake sediments have surface layers enriched in Fe. It is hypothesized here, using field and laboratory evidence, that reduction and mobilization of Mn from the nodule surface during periods of anoxic sediment cover produce the high Fe layers observed in the nodules.

Oneida Lake nodules have been the subject of several investigations²⁻⁶. Mn phases present are 10 Å manganite (todorokite) and δMnO_2 ; Fe phases are goethite and X-ray amorphous ferric oxides and hydroxides^{4.5}. The nodules exhibit a discontinuous growth pattern characterized by periods of rapid growth interrupted by periods of no growth or erosion⁶. The nodules are most abundant in the 5-10 m deep central area of the lake where $\sim 25\%$ of the bottom may be covered with 1-20 cm diameter diskoidal nodules⁵.

The Mn/Fe ratio of freshly precipitated (<5-yr old) ferromanganese deposits in Oneida Lake is in the range 2.7-3.8 (Table 1). These thin deposits (<1-3 mm) show no Fe-rich layers. The surface Mn/Fe ratios of most Oneida nodules exposed to the Lake water are in this same range (ref. 6 and Table 2). These data suggest that freshly precipated Oneida ferromanganese deposits including nodule surfaces have a Mn/Fe ratio >2; yet in bulk composition the nodules have Mn/Fe ratios of 1.8 with some layers having ratios as low as 0.07 (ref. 5 and Table 2).

Extensive wind-induced mixing prevents the bottom waters of Oneida Lake from becoming anoxic as many eutrophic lakes do in the summer⁷. Although there is no long-term record of sediment deposition on the central shoals where nodules lie unconformably on a weathering Silurain shale, seasonal events may produce fine-grained sediment cover on these nodules depleating O2 and leading to the reduction of surficial MnO2. In anoxic sediments (those containing $\leq 3 \,\mu\text{mol O}_2 \,\text{kg}^{-1}$) MnO₂ reduction is largely completed before Fe₂O₃ reduction begins⁸. These studies have been confirmed in Oneida Lake sediments¹⁰. Thus nodules buried in sediments should have Mn mobilized from their surfaces in preference to Fe. The Mn may diffuse into the nodule or the adjacent sediment pore water. At site 123 in the lake (see Table 2), such a fine cover was observed during June 1977 but not in August 1977; the sediment cover was absent in August 1978 but present in June 1979. This sediment consisted primarily of organic-rich diatom shells, probably resulting from periods of high productivity in the near-surface waters of the lake⁷. Nodules covered by these anoxic sediments should lose Mn and become enriched in Fe on their surfaces. Removal of the sediment cover by oxidation and winowing would leave a nodule surface enriched in Fe available to receive more MnO₂.

The degree of surficial reduction depends directly on the length of time the nodule is covered and the O_2 demand of the sediment. In extreme cases most of the Mn may be removed from a considerable thickness of nodule producing a major Fe-rich band. In other cases Mn accretion may continue relatively uninterrupted for many years to produce a thick Mn-rich band.

There are two sets of data which suggest that these conditions produce the banded structure of the nodules. The first are measurements of low Mn/Fe ratios ranging from 0.07 to 0.31 (Table 2) on the surfaces of nodules recovered from beneath 15-25 cm of sediment in the lake. Below this surface layer, a Mn-rich layer is often present with Mn/Fe ratios of 2-4 (Table 2). Fe-rich layers seem to be forming on these buried nodules. Second, surface depletions of Mn were produced in laboratory experiments by burying nodules in lake sediments for 6 months. In all cases a 1-mm thick layer rich in Fe and depleted in Mn was produced on the nodule surface (Table 2). When the burial

Table 1 Mn/Fe ratios on different substrates in Oneida Lake

Substrate	Distance above sediment-water interface	Estimated age of deposit	Mn%	Mn/Fe
Plastic bottle	2 m	< 2 weeks	22	3.8
Aluminium can	0-4 cm	< 2 yr	18	2.7
Glass bottle	0-4 cm	< 5 yr	21	3.1
Mussell shell	0-6 cm	< 3 yr	19	3.7

Table 2 Manganese and iron in Oneida Lake nodules Site no. Sample description (thickness) Mn% Mn/Fe 129 Buried in 15 cm of sediment Hard surface layer (1 mm) 3 44 0.24Friable layer below surface (2 mm) 12.5 2.8 129 Buried in 15 cm of sediment 4 89 Hard surface layer (1 mm) 0.31 Friable layer below surface (3 mm) 13.0 3.45 Buried in 15 cm of sediment Hard surface laver (1 mm) 0.860.07 Friable layer below surface (2 mm) 7.21 1.16 Surficial nodule 129 Friable surface material (1 mm) 17.8 3.03 Friable material just below surface (3 mm) 2.63 Surficial nodule, appeared highly weathered Very soft surface material 9.27 2.08 Very hard layer just below surface 1.65 0.10Buried in 25 cm of sediment Flaky surface material (2 mm) 2 79 0.13Harder material below flaky surface 4.90 0.25 (2 mm)123 Surficial nodule, appeared weathered 4.66 0.18 Hard layer from surface (1 mm) Friable material below surface (1 mm) 19.4 1.96 Buried in sediment in lab for 6 months Surface layer (1 mm) 9.26 0.27 Layer below surface (3 mm) 11.01 0.45 Buried in sediment in lab for 6 months Surface layer (1 mm) 10.97 0.44 1st layer below surface (1 mm) 14.86 0.56 2nd layer below surface (2 mm) 32.33 3.7

These sites are 20-30 m south of New York State Barge Canal buoys bearing the same number. Each is a shoal area about 10 m deep with abundant nodules.

13.09

32.97

0.70

6.1

123 Buried in sediment in lab for 6 months

Layer below surface (2 mm)

Surface layer (1 mm)

experiments were conducted in covered glass jars, a ring of MnO_2 appeared on the glass at the sediment-water interface. In jars of similar sediment but without nodules, no ring of MnO_2 appeared.

The results of these nodule burial experiments are unequivocal. A skin rich in Fe and depleted in Mn is produced by burying a nodule in lake sediments for several months. Apparently MnO₂ on the nodule surface accepts electrons from carbon oxidation in the sediments and is mobilized as Mn²⁺. In the jar experiments some of this Mn²⁺ diffused to the sediment surface where it was reoxidized to MnO₂. Because the jars with the sediment but no nodules showed no MnO₂ ring, this Mn must have come from the nodules. No similar Fe mobilization was evident.

Besides explaining the Mn-Fe banding of the nodules, the hypothesis answers an additional question concerning the Oneida nodules, namely their episodic growth. It has been shown⁶ that periods of rapid growth (>1 mm per 100 yr) are separated by growth hiatuses which may last 500-1,000 yr. These growth hiatuses may in fact reflect periods of extensive chemical erosion of the nodules during which Mn is removed selectively and a lag deposit rich in Fe forms on the nodule surface. These hard, Fe-rich layers provide structural integrity for the nodules. The Mn-rich sections are cut easily with a steel blade, but some Fe-rich layers have a hardness > 6 enabling the nodules to resist erosion. The partial dissolution of the nodules thus seems to be instrumental in producing a framework strong enough to become an important component of the lake sediments.

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- 1. Burns, R. G. and Brown, B. A. in Ferromanganese Deposits on the Ocean Floor (ed. Horn, D. Burns, R. O. and Blowii, B. A. In Paramagnature R.) 51 (National Science Foundation, Washington DC, 1972). Dean, W. E. Proc. 13th Conf. Great Lakes Res. 217–226 (1970). Krishnaswami, S. & Moore, W. S. Nature 243, 114–116 (1973).

- Ghosh, S. K. thesis, Syracuse Univ. (1975).
 Dean, W. E. & Ghosh, S. K. J. Res. U.S. geol. Surv. 6, 231-240 (1978).
- 6. Moore, W. S., Dean, W. E., Krishnaswami, S. & Borole, D. V. Earth planet. Sci. Lett. 46, 191-200 (1980).
- 7. Mills, E. L., Forney, J. L., Clady, M. D. & Schaffner, W. R. in Lakes of New York State Vol. 2 (ed. Blomfield, J. A.) 367-451 (Academic, New York, 1978).

 Froelich, P. N. et al. Geochim, cosmochim. Acta 43, 1075-1090 (1979).
- Klinkhammer, G. P. Earth planet. Sci. Lett. 49, 81-101 (1980).
- 10. Dean, W. E., Moore, W. S. & Nealson, K. H. Chem. Geol. (in the press).

N₂O exchange between a grassland soil and the atmosphere

J. C. Ryden

The Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR, UK

The recognition that NO, derived from atmospheric N2O, is an important factor in the destruction of stratospheric ozone1, and that atmospheric N2O may reduce radiative heat loss from the Earth's surface², has stimulated interest³⁻⁶ in the global sources and sinks for N2O. One possibility is that increasing use of industrially fixed N in agricultural production increases loss of N2O to the atmosphere. Until recently, however, field data for the evaluation of this concept were not available. Even now, only a few longer-term studies of N2O loss from representative agricultural land permit estimates of loss over a complete year or a cropping season. In the present study, field measurements of N2O exchange over 12 months between a grassland soil and the atmosphere indicated that the soil acts as both a source and a sink for atmospheric N2O, depending on soil conditions and the amount of nitrogenous fertilizer applied.

The measurements of N₂O exchange (as described in Fig. 1) were made in duplicate in a 10×10 m subplot within a 0.4-ha plot sown to perennial ryegrass (Lolium perenne L.) and receiving 250 kg N ha⁻¹ yr⁻¹ as ammonium nitrate. The field plots formed part of a long-term study of nitrogen fertilizer application to grassland by ICI Ltd at Bracknell, Berkshire, UK. Quadruplicate measurements of N2O exchange were also made on an unfertilized control strip alongside the 250 kg N ha treatment. The soil at the site is a loam in the Wickham series overlying London clay7 (Ochraqualf; US Comprehensive Soil Classification). Some properties of the upper 20 cm of the profile are: pH = 6.0-6.5; organic $C = 34.7 \text{ mg g}^{-1}$; total N = 2.3 mg g^{-1} ; bulk density = 1.06 g cm^{-3} .

Nitrous oxide exchange varied appreciably with time of year and prevailing soil conditions. For the 250 kg N ha⁻¹ treatment (Fig. 1) there was a net loss of N_2O from the soil, weekly mean rates of N_2O loss being as high as 245 ng N m⁻² s⁻¹ (1 ng N m⁻² s⁻¹ = 8.64×10^{-4} kg N ha⁻¹ day⁻¹) after the third fertilizer application which was made during a period of frequent rain. The highest rates of N₂O loss were observed when the following conditions prevailed simultaneously in the upper 20 cm of the soil profile; water content >20% by weight (air-filled porosity <38% of an undisturbed soil volume), nitrate and ammonium contents >10 µg N per g soil and temperature >10 °C. Additional measurements indicated that the highest rates of N₂O loss were also associated with water content >35% in the upper 2.5 cm of the soil profile.

Low rates of N₂O loss (<12.0 ng N m⁻² s⁻¹) were observed following the first and fourth fertilizer applications. These periods of low N2O loss coincided with appreciable drying of the soil. Low rates of N₂O loss were also observed during the first 3 weeks of the study even though the soil was at field capacity (water content 28-30%); however, soil temperature was ≤5 °C and nitrate content $<1 \mu g N g^{-1}$.

The most striking feature of the data in Fig. 1 is that the 250 kg N ha-1 treatment acted as both a source of and a sink for atmospheric N2O. Sink activity as high as 11.6 ng N m⁻² s⁻¹ has been measured, although weekly mean values (Fig. 1) never exceeded 5.8 and were generally between 1.2 and $3.5 \text{ ng N m}^{-2} \text{ s}^{-1}$. The prevailing soil conditions during periods of sink activity were moderate to high (>20%) soil water content, very low nitrate content ($\leq 1~\mu g~N~g^{-1}$) and temperatures above 5-8 °C (Fig. 1). Sink activity was observed before fertilizer application when soil temperature increased above 5 °C. Sink activity resumed 3 weeks after the second fertilizer application and 2 weeks after the third application when soil nitrate content fell to low levels and water content remained

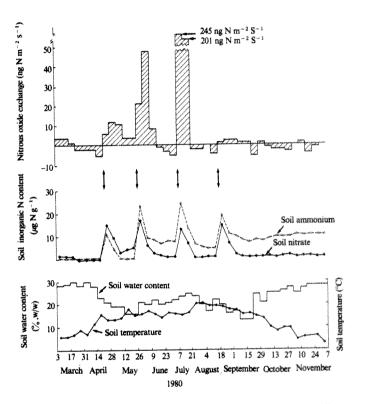


Fig. 1 Nitrous oxide exchange, soil nitrate and ammonium content (0-20 cm), soil moisture content (0-20 cm) and soil temperature (2 cm) in grassland receiving 250 kg N ha⁻¹ yr⁻¹ as ammonium nitrate. Fertilizer was applied in four equal amounts on the dates indicated (\$). The sward was cut and herbage removed immediately before each fertilizer application and on 19 September N2O exchange was measured using a modified version of the soil cover technique described in ref. 28. The cover had a removable lid, enclosed an air volume of 7.5 dm³ and covered an area of 0.075 m². The air flow (500 cm³ min⁻¹) through the cover was allowed to equilibrate with the soil surface for at least 1 h before starting the N2O exchange measurement. N₂O exchange was determined for periods of 3 h by comparing the amount of N₂O in air drawn through the cover with that in an equivalent flow of air drawn from the free atmosphere at the same height as the air entry port for the cover. The rate of air flow used to sweep the cover must equal that used to sample the free atmosphere. Accurate matching of flow rates was achieved using stainless steel needle valves and air flow tubes each of which was calibrated to 500 cm³ min⁻¹ ± 1% using a wet gas flowmeter. The air flow control gave a standard error of ±0.005 p.p.m. (v/v) for 10 simultaneous determinations of an atmospheric N2O concentration of 0.320 p.p.m. The mean atmospheric N2O concentration during the study period was 0.324 ± 0.013 p.p.m. for 160 determinations. A detection limit of 1.2 ng N m $^{-2}$ s $^{-1}$ for N2O exchange was obtained. Measurements of N2O exchange were made on at least 3 days per week between 3 March and 19 September. During periods following fertilizer application or rain, measurements were made on 5 or 6 days per week. After 19 September measurements were made on 2 days per week. To facilitate handling of large numbers of samples, soil nitrate and ammonium content was determined by extraction with 2 M KCl after rapidly drying soils in a forced-air oven at 70 °C. Soil water content was determined gravimetrically on the same samples. Soil nitrate contents determined using this procedure were not significantly different from those determined by extraction of field moist soil. Drying increased ammonium content by 2-4 µg N per g soil.

>20% as a result of persistent rain. Significantly, in a companion treatment receiving 500 kg N ha-1 yr-1 no sink activity was observed—soil nitrate content remained above 2 µg N g throughout the study.

How soil water content affects source or sink activity is illustrated in the 3-week periods following 28 July and 22 September when sink activity ceased as the soil passed through a drying phase. A similar effect was observed in November when the soil passed through periods when its temperature fell below 5 °C. The oscillation between low rates of N₂O loss and sink activity in response to changes in soil temperature continued throughout the winter until the first fertilizer application in mid-March 1981 when significant loss of N₂O resumed.

The unfertilized control strip invariably acted as a sink for N₂O at times when soil water content exceeded 20% (Table 1). Sink activity ceased only when the soil dried to lower water content or when its temperature fell below 5 °C (Table 1; Fig. 1). As with the 250 kg N ha⁻¹ treatment, sink activity was associated with persistently low soil nitrate content, weekly mean and individual values never exceeding 1 µg N g⁻¹ (Table 1).

Measurements made concurrently with those reported in Fig. 1 and Table 1 indicated that denitrification was the major process determining both source and sink activity for N2O in the present study. Loss of N2O was always associated with a larger denitrification loss measured using the C₂H₂-inhibition technique⁸ at other areas of the same plot. Rates of N₂O loss from C₂H₂-treated areas were two to seven times greater than those measured in the absence of C₂H₂ (Fig. 1). Production of N₂O during denitrification in soils is favoured by high nitrate content, temperature conducive to high respiratory oxygen demand, and water contents leading to restricted aeration and the development of anaerobic microsites⁹. Maximum N₂O loss was observed (Fig. 1) when such conditions prevailed. During periods of sink activity there was also a small net loss of N (generally <12 ng N m⁻² s⁻¹) from C_2H_2 -treated areas. Sink activity was only observed in conditions conducive to microbial reduction of N2O; namely when the nitrate available for reduction during denitrification was essentially exhausted and when reductive stress was placed on the system^{10,11} with soil water content at or above 20% and temperatures above 5-8 °C.

Some workers have found that N2O loss may also arise from nitrification, particularly in calcareous soils with high ammonium content¹². Following the application of fertilizer on 7 July 1980, the rates of N₂O loss measured 12-24 h after termination of C₂H₂ treatment were not significantly different (99% confidence level; six replicates) from those measured in areas that had not been treated with C₂H₂ (six replicates). For example, on 11 July, areas treated with C₂H₂ yielded N₂O at

Table 1 Weekly mean rates of N2O exchange between the atmosphere and a grassland soil receiving no fertilizer nitrogen

Week (1980)	Rate of N ₂ O exchange (ng N m ⁻² s ⁻¹)	Soil nitrate content (0-20 cm) (µg N per g soil)	Soil water content (0-20 cm) % (w/w)
18-24 August	-3.1	0.6	20.1
25-31	-1.7	0.8	18.5
1-7 September	-1.2	0.6	19.0
8-14	tr*	tr†	14.1
15-21	tr	0.7	15.0
22-28	-2.9	1.0	22.5
29-5	tr	tr	19.5
6-12 October	-2.3	0.4	20.1
13-19	-1.2	tr	22.2
20-26	-1.2	tr	23.0
27-2	-2.3	0.9	25.2
3-9 November	tr	0.4	23.8
10-16	tr	0.6	24.4
17-23	-4.1	0.9	24.8
24-30	tr	0.5	23.7
1-7 December	tr	0.6	25.2

 $^{^*}$ <+1.2, >-1.2 ng N m⁻² s⁻¹.

 $493 \pm 135 \text{ ng N m}^{-2} \text{ s}^{-1}$. Other areas that had received C_2H_2 treatment 16 h previously yielded N_2O at 325 ± 24 ng N m⁻² s⁻¹ a rate not significantly different from that $(332\pm58~{\rm ng}~N~m^{-2}~s^{-1})$ for areas that had never been treated with C_2H_2 . As low concentrations of C_2H_2 also inhibit nitrification, an effect which persists for several days after removal of C₂H₂ (ref. 13), it seems unlikely that nitrification made an appreciable contribution to N₂O loss from this non-calcareous soil even during the periods of high soil ammonium content following fertilizer applications.

The rates of N₂O loss observed in the present study are within a similar range to that reported¹⁴⁻²¹ for arable and grassland systems, although in only one such study¹⁷ has sink activity been observed. Nevertheless, there is direct²² and indirect^{10,23,24} evidence that soil may act as a sink for atmospheric N₂O. Conditions favourable to sink activity 10,11 may be expected to develop rapidly in moist to wet soils under grass. The dense root mass characteristic of such soils rapidly depletes nitrate in the soil solution and provides potential for appreciable oxygen demand as a result of root respiration and microbial degradation of organic matter. The observation that sink activity rarely exceeded mean weekly rates of $5.8~\rm ng~N~m^{-2}~s^{-1}$ (Fig. 1; Table 1), irrespective of fertilizer application, suggests that sink activity was limited more by the ease of diffusion of N2O from the atmosphere to zones of N₂O depletion within the soil, than by the potential for N_2O reduction per se.

In the present study, $\sim 1.3\%$ of the fertilizer N applied at a rate of 250 kg N ha⁻¹ yr⁻¹ was lost between 3 March 1980 and 2 March 1981. This proportion is in reasonable agreement with the estimate made by the Council for Agricultural Science and Technology³ for annual N₂O loss from fertilized land but is at least one order of magnitude lower than that implied by other models of N₂O dynamics⁴⁻⁶. The proportion of fertilizer N lost as N₂O in the present study is of the same order as that observed during complete cropping seasons elsewhere 14-16,18

The fact that sink activity for N₂O (Fig. 1; Table 1) was associated with a small net loss of N arising from denitrification (discussed above) suggests that denitrification in soils with low concentrations of inorganic N, despite high total N content, contributes little if any N₂O to the atmosphere. However, additional N₂O may be lost following conversion of grassland to arable production when mineralization of stored fertilizerderived N may produce elevated concentrations of inorganic N. Nevertheless, even in agricultural systems assumed to be at equilibrium with respect to reserves of soil N, losses of N as N₂O were never greater than 5-7% of the fertilizer N applied even at rates as high as 680 kg N ha⁻¹ yr⁻¹ (ref. 15).

The present and previous studies¹⁴⁻²⁰ suggest that forecasts of

N₂O losses from industrially fixed N may have been appreciably overestimated. Furthermore, sink activity for N₂O has now been observed in grassland soils (present study), arable soils¹⁷ and lake, estuarine and marine sediments²⁵⁻²⁷. The indications that N₂O source strength from industrially fixed N may have been overestimated suggest that the sink strength of the pedosphere and hydrosphere for atmospheric N₂O may be more significant than previously assumed.

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- Crutzen, P. I. O. Jl R. met. Soc. 96, 320-325 (1970).
- Wang, W. C. & Sze, N. D. Nature 268, 589-590 (1980).
- Council for Agricultural Science and Technology Effect of Increased Nitrogen Fixation on Stratospheric Ozone (Rep. No. 53, Iowa State University, Ames, 1976).
- McEiroy, M. B., Wofsy, S. C. & Yung, Y. L. Phil. Trans. R. Soc. B277, 159-181 (1977). Hahn, J. & Junge, C. Z. Naturforsch. 32a, 190-214 (1977). Hahn, J. Phil. Trans. R. Soc. A290, 495-504 (1979).
- Jarvis, M. G., Hazelden, J. & Mackney, D. Soils of Berkshire (Soil Survey of England and Wales, Harpenden, 1979).
- Ryden, J. C., Lund, L. J., Letey, J. & Focht, D. D. Soil Sci. Soc. Am. J. 43, 110-118 (1979). Focht, D. D. & Verstraete, W. Adv. Microbial Ecol. 135-214 (1977).
- 10. Blackmer, A. M. & Bremner, J. M. Geophys. Res. Lett. 3, 739-742 (1976).

 $t < +0.1 \mu g N per g soil.$

- 11. Bremner, J. M. in Nitrogen in the Environment Vol. 1 (eds Nielsen, D. R. & MacDonald, J. G.) 474-491 (Academic, New York, 1978)
- Bremner, J. M. & Blackmer, A. M. Science 199, 295-297 (1978).
- Walter, H. M., Keeney, D. R. & Fillery, I. R. Soil Sci. Soc. Am. J. 43, 195-196 (1979).
 Hutchinson, G. L. & Mosier, A. R. Science 205, 1125-1127 (1979).

- Ryden, J. C. & Lund, L. J. J. envir. Qual. 9, 387–393 (1980).
 McKenney, D. J., Shuttleworth, K. F. & Findlay, W. I. Can. J. Soil Sci. 60, 429–438 (1980).
- Bremner, J. M., Robbins, S. G. & Blackmer, A. M. Geophys. Res. Lett. 7, 641-644 (1980).
 Burford, J. R., Dowdell, R. J. & Cress, R. J. Sci. Fd Agric. 32, 219-223 (1981).
- Conrad, R. & Seiler, W. Atmos. Envir. 14, 555-558 (1980).
 Breitenbeck, G. A., Blackmer, A. M. & Bremner, J. M. Geophys. Res. Lett. 7, 85-88 (1980).
- Denmead, O. T., Freney, J. R. & Simpson, J. R. Soil Sci. Soc. Am. J. 43, 726-728 (1979).
 Cicerone, R. J., Shetter, J. D., Stedman, D. M., Kelly, T. J. & Liu, S. C. J. geophys. Res. 83, 3042-3050 (1978).
- Letey, J., Hadas, A., Valoras, N. & Focht, D. D. J. envir. Qual. 9, 232-235 (1980).
- Delwiche, C. C., Bissell, S. & Virginia, R. in Nitrogen in the Environmen. D. R. & MacDonald, J. G.) 459-476 (Academic, New York, 1978).
- Chan, Y. K. & Knowles, R. Appl. envir. Microbiol. 37, 1067-1072 (1979).
 Elkins, J. W., Wofsy, S. C., McElroy, M. B., Kolb, C. E. & Kaplin, W. A. Nature 275, 602-606 (1978).
- Cohen, Y. Nature 272, 235-237 (1978).
- 28. Ryden, J. C., Lund, L. J. & Focht, D. D. Soil Sci. Soc. Am. J. 42, 731-738.

Pseudogenes as a paradigm of neutral evolution

Wen-Hsiung Li, Takashi Gojobori & Masatoshi Nei

Center for Demographic and Population Genetics. The University of Texas at Houston, Houston, Texas 77025, USA

On the neutral mutation hypothesis 1-3, the rate of nucleotide substitution is expected to be higher for functionally less important genes or parts of genes than for functionally more important genes, as the latter would be subject to stronger purifying (negative) selection²⁻⁴. On the other hand, selectionists believe that most nucleotide substitutions are caused by positive darwinian selection^{5,6}, in which case the rate of nucleotide substitution in functionally unimportant genes or parts of genes^{2,7} is expected to be relatively lower because the mutations in these regions of DNA would not produce any significant selective advantages. Kimura⁸ and Jukes⁹ have argued that the higher substitution rate observed at the third positions of codons than at the first two positions supports the neutral mutation hypothesis, as most third-position substitutions are synonymous and do not change the amino acids encoded, although others^{5,10} have discussed the possibility that third-position substitutions are subject to positive darwinian selection. Recently, Kimura¹ noted that the mouse globin pseudogene, $\psi \alpha 3$, evolved faster than the normal mouse $\alpha 1$ gene, although he did not compute the substitution rate. Here, we present a method of computing the rate of nucleotide substitution for pseudogenes, and report that the three recently discovered pseudogenes show an extremely high rate of nucleotide substitution. As these pseudogenes apparently have no function, this finding strongly supports the neutral mutation hypothesis.

A pseudogene is a DNA segment with high homology with a functional gene but containing nucleotide changes such as frameshift and nonsense mutations that prevent its expression. (Some authors 12 have argued possible functions of pseudogenes, but their arguments are not substantiated.) Pseudogenes seem to have been produced by the nonfunctionalization of duplicate genes. A complete nucleotide sequence is now available for three pseudogenes (mouse $\psi \alpha 3$, human $\psi \alpha 1$ and rabbit $\psi \beta 2$ in the globin gene families)¹³⁻¹⁵. Figure 1 shows a probable evolutionary scheme for mouse pseudogene $\psi \alpha 3$ ($M\psi \alpha 3$), mouse functional gene $\alpha 1 (M\alpha 1)^{16}$ and rabbit functional gene $\alpha (R\alpha)^{16}$ in which O denotes the point of duplication leading to $M\psi\alpha$ 3 and $M\alpha 1$.

Let d_{ABi} , d_{ACi} and d_{BCi} be the numbers of nucleotide substitutions per site at the *i*th position of codons (i = 1, 2 or 3) between $M\psi\alpha$ 3 and $M\alpha$ 1, between $M\psi\alpha$ 3 and $R\alpha$, and between $M\alpha$ 1 and $R\alpha$, respectively, and let l_i , m_i and n_i be the numbers of nucleotide substitutions per site between O and $M\psi\alpha$ 3, between O and $M\alpha$ and between O and $R\alpha$, respectively. We then have $d_{ABi} = l_i + m_i$, $d_{ACi} = l_i + n_i$, $d_{BCi} = m_i + n_i$. Therefore, l_i , m_i and n_i can be estimated by

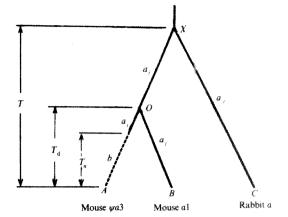


Fig. 1 Plausible phylogenetic tree for mouse $\psi \alpha 3$, mouse $\alpha 1$ and rabbit a. T denotes the divergence time between mouse and rabbit, T_d the time since duplication of mouse $\psi \alpha 3$ and $\alpha 1$, and T_n the time since nonfunctionalization of mouse $\psi \alpha 3$. a, denotes the rate of nucleotide substitution per site per year at the ith position of codons in the normal globin genes and b the rate of substitution for mouse pseudogene $\psi \alpha 3$. The proportion of nucleotide differences is 51/354 between mouse $\psi \alpha 3$ and $\alpha 1, 84/423$ between mouse $\alpha 1$ and rabbit α , and 91/354 between mouse $\psi \alpha 3$ and rabbit α (see Table 1)

$$l_i = (d_{ABi} + d_{ACi} - d_{Bci})/2 \tag{1a}$$

$$m_i = (d_{ABi} - d_{ACi} + d_{BCi})/2$$
 (1b)

$$n_i = (-d_{ABi} + d_{ACi} + d_{BCi})/2$$
 (1c)

Pseudogenes often have deletions and insertions. nucleotides involved in these changes should be eliminated from data analysis, because we are interested only in nucleotide substitution. This can be done by aligning a pseudogene with its homologous functional gene for the coding regions. In our study we followed the alignments given by the original authors 13-15. In mouse $\psi \alpha 3$, however, we excluded the 30 nucleotides that have been aligned with the nucleotides starting from positions 91 to 120 of the functional mouse $\alpha 1$. In this region there were 21 mismatches, including one sequence of 8 mismatches and another of 7 mismatches. This pattern was very different from that of other parts of the gene and probably occurred through insertion rather than nucleotide substitution. At any rate, after these alignments, we computed the proportion of different nucleotides between homologous genes (Table 1), and from this proportion (p) the total number of nucleotide substitutions per site (d_{ABi}, d_{ACi}) or d_{BCi} was estimated by $d = -(3/4) \ln [1 - (4/3)p]$ (ref. 18). For computing d we also

Table 1 Proportions of nucleotide differences between genes for the three positions of codons

	•		
Genes compared	First position	Second position	Third position
$M\psi\alpha$ 3 with: $M\alpha$ 1	12/118	17/117	22/119
$H\alpha$	19/118	23/117	47/119
Rlpha	21/118	22/117	48/119
$H\psi\alpha$ 1 with: $H\alpha$	30/133	26/132	42.5/133
M α 1	34/133	31/132	49/133
Rlpha	39/133	32/132	51/133
$M\alpha$ 1 with: $H\alpha$	13/141	14/141	54.5/141
Rlpha	16/141	15/141	53/141
$H\alpha$ with: $R\alpha$	18/141	14/141	31/141
$R\psi\beta$ 2 with: $R\beta$ 1	26/146	19/145	34/145
Нβ	31/146	24/145	42/145
$M\beta$	34/146	31/145	43/145
$R\beta$ 1 with: $H\beta$	9/146	6.5/146	32/146
Мβ	21.5/146	16/146	46/146

 $M\psi\alpha 3$ = mouse $\alpha 3$ pseudogene; $M\alpha 1$ = mouse $\alpha 1$; $H\alpha$ = average for human $\alpha 1$ and $\alpha 2$; $R\alpha = \text{rabbit } \alpha$; $H\psi\alpha 1 = \text{human } \alpha 1$ pseudogene; $R\psi\beta 2$ = rabbit $\beta 2$ pseudogene; $R\beta 1$ = average for rabbit $\beta 1$ allele 1 and allele 2; $H\beta$ = human β , $M\beta$ = mouse β major. The 30 bases near the middle of $M\psi\alpha 3$ are excluded from the comparison (see text).

Table 2 Numbers of nucleotide substitutions per site at the first, second and third positions of codons between O and A, between O and B and between O and C, where O is the point of duplication leading to sequences A and B in Fig. 1

Pseudogene			Between O and A		Between O and B		Between O and C				
(Sequence A)	Sequence B	Sequence C	1	2	3	1	2	3	1	2	3
Mouse $\psi \alpha 3$	Mouse α 1	Human α , rabbit α	0.095	0.137	0.125	0.014	0.025	0.088	0.097	0.086	0.446
Human ψα 1	Human α	Mouse $\alpha 1$, rabbit α	0.246	0.205	0.268	0.022	0.024	0.148	0.097	0.083	0.254
Rabbit ψβ2	Rabbit β1	Human β , mouse β^{maj}	0.184	0.140	0.159	0.020	0.004	0.122	0.086	0.080	0.216

used Kimura's formula¹¹, but the values were not much different.

In Fig. 1 we used rabbit α as a third gene, but human α 1 or α 2 can also be used for this purpose, as their nucleotide sequences are known^{14,19}. We have therefore computed the d values for each of these genes and used the averages of the values to compute l_i , m_i and n_i (Table 2) and the other quantities to be given later. Table 2 also includes the results for human pseudogene $\psi \alpha$ 1 and rabbit pseudogene $\psi \beta$ 2. It is seen that in the evolution of functional genes (between O and B and between O and C), the third position of codons changes several times faster than the first two positions, which evolve at almost the same rate, but in the line leading to a pseudogene (between O and A) the rates of change for the three positions are about the same and are higher than those between O and B. Clearly, pseudogenes evolve faster than functional genes.

The above computation does not give the substitution rate for pseudogenes. To compute this rate, we must know the time since nonfunctionalization of a pseudogene. Let us again use the three genes in Fig. 1 as an example. In this figure the time (T) since divergence between $M\alpha 1$ and $R\alpha$ is known to be about 80 Myr, but the time (T_a) since duplication of $M\psi\alpha 3$ and $M\alpha 1$ and the time (T_n) since nonfunctionalization of $M\psi\alpha 3$ must be estimated. We estimate these times and the rates of nucleotide substitution for the functional genes and pseudogenes simultaneously. Let a_1 , a_2 and a_3 be the rates of nucleotide substitution per site per year at the first, second and third positions of codons in the functional genes, respectively. Once a gene becomes nonfunctional, the rate of nucleotide substitution is expected to be the same for all of the three positions, and we denote the rate by b. From Fig. 1 we obtain

$$d_{ABi} = 2a_i T_d + (b - a_i) T_n \tag{2}$$

$$d_{ACi} = 2a_i T + (b - a_i) T_n \tag{3}$$

$$d_{BCi} = 2a_i T (4)$$

As we know T, a_i can be estimated by $d_{BCi}/(2T)$. We also note

$$y_i \equiv d_{ACi} - d_{BCi} = bT_n - a_i T_n \tag{5}$$

Therefore, from equations (2) and (5), T_d can be estimated by $(\sum d_{ABi} - \sum y_i)/(2\sum a_i)$, where \sum stands for the summation over *i*.

To estimate T_n and b, we can use equation (5) and apply the standard least-squares method, as there are two unknowns and three equations. However, note that essentially the same results are obtained by the following simple formulae

$$T_n = (y_{12} - y_3)/(a_3 - a_{12}) \tag{6}$$

$$b = (a_3 y_{12} - a_{12} y_3) / (y_{12} - y_3)$$
 (7)

where $y_{12} = (y_1 + y_2)/2$ and $a_{12} = (a_1 + a_2)/2$. Expressing equations (6) and (7) in terms of l_i , m_i and n_i , we have obtained approximate formulae for the standard errors of T_n and b (not

shown). In practice, T_n obtained by equation (6) may be larger than T_d . In this case, we set $T_d = T_n$ because by definition $T_n \le T_d$. When $T_n = T_d$, equation (5) gives $b = (\sum a_i T_d + \sum y_i)/T_d$.

Table 3 shows the results for a_i , T_d , T_n and b. Interestingly, each of a_1 , a_2 and a_3 is nearly the same for the three groups of genes studied. However, a_3 is about four times larger than a_1 and a_2 . These estimates are similar to those obtained by Kimura⁸ and Jukes⁹ and support these authors' conclusion that the rate of synonymous substitution is higher than the rate of non-synonymous substitution. The b values in Table 2 indicate that the rate of nucleotide substitution in pseudogenes is even higher than the rate at the third positions of codons in the functional genes. The average rate of 4.6×10^{-9} is one of the highest rates of nucleotide substitutions so far estimated. Only two other estimates are comparable with this value. One is the rate (7.0×10^{-9}) for the synonymous substitution in the Cpeptide region of the preproinsulin genes²⁰, and the other is that (6.2×10^{-9}) estimated from amino acid sequence data for the rapidly evolving residues of fibrinopeptides²¹. These peptides are believed to have no biological function except for holding other polypeptides that will later form a protein. Our finding clearly indicates that functionally less important genes evolve faster than functionally more important genes, and thus supports the neutral mutation hypothesis. Furthermore, comparison of a_3 and b suggests that the third-position substitutions in the globin genes are subject to purifying selection.

Our estimate (T_d) of the time since gene duplication is 27 Myr for mouse $\psi \alpha 3$ and $\alpha 1$, 49 Myr for human $\psi \alpha 1$ and α , and 44 Myr for rabbit $\psi \beta 2$ and $\beta 1$. These estimates are somewhat smaller than those (30, 60, 55 Myr, respectively) obtained by Maniatis and his associates ^{14,15}, mainly because these authors assumed that the substitution rate for the pseudogenes is the same as that for the synonymous changes in functional globin genes. This assumption seems to be incorrect, as the former rate is about twice as great as the latter rate in our study. If our estimates of the times of gene duplication are reliable, the mouse $\psi \alpha 3$ diverged from the $\alpha 1$ gene about 27 Myr ago and became a pseudogene about 4 Myr later. On the other hand, the human $\psi \alpha 1$ was duplicated from the α gene about 49 Myr ago and became nonfunctional about 4 Myr later. The rabbit $\psi \beta 2$ gene became nonfunctional almost immediately after it was duplicated from the $\beta 1$ gene about 44 Myr ago.

We have studied the evolutionary changes of pseudogenes as a paradigm of neutral evolution because in these genes the fate of new mutations in a population is determined almost entirely by genetic drift under the neutral mutation hypothesis. In practice, most genes in the genome would have some biological function and thus be subject to a varying degree of purifying selection. Even in these genes, however, there may be a large

Table 3 Times since gene duplication (T_d) , times since nonfunctionalization (T_n) and rates of nucleotide substitution per site per year (b) for pseudogenes mouse $\psi \alpha 3$, human $\psi \alpha 1$ and rabbit $\psi \beta 2$

Pseudogene (Sequence A)	Sequence B	Sequence C	$a_1 \times 10^{-9}$	$(\times 10^{-9})$	$(\times 10^{-9})$	$\frac{T_d}{(\mathrm{Myr})}$	T_n (Myr)	$b \times 10^{-9}$
Mouse $\psi \alpha 3$ Human $\psi \alpha 1$ Rabbit $\psi \beta 2$	Mouse $\alpha 1$ Human α Rabbit $\beta 1$	Human α , rabbit α Mouse α 1, rabbit α Human β , mouse β ^{maj}	0.69 ± 0.26 0.74 ± 0.27 0.71 ± 0.27	0.69 ± 0.26 0.67 ± 0.26 0.51 ± 0.22	3.32 ± 0.73 2.51 ± 0.61 2.09 ± 0.51	27±6 49±8 44±8	23 ± 19 45 ± 37 44*	5.0 ± 3.2 5.1 ± 3.3 3.6*
Average			0.71	0.62	2.64			4.6

 a_1 , a_2 and a_3 refer to the rates of nucleotide substitutions for the first, second and third positions of codons in the functional genes, respectively. * We were unable to compute a proper standard error for this estimate, because T_n was assumed to be equal to T_d (see text).

number of mutant forms (nucleotide sequences) that are equally functional and fit in adaptation. All these mutant forms will be a source of neutral evolution.

This work was supported by grants from the NIH and NSF. Note added in proof: After submission of this paper, we learned that Miyata and Yasunaga²² estimated the rate of nucleotide substitution for mouse $\psi \alpha 3$. Their estimate is higher than ours, because they did not exclude the middle part of the sequence.

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- Kimura, M. Nature 217, 624-626 (1968). King, J. L. & Jukes, T. H. Science 164, 788-798 (1969).
- Kimura, M. & Ohta, T. Proc. natn. Acad. Sci. U.S.A. 71, 2848–2852 (1974). Dickerson, R. E. J. malec. Evol. 1, 26–45 (1971).

- Dickerson, K. E. J. molec. Evol. 1, 26-45 (1971).
 Clarke, B. Science 168, 1009-1011 (1970).
 Milkman, R. Trends biochem. Sci. 1, N152-N154 (1976).
 Jukes, T. H. & King, J. L. Nature 231, 114-115 (1971).
 Kimura, M. Nature 267, 275-276 (1977).
 Jukes, T. H. J. molec. Evol. 11, 207-209 (1978).
 Pithese, T. H. J. molec. Evol. 11, 207-209 (1978).

- Richmond, R. C. Nature 225, 1025-1028 (1970).
 Kimura, M. J. molec. Evol. 16, 111-120 (1980).
- 12. Proudfoot, N. J. Nature 286, 840-841 (1980).
- Prouutoot, N. J. Nature 280, 840-841 (1980).
 Nishioka, Y., Leder, A. & Leder, P. Proc. natn. Acad. Sci. U.S.A. 77, 2806-2809 (1980).
 Proudfoot, N. J. & Maniatis, T. Cell 21, 537-544 (1980).
 Lacy, E. & Maniatis, T. Cell 21, 545-553 (1980).
 Nishioka, Y. & Leder, P. Cell 18, 875-882 (1979).
 Heindell, H. C. et al. Cell 15, 43-54 (1978).

- 18. Jukes, T. H. & Cantor, C. H. Mammalian Protein Metabolism (ed. Munro, H. N.) 21-123 (Academic, New York, 1969).
- Michelson, A. M. & Orkin, S. H. Cell 22, 371-377 (1980). Perler, F. et al. Cello 20, 555-566 (1980).
- Kafatos, F. C. et al. Proc. natn. Acad. Sci. U.S.A. 74, 5618-5622 (1977)
- 22. Miyata, T. & Yasunaga, T. Proc. natn. Acad. Sci. U.S.A. 78, 450-453 (1981).

Gait and the energetics of locomotion in horses

Donald F. Hoyt* & C. Richard Taylor

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, USA

It seems reasonable that quadrupeds should change gait from a walk to a trot to a gallop in such a way as to minimize their energy consumption, as human beings are known to change from a walk to a run at a particular speed (2.4 m s^{-1}) below which walking requires less energy than running and above which the opposite is true. Thus by changing gait, human beings keep the energy cost of locomotion to a minimum as their speed increases. One reason this relation holds is that in humans, metabolic rate increases curvilinearly with walking speed¹. If metabolism were a curvilinear function of speed within each of the gaits used by quadrupeds, it would support the hypothesis that they also change gait to minimize energetic cost. There is an old controversy about whether metabolic rate increases linearly or curvilinearly in running humans^{1,2} but all previous reports have suggested that metabolic rate increases linearly with speed in quadrupeds. Extended gaits were an important experimental tool in the study of human gait changes; thus we have trained three small horses (110-170 kg) to walk, trot and gallop on a motorized treadmill, and to extend their gaits on command. We report here that, using measurements of rates of oxygen consumption as an indicator of rates of energy consumption, we have confirmed that the natural gait at any speed indeed entails the smallest possible energy expenditure.

Rate of oxygen consumption increased curvilinearly with speed for walking and trotting (Fig. 1). We were unable to obtain sufficiently high galloping speeds to evaluate whether rate of oxygen consumption also increased curvilinearly during a gallop. Transitions between gaits normally occurred at the speeds where the curves intersected and oxygen consumption was the same for the two gaits. When the gaits were extended beyond their normal range of speeds, oxygen consumption was higher in the extended gait than in that which the animal would normally be using. For example, horse B normally walked at a speed of 1.25 m s⁻¹. When trotting at 1.25 m s⁻¹, the rate of oxygen consumption was 1.5 times that measured during a walk.

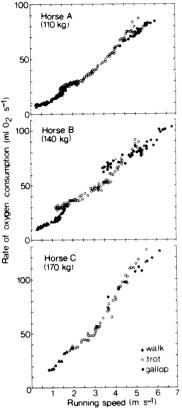


Fig. 1 Steady-state rate of oxygen consumption as a function of speed for a walk, a trot and a gallon in three small horses. The horses normally changed from walk to trot, or trot to gallop at the point where rate of oxygen consumption was the same in the two gaits. We were able to train the animals to extend their gaits to speeds where they would have normally used a different gait. The data from the extended gaits accentuated the curvilinear nature of the relationship between rate of oxygen consumption and speed within a gait. Linear regression provided a good fit of the data if we omitted measurements made when animals used extended gaits. The least-squares regressions and coefficients of determination (r^2) are: horse A, $V_{\rm O2} = -1.22 + 14.4 \cdot v$, $r^2 = 0.99$; B, $V_{\rm O2} = 2.68 + 15.0 \cdot v$, $r^2 = 0.98$; C, $V_{\rm O2} = -8.61 + 22.2 \cdot v$, $r^2 = 0.98$ where $V_{\rm O2}$ is ml O₂ s⁻¹ and v is velocity in m s⁻¹ We considered that a steady-state oxygen consumption was achieved when we recorded a constant rate of oxygen consumption for at least 5 min. At the speeds used here, steady-state was never reached during the first 6 min of measurement, thus all measurements at a given speed were made for at least 11 min. During these measurements, air temperature averaged 21 °C and air speed was approximately matched to tread speed. The animals were loosefitting masks which allowed capture of all expired gases. Rates of gas flow through the mask were varied from 1501 min⁻¹ to 1,3001 min⁻¹ during different runs to produce excurrent oxygen concentrations between 20.6 and 20.1%. During the runs the laboratory was ventilated by a large fan and ambient oxygen concentrations remained constant at ~20.9%. Previous studies of horses, including pony B of ref. 4, found no change in blood lactate levels when animals ran on a level treadmill at speeds up to 10 m s⁻¹; therefore we conclude that there is no anaerobic contribution to energy consumption at speeds $<7 \,\mathrm{m\,s^{-1}}$.

There was a speed for each gait where the amount of oxygen used to move a given distance (rate of exygen consumption divided by speed) reached a minimum value (Fig. 2). This speed represents one that is energetically optimal for each gait. The minimum values were about the same for a walk, a trot and a gallop. Therefore, the amount of energy consumed by a horse to move a given distance is the same at these optimal speeds.

To determine whether animals normally moved at energetically optimal speeds, we measured speeds and gaits of a horse as it moved freely over a marked grid. We found that the horse selected speeds within each gait around the energetically optimal speed (Fig. 2). Pennycuick³ has observed that migrating African animals use only a restricted range of speeds within each gait. This suggests that these animals might also be using an energetically optimal speed for each gait.

Linear regression of the data provides a reasonable fit to the relationship between oxygen consumption and speed if one disregards the data obtained when the animals had abnormally extended gaits $(r^2 = 0.98)$. In fact the curvilinear relationship

^{*}To whom correspondence should be addressed at: Department of Biological Sciences, California State Polytechnic University, Pomona, California 91768, USA.

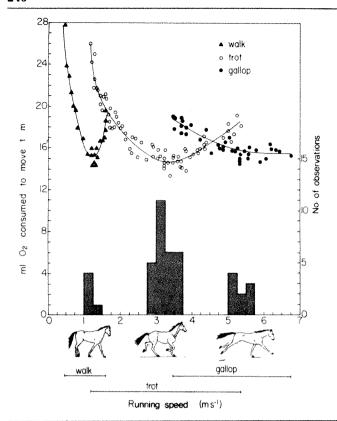


Fig. 2 The oxygen cost to move a unit distance (rate of oxygen consumption divided by speed, calculated from data in Fig. 1) declined to a minimum and then increased with increasing speed in a walk and trot. It also declines to a minimum in a gallop, but we were unable to obtain sufficiently high galloping speeds to observe any increase at high galloning speed if it occurred. The minimum oxygen cost to move a unit distance was almost the same in all three gaits. The histogram shows gaits where horse B was allowed to select her own speed while running on the ground. She chose three speeds which coincided with the energetically optimal speed for each gait. On a motorized treadmill, the animal must move at the speed of the tread, but note that when running on the ground, there were ranges of speeds which the animal never used for any sustained period.

can only be observed when great care is taken to avoid any source of variability in the data. Therefore, it seems useful to continue to use a single linear relationship between rate of oxygen consumption and speed for comparative studies of energetics of locomotion.

We conclude that horses, like humans, change gait and select speed within a gait in a manner that minimizes energy consumption. Observations of speeds used by migrating African animals³ suggest that this finding may also apply generally to terrestrial animals.

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- 1. Margaria, R. Atti. Accad. naz. Lincei Rc. 6, 7 (1938)
- Knuttgen, H. G. Acta physiol. scand. **52**, 366-371 (1961). Pennycuick, C. J. J. exp. Biol. **63**, 775-799 (1975).
- Seeherman, H. J., Taylor, C. R., Maloiy, G. M. O. & Armstrong, R. B. Resp. Physiol. (in the

Gene expression in visna virus infection in sheep

Michel Brahic, Linda Stowring, Peter Ventura & Ashlev T. Haase

Infectious Disease Section, Veterans Administration Medical Center and Departments of Medicine and Microbiology, University of California, San Francisco, San Francisco, California 94121, USA

Visna is a slow degenerative disease of the central nervous system (CNS) of sheep caused by a nontumorigenic retrovirus'. During the course of this disease, visna virus establishes a persistent infection of the CNS, lung and haematopoietic system, despite a specific humoral and cellular immune response. We have studied visna virus life cycle at the single-cell level in choroid plexus of experimentally infected animals, using a very sensitive and quantitative in situ hybridization assay2. We report here that although proviral DNA is synthesized in significant amounts, its expression is blocked at the transcriptional level. This restriction of proviral DNA transcription offers an explanation for the slowness of the disease and the persistence of the infection.

Two aspects of visna infection are relevant to the question of slowness and persistence. First, the virus is predominantly cellassociated, especially in the blood; second, the amount of virus present at any time during the course of the disease is minimal³. This limitation in virus growth is not a consequence of the immune response because it is observed before virus-specific antibodies can be detected, and because immunosuppression of infected animals does not lead to a higher titre of virus4. Restricted virus replication therefore seems to be an intrinsic property of the virus-cell interaction in the tissues of the animal (in vivo). This in vivo situation is in sharp contrast to virus replication in ovine cells grown in vitro, where the virus grows to high titre and causes a characteristic cytopathic effect that culminates in cell death 48-72 h after infection.

The first clue to the mechanism of restriction of replication in vivo was revealed by studies which showed that proviral DNA can be detected by in situ hybridization in a significant fraction

of the cells in the choroid plexus, but that only an occasional cell contains the major structural viral polypeptide p30 as detected by immunofluorescence⁵. This restriction of proviral DNA expression provided an explanation for the persistence of the infection: the immune defence mechanisms are unable to eradicate the infection because the virus persists intracellularly in cells that do not express viral antigens. Therefore, an understanding of the mechanism of restriction of viral gene expression is central to the study of visna pathogenesis.

We chose to study viral replication in the choroid plexus because histopathological lesions of visna always predominate in this tissue as well as in the periventricular areas of the brain³, and because persistent infection of the choroid plexus has been clearly documented by isolation studies³. Although it is a relatively simple structure, the choroid plexus consists of various cell types (cuboidal epithelium cells, fibroblasts, endothelial cells and smooth muscle cells) interspersed with large numbers of inflammatory cells characteristic of visna lesions. Because of this complex setting and the possibility of local variations in the extent of infection, virus replication must be studied at the single-cell level. Viral protein synthesis was assessed by immunofluorescence using an indirect assay and the labelled avidin-biotin method of detection⁶. Virus nucleic acids were quantitated in single cells in histological sections by in situ hybridization² modified for the detection of proviral DNA as described in Fig. 1 legend. This assay will detect 1 to 2 copies of viral RNA and 10 copies of proviral DNA per cell after three weeks of autoradiographic exposure (10 grains per cell above background).

Three American lambs were inoculated intracranially with 10° plaque-forming units (PFU) of visna virus strain 1514. The inoculum (1 ml) was deposited in the vicinity of the lateral ventricule. A large inoculum was chosen to infect a maximum number of cells at the site of inoculation. The animals were killed between 7 and 15 days after inoculation, and the choroid plexi removed. Inflammatory lesions typical of visna were observed in all cases. A portion of choroid plexus was frozen, subsequently sectioned in a cryomicrotome and assayed for viral nucleic acids and proteins. Examples of the results obtained by in situ hybridization are shown in Fig. 2. Proviral DNA and viral RNA were readily detected in these sections. In all cases where the cells could be unambiguously identified, viral nucleic acids were present only in cuboidal epithelium cells; they were not found in vessels, connective tissue cells or inflammatory mononuclear cells.

The fraction of choroid plexus cells carrying proviral DNA and viral RNA was determined (Table 1); values range between

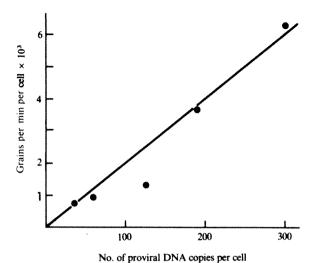
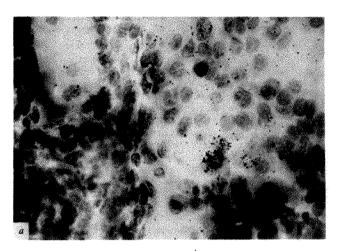


Fig. 1 Relationship between the amount of proviral DNA per cell and the number of autoradiographic grains developed after in situ hybridization. Sheep choroid plexus cells grown in vitro were infected with 3 PFU of visna virus per cell. At various times after infection (10, 14, 23, 48 and 72 h), the cells were collected and subjected in parallel to in situ hybridization and determination of proviral DNA content by liquid hybridization9. For in situ hybridization microscope slides were treated with bovine serum albumin, Ficoll and polyvinyl pyrrolidone as described elsewhere² cells were deposited on treated slides using a cytocentrifuge, air dried for a few minutes and fixed for 4 min at -20°C in a mixture of methanol and acetone (1:2, v/v). The slides were treated with HCl, heat and proteinase K as described elsewhere². RNA was eliminated by treating the slides for 30 min at 37°C with 100 µg ml of RNase A and 10 units ml⁻¹ of RNase T_1 in $2 \times SSC$ (0.3 M NaCl, 0.03 M Na citrate, pH 7.5), followed by two washes in $2 \times SSC$ and dehydration in graded ethanol. To ensure maximal retention of viral DNA, the slides were refixed in 5% formaldehyde at room temperature for 2 h, then washed twice in 2×SSC and dehydrated in graded ethanol. DNA denaturation was achieved at 65°C for 15 min in 95% deionized formamide, 0.1×SSC followed by quenching in ice-cold 0.1×SSC. The slides were washed twice more in 0.1 × SSC and dehydrated in graded ethanol. Hybridization in situ was as described elsewhere² except that the concentration of cDNA was 0.8 ng μl^{-1} . Washing of the slides, dipping in NTB-3 Kodak emulsion and exposure was as described elsewhere². The slides were exposed for 3 weeks (10-h infected cells), 11 days (14 and 24-h infected cells) and 48 h (48 and 72-h infected cells). An average of 1,500 grains were counted for each slide; the extent of in situ hybridization was expressed as number of grains per min of exposure time per nucleus. Uninfected cells were processed in parallel for the determination of background. The background was 1.7×10^{-4} grains per min per nucleus.

1 and 3%. For a given animal the same fraction of cells contained proviral DNA and viral RNA. Table 1 also shows averaged values of proviral DNA and viral RNA content per cell. The distribution of values for individual cells is shown in Fig. 3. On average, infected cells contained 60–70 copies of proviral DNA per cell. Although the values are spread over a relatively broad range, cells containing levels of proviral DNA comparable to those observed at the end of the permissive cycle (200–300 copies per cell (B. Traynor, personal communication)) were extremely rare (Fig. 3). The average content of viral RNA in the tissues varied between 100 and 180 copies of 35S RNA genome equivalents, and the distribution of RNA content among cells was relatively narrow, with almost no cells containing more than 500 copies. These values are almost two orders of magnitude below the level of RNA synthesis observed at the end



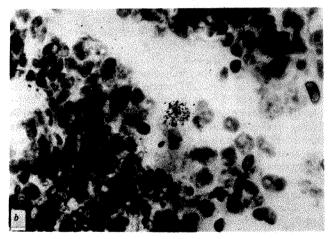


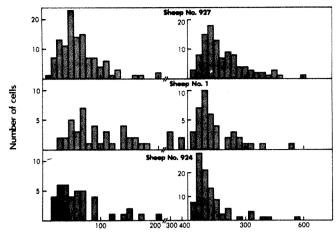
Fig. 2 Detection of proviral DNA (a) and viral RNA (b) in sections of choroid plexus from experimentally infected sheep. The figure shows one representative field in each case. Exposure times were 3 weeks (a) and 48 h (b).

Table 1 Numbers of choroid plexus cells carrying proviral DNA and viral RNA

		Viral DNA No. of positive cells	Viral RNA No. of positive cells	Avera	% Of cells		
Sheep no.	Days after inoculation	Total no. of cells (%)	Total no. of cells (%)	Viral DNA	Viral RNA	containing viral proteins	
927	7	$142/1.5 \times 10^4 (0.9)$	$325/2.5 \times 10^4 (1.3)$	61	177	0	
1	10	$156/1.5 \times 10^4 (1.0)$	$394/2.5 \times 10^4 (1.6)$	74	129	0.0025	
924	15	$429/1.5 \times 10^4 (2.8)$	$684/2.5 \times 10^4 (2.7)$	61	105	0	

The number of cells positive for viral DNA was measured after 6 weeks of autoradiographic exposure, and for viral RNA was measured after 3 weeks of exposure. Total number of cells was estimated from the number of cells within the area of a reticle and the ratio of the area of the reticle to that of the section. Percentage of cells containing yiral proteins was determined by immunofluorescence using anti-p30 serum.

* One copy corresponds to a MW of 6×10^6 double-stranded provinal DNA or 3×10^6 single-stranded viral RNA.



Proviral DNA (copies per cell)

Viral RNA (copies per cell)

Fig. 3 Distribution of proviral DNA and viral RNA content among choroid plexus cells in vivo. The choroid plexus of three experimentally infected animals were examined by in situ hybridization. The autoradiographic exposure times were 3 weeks and 48 h for proviral DNA and viral RNA detection, respectively. In each case a representative section (~12 mm²) was systematically scanned under the microscope at a magnification of ×1,000. The number of grains per cell was counted for each positive cell. The number of genome copies per cell was determined from calibration curves (see Fig. 1 and ref. 2). The histograms were constructed by grouping copy numbers to the nearest multiple of 10.

of a permissive cycle (=10,000 copies per cell⁷). Hybridization to proviral DNA was totally dependent on denaturation and was sensitive to pretreatment with DNase, therefore ruling out the possibility that the hybrids observed in the DNA assay were due to residual viral RNA. Immunofluorescence was carried out on subjacent sections of choroid plexus from the same animals; no convincingly positive cells were detected in two animals. A positive cell was occasionally found in the choroid plexus of sheep no. 1 (Table 1).

This quantitative analysis of viral replication in the choroid plexus demonstrates the following: (1) visna virus infects cuboidal epithelial cells. (2) Despite a relatively large inoculum deposited in the vicinity of the choroid plexus, the proportion of cuboidal choroid plexus cells infected is relatively low (=1%). (3) The level of viral RNA expressed in these cells is minimal. Infected cells constitute a fairly homogeneous population of cells containing 100-200 viral genome equivalents. In this respect these cells are similar to in vitro infected permissive cells arrested at a very early stage of the viral life cycle. Although they contain relatively high levels of proviral DNA (60-70 copies per cell), their RNA content is about two orders of magnitude lower than during a permissive viral replication cycle. This shows that the restriction of proviral DNA expression is regulated, at least in part, at the transcriptional level. (4) Choroid plexus cells do not contain viral structural proteins as determined by immunofluorescence.

These results represent a new step in the understanding of visna pathogenesis. Two characteristic aspects of this disease are the low level of virus expression and the persistence of the infection for long periods of time in spite of an immune response. Here we have documented the restriction of virus expression at the single-cell level. Minimal virus production is due to the limitation of the infection to a small fraction of the cells and the existence in these cells of a block at the level of proviral DNA transcription. We propose that restricted proviral DNA expression plays a part in two aspects of visna pathogenesis. First, it provides a damping mechanism which prevents the rapid spread of the infection and its expression as an acute disease and also limits the damage caused by viral replication to individual cells. (Indeed cell necrosis is conspicuously absent in visna, particularly during the early incubation period⁸.) Second, it allows the virus to persist: cells which are not killed as a result of the infection and which do not express viral antigens at their

surface provide the virus with a site where it can persist without being detected and eradicated by the immune defence mechanisms.

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- 1. Brahic, M. & Haase, A. T. in Comparative Diagnosis of Viral Diseases Vol. 3 (ed. Kurstak, E) (Academic, New York, in the press).
- Brahic, M. & Haase, A. T. Proc. natn. Acad. Sci. U.S.A. 75, 6125-6129 (1978).
 Petursson, G., Nathanson, N., Georgsson, G., Panitch, H. & Paisson, P. A. Lab. Invest. 35, 402-412 (1976)
- Panitch, H., Petursson, G., Georgsson, G., Palsson, P. A. & Nathanson, N. Lab. Invest. 35, 452-560 (1976)
- Haase, A. T., Stowring, L., Narayan, O., Griffin, D. & Price, D. Science 195, 175-177
- Guesdon, J., Ternynck, T. & Avrameas, S. J. Histochem. Cytochem. 27, 1131-1139 (1979). Brahic, M., Filippi, P., Vigne, R. & Haase, A. T. J. Virol. 24, 74-81 (1977).
- Georgsson, G., Palsson, P. A., Panitch, H., Nathanson, N. & Petursson, G. Acta neuropath.
- 9. Haase, A. T. & Varmus, H. E. Nature new biol. 245, 237-239 (1973).

Apparent reversion of stable in vitro genetic markers detected in tumour cells from spontaneous metastases

J. Dennis, T. Donaghue, M. Florian & R. S. Kerbel

Cancer Research Laboratories, Department of Pathology, Queen's University, Kingston, Ontario, Canada K7L 3N6

The evolution towards more aggressive and autonomous behaviour of many cancerous tumours, often referred to as tumour progression¹, is thought to stem from the development of heterogeneity within the tumour cell population, combined with the continuous selection of progressively more malignant cellular phenotypes^{2,3}. During the course of the disease, the tumour cells show multiple phenotypic changes in a stepwise, but apparently random fashion, becoming more anaplastic, increasingly independent of growth controls and more metastatic4. Several laboratories, including our own, have analysed aspects of tumour heterogeneity and cancer metastasis by selecting and studying the properties of lectin-resistant (Lec^R) membrane mutant tumour sublines⁵⁻⁸; in a few cases, such variants have been claimed to be less tumorigenic^{5,6} or metastatic^{7,8} than the parental cells from which they were derived. We have attempted to study the factors involved in the reestablishment of tumour heterogeneity by monitoring the stability in vivo of Lec^R phenotypes of metastatic tumour cells after injection of cloned Lec^R tumour cells. We now report that spontaneous metastases arising after a subcutaneous (s.c.) injection of cells from variant tumour lines selected from a highly metastatic DBA/2 mouse tumour known as MDAY-D29, and which were stably resistant in tissue culture to wheat germ agglutinin (WGA), no longer carry the WGA-resistant (WGAR) phenotype. The results demonstrate that WGA tumour cells do not metastasize, but rather, 'revertants' for the WGAR phenotype, which presumably were generated in vivo after injection, were the cells actually capable of metastatic

The cloned WGAR variant subline MDW4 was selected from the parental MDAY-D2 tumour line by its ability to grow in normally toxic concentrations (for example, 20-50 µg ml⁻¹) of WGA⁵. We have previously shown that MDW4 carries a second phenotypic marker-a deficiency of fucose salvage (fucose salvage caused by a block in the conversion of fucose-1-P to GDP-fucose⁸. These phenotypes seemed to be remarkably stable in MDW4 cells maintained in tissue culture for more than 2.5 yr (Fig. 1). A second variant cell line, MDOW-2b, was sequentially selected for its ability to grow in high concentrations of the toxic membrane-active drug ouabain⁹ (3 mM)

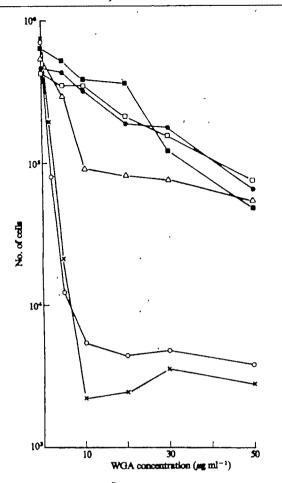


Fig. 1 Stability of the WGA[®] phenotype of the MDW4 tumour clone maintained in tissue culture. Tumour cells were grown in tissue culture in α-MEM (Flow) plus 10% fetal calf serum with subculturing every 3–5 days. The variant was periodically tested for the ability to grow in the presence of WGA; Δ, 10 July 1978; ∰, 6 December 1978; ∭, 10 June 1980; □, 1 October 1980. MDW4 cells were seeded at 3×10³ cells mi⁻¹ in the presence of 0, 2, 5, 10, 20, 30 and 50 μg mi⁻¹ WGA and were grown in ethn until those seeded in the absence of WGA had reached confluence (7–9 days), after which cell numbers were determined in each culture. The revertant, WGA-sensitive (WGA[®]) phenotype of the tumour lines obtained from metastasses and maintained in tissue culture also remained unchanged for the reverted phenotypes after more than 6 months MDW4-22a cells were tested on 22 August 1980 (O) and again on 10 January 1981 (×) The WGA[®] profile of the parental MDAY-D2 cells is shown in Fig. 2, and has also been described previously[§].

and WGA (20 µg ml⁻¹). MDOW-2b did not possess the fucose salvage⁻ phenotype.

As reported previously⁸, WGA^R variants of MDAY-D2 grew and metastasized in an altered way in the syngeneic DBA/2 host. Thus, a s.c. injection of 10³ MDAY-D2 tumour cells produced visible foci of tumour growth in the liver within 2 weeks of injection. In contrast, no liver metastases were evident 2 weeks after a s.c. injection of 105 cells of either MDW4 or MDOW-2b. The number of metastatic nodules, at death, in the livers of mice which had received 10⁵ MDW4 cells s.c. ranged from 0 to 90, whereas mice which died after injection with the same dose of MDAY-D2 cells had almost complete liver replacement with tumour tissue (that is, the number of tumour foci were excessive and not discrete, and therefore not easily counted). An intravenous (i.v.) injection of 103 MDW4 cells results in no tumour takes, whereas i.v. injection of as few as 10 MDAY-D2 cells results in widespread metastases, similar to that observed after s.c. injections. Mice succumbed to the WGAR variants in ~60 days, while the parental tumour MDAY-D2 killed the mice in 35 days (Table 1). We concluded from these results that the WGAR variants, although still malignant, were less so than the parental MDAY-D2 tumour.

Due to the potential ability of tumour cells to change phenotypes and become progressively more malignant in animals¹⁰, it

was not immediately clear whether the few metastases produced by a s.c. injection of the WGAR variants was indicative of an inherently less metastatic tumour line, or of an essentially non-metastatic line which, as a result of the generation and selection of more malignant variants in vivo, showed limited metastasis of these new variants. If ouabain-resistant (Oua"), WGAR and/or fucose salvage phenotypes were related to a reduction in the metastatic potential of the tumour cells, then from the first hypothesis we could predict that metastases would retain the same phenotypes as the injected cells. Conversely, the latter hypothesis would predict that metastases would show a high incidence of revertant phenotypes. To examine this question, solid tumours at the site of injection and individual spontaneous metastases from DBA/2 mice which had been injected with either MDW4 or MDOW-2b cells were removed and re-established as tissue culture lines. The tumour cells were removed from single, discrete metastatic foci obtained from liver, kidney, brain, spleen or intestine.

Tumour cells at the site of injection and metastases resulting from a s.c. injection of MDOW-2b tumour cells retained their Oua^R phenotype but were revertant for WGA^R (Fig. 2). Retention of the OuaR phenotype indicated that the tumour nodules originated from the injection of MDOW-2b cells and were not new tumours, induced in situ11. Similarly, all metastases resulting from s.c. MDW4 cells were revertant for the WGA^R and fucose salvage phenotypes, two characteristics of tumour cells which have previously been suggested to influence metastatic capacity 6-8,12 (Table 1). The reverted phenotype of the tumour lines derived from metastases were stable as they remained unchanged after more than 6 months in tissue culture (Fig. 1). Furthermore, injection of the cultured tumour cells established from metastatic foci resulted in rapid tumour growth (27-35 days for 50% mortality) and the same metastatic pattern of liver replacement observed with the parental tumour MDAY-D2 (Table 1).

In contrast to the metastases, not all the 'primary' s.c. growing solid tumours were complete revertants for both WGAR and fucose salvage" (Table 1). An inoculum of 102 MDW4 cells produced a solid tumour which was a 'complete revertant' (MDW4-11) while inocula of 10⁴ and 10⁵ MDW4 cells produced tumour cell populations at the site of injection which seemed to be only partially reverted for either the WGAR or fucose salvage phenotypes. The finding that injection of a low dose of MDW4 cells resulted in a solid tumour which was a complete revertant (MDW4-11), while higher doses of MDW4 produced tumours at the site of inoculation which were only partially reverted for the WGAR and fucose salvage" phenotypes (a heterogeneous population of cells), suggests, but does not prove, that the revertants did not exist in the original inoculum of MDW4 cells, but arose later in the animals and were selected as a consequence of their superior ability to survive in vivo. Hence, it seemed that the MDW4 metastases were revertant for both WGAR and fucose salvage, even in the cases where the solid tumour displayed only a partially reverted phenotype; this suggests that the cells capable of metastasizing consisted of a small subpopulation of tumour cells originating at the site of inoculation and displaying revertant phenotypes.

The results of the following experiment support this interpretation (Table 2). Intravenous injection of MDW4 cells, as already stated, even when as many as 10^5 cells are given, does not result in any tumour takes (Table 2). The same is true when nude mice are used as recipients (data not shown). However, if one mixes as few as 10 cells from a metastasis (designated MDW4-22a)—which arose in a DBA/2 mouse after s.c. injection of MDW4 cells—with 10^5 MDW4 cells, and injects the mixture i.v., 75% (n=4) of the animals rapidly develop metastases and die within 4 weeks. In view of these results and the fact that the s.c. injection of as few as 100 MDW4 cells leads to WGA-sensitive metastases (Table 1), it is difficult to argue that pre-existing cells revertant for the WGA²² phenotype in the MDW4 cell line were entirely responsible for the reported findings.

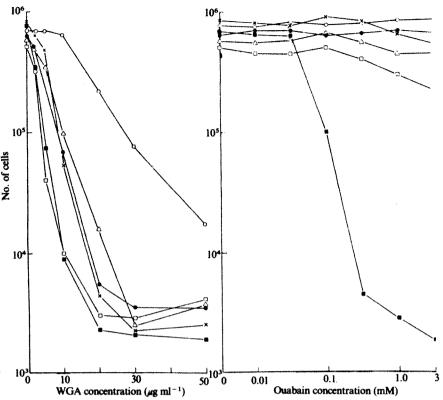


Fig. 2 WGA and ouabain sensitivity of MDAY-D2, MDOW-2b and of cell lines established from spontaneous metastases removed from the liver of an MDOW-2b-bearing mouse. DBA/2 mice were given a s.c. injection of 10⁵ MDOW-2b cells followed 60 days later by removal of tumour tissue from the site of injection (MDOW-11) and three clearly discrete metastatic nodules found in the liver (MDOW-21a, MDOW-21b, MDOW-21c). Tumour tissue removed from the mouse was minced, trypsin treated and the resulting cell suspension placed in tissue culture for at least 3 weeks to allow tumour cells to outgrow host-derived cells. The established tissue culture lines were then tested for the ability to grow in the presence of either WGA (left panel) or ouabain (right panel) as described in Fig. 1 legend; ■, MDAY-D2; ○, MDOW-2b; ×, MDOW-11; △, MDOW-21a; □, MDOW-21b; ●, MDOW-21c.

Several mechanisms can be envisaged for the generation of the revertant and more malignant phenotypes—classical genetic mechanisms of back mutation, acquisition or loss of genetic material by the tumour cells, stable regulatory changes in gene expression or somatic cell hybridization in vivo between a tumour cell and a host cell. The last possibility has been demonstrated previously¹³ but seemed unlikely in the present situation because Oua^R is a co-dominant marker⁹, such that a somatic

hybrid between Oua^R and a wild-type cell results in a partial loss of Oua^R in the resultant hybrid⁹, an event not observed for the metastases of MDOW-2b. We are now carrying out experiments to examine this possibility in more detail, and the other potential mechanisms involved in the generation of the revertant phenotypes in vivo.

The results demonstrate a need to examine the progeny, especially metastases, arising from an injection of selected

Table 1 WGAR and fucose salvage" phenotypes of subcutaneous and metastatic tumour nodules

Cell line	Source	WGA resistance (mg ml ⁻¹)	³ H-fucose/ ³ H-leucine* (c.p.m./c.p.m.×10 ⁻¹)	50% mortality† (days)	Liver metastases at death‡
MDW4-11	1°, 10 ² s.c.	2	1.70 ± 0.30 (195)	33	С
MDW4-21a	2°, liver	2	Not tested	30	C
MDW4-21b	2°, liver	2	1.91 ± 0.56 (220)	35	C C
MDW4-21c	2°, liver	2	1.70 ± 0.28 (195)	32	C
MDW4-12	1°, 10 ⁴ s.c.	2	0.38 ± 0.07 (44)	29	С
MDW4-22a	2°, liver	2	0.73 ± 0.18 (84)	30	C
MDW4-22b	2°, liver	2	0.70 ± 0.35 (81)	27	C C C
MDW4-22c	2°, kidney	2	$0.84 \pm 0.16 (97)$	31	C
MDW4-22d	2°, brain	2	0.71 ± 0.07 (82)	30	C
MDW4-14	1°, 10 ⁴ s.c.	2	0.08 ± 0.03 (9)	32	С
MDW4-24a	2°, liver	2	$1.16 \pm 0.11 (133)$	34	C
MDW4-24b	2°, liver	2	2.05 ± 0.89 (236)	34	C
MDW4-24c	2°, liver	2	1.84 ± 0.37 (211)	35	С
MDW4-13	1°, 10 ⁵ s.c.	43	$0.26 \pm 0.1 (30)$	30	C
MDW4-23a	2°, liver	2	1.37 ± 0.25 (157)	31	C C
MDW4-23b	2°, liver	2	$1.27 \pm 0.28 (146)$	29	C
MDW4-23c	2°, spleen	2	$1.18 \pm 0.09 (136)$	33	C
MDW4-23d	2°, gut	2	$1.33 \pm 0.17 (153)$	30	C
MDAY-D2	Tissue culture line	5	$0.87 \pm 0.21 (100)$	35	C
MDW4	Tissue culture line	50	0.03 ± 0.01 (3)	63	N(3-90)

DBA/2 mice were given a s.c. injection of the WGA^R, fucose salvage⁻ tumour cells MDW4. Seven to ten weeks after the injection of 10², 10⁴ or 10⁵ MDW4 cells, tumour tissue at the site of injection (1°) and three or four secondary metastatic nodules (2°) were removed, minced with scissors, trypsin treated to make a cell suspension and established as tissue culture lines. The cell lines derived from metastases (2°), which are listed below each primary (1°), were removed from liver, lung, spleen, brain, kidney or intestine of the same mouse. The concentration of WGA necessary to cause a 90% inhibition of tumour cell growth in tissue culture was determined as described in Fig. 1 legend.

* Tumour cells, 10^5 in $200 \,\mu$ l of culture medium, were pulse-labelled with $1 \,\mu$ Ci of L- $[6-^3H]$ fucose (27 Ci mmol $^{-1}$) and with $1 \,\mu$ Ci of L- $[4, 5-^3H]$ leucine (60 Ci mmol $^{-1}$) in parallel for 16 h at 37 °C. Acid-precipitable material was washed three times in 10% trichloroacetic acid followed by solubilization and determination of radioactivity. The ratio of fucose to leucine was determined for each cell line and the numbers in parentheses are 3H -fucose to 3H -leucine ratios expressed as a percentage of the 3H -fucose to 3H -leucine ratio for MDAY-D2. The results are the mean of three determinations performed in duplicate $\pm s.d.$

† Twelve DBA/2 mice were given s.c. injections of 10⁵ MDW4 or MDAY-D2 cells and the number of days required for 50% of the animals to succumb to the tumours was recorded. For each of the 1° and 2° tumour cell lines, four DBA/2 mice were used for the 50% mortality determinations. The tumour lines were maintained in tissue culture for at least a month before assessing their malignancy.

‡ Tumour infiltration of the liver was examined at death and described as confluent with tumour (C) or infiltrated with a limited number of nodules (N); the range is indicated in parentheses.

Table 2 Tumour takes in DBA/2 mice injected intravenously with MDW4 and cells from MDW4 metastases

Tumour cells injected	Tumour takes per no. of mice injected
MDW4 (10 ⁵)	0/8
$MDW4 (10^5) + MDW4-22a (10)$	3/4
$MDW4 (10^5) + MDW4-22a (10^2)$	4/4
MDW4-22a (10 ²)	4/4

DBA/2 mice were given a single i.v. injection of MDW4, MDW4-22a or a mixture of the two cell lines, and tumour growth was determined at death by autopsy. Animals with tumour died 25-35 days after tumour injection. Those that survived were found to be tumour free 4-5 months after inoculation. MDW4-22a refers to a cell line established from a liver metastasis which had been removed from an animal previously injected s.c. with MDW4 cells. The numbers in parentheses refer to the number of cells injected i.v.

tumour variants to determine whether or not they are phenotypically the same as the cells injected, especially when the selecting agent seems to influence the malignant potential of the variant (WGA). Tumour variants selected in vitro which display a decreased malignant potential are apparently subject to the forces which generate tumour heterogeneity in vivo. Consequently, revertant and more malignant phenotypes may

arise relatively quickly when the cells are injected into animals. Our work extends the results of Tao and Burger⁶, who have shown that WGAR variant tumour cells are often poorly metastatic. We have demonstrated that the WGAR variants may be non-metastatic and that the WGAR tumour cells must undergo phenotypic alterations, including a reversion of WGAR, before metastasis can occur.

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- Prehn, R. T. Adv. Cancer Res. 23, 203-236 (1976).
- Nowell, P. C. Science 14, 23-28 (1976). Klein, G. & Klein, E. Transplantn Proc. 9, 1095-1104 (1977). Poste, G. & Fidler, I. J. Nature 283, 139-146 (1980).
- Kerbel, R. S. Am. J. Path. 97, 609-622 (1979)
- Tao, W.-W. & Burger, M. M. Nature 270, 437-438 (1977).
- Reading, C. L., Belloni, P. N. & Nicolson, G. J. J. nam. Cancer Inst. 64, 1241-1249 (1980). Dennis, J. W. & Kerbel, R. S. Cancer Res. 41, 98-104 (1981).
- Baker, R. M. et al. Cell 1, 9-21 (1974).
- Daker, N. M. et al. Cell 1, 7-21 (1974). Schirmacher, V. Immunobiology 157, 89-98 (1980). Kerbel, R. S., Florian, M., Man, M. S., Dennis, J. & McKenzie, I. F. C. J. natn. Cancer Inst. 64, 1221-1231 (1980).
- Vischer, P. & Reutter, W. Eur. J. Biochem. 84, 363-368 (1978).
 Wiener, F., Fenyö, E. M. & Klein, G. Nature new Biol. 238, 155-159 (1972).

A single dominant gene determines susceptibility to a leukaemogenic recombinant retrovirus

Robert S. Schwartz & Raman H. Khiroya

Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111, USA

The production of recombinant retroviruses is an important episode in the natural history of thymic leukaemia in AKR and HRS/J (hr/hr) mice^{1,2}. These viruses apparently originate from ecotropic and xenotropic precursors in the late preleukaemic stage of the disease. Analyses of their structural proteins^{3,4} and genomic oligonucleotides^{3,5} indicate that they arise by recombination of env genes of the precursor viruses. This event leads to a viral envelope glycoprotein (gp70) with some peptides that have features of the gp70 glycoproteins of ecotropic and xenotropic viruses, and others that are unique for each recombinant virus^{3,4,6}. The former property explains the broad host range of recombinant viruses, and hence their designation as dual tropic1 or polytropic² viruses. It has been postulated that the unique aspect of each recombinant's gp70 determines the phenotypes of leukaemic cells7. Polytropic viruses may be highly thymotropic. Their systemic administration results in an infection that confines itself virtually to the thymus^{3,8}. Moreover, these viruses are leukaemogenic whereas their precursors are not, or only weakly so^{3,8,9}. The leukaemogenicity of polytropic viruses is, however, restricted to certain inbred strains of mice^{3,8}. The HRS/J isolate PTV-1 is leukaemogenic in HRS/J and CBA/J mice, but not in SWR/J or NIH/Swiss mice³. The experiments described here demonstrate that a single dominant gene permits infection of thymocytes by a leukaemogenic polytropic virus. CBA/J mice, which develop thymic leukaemia after infection by this virus, posesses this gene, whereas leukaemia-resistant NFS mice lack it.

Figure 1 shows the results when newborn mice were inoculated with 10³-10⁴ focus-forming units (FFU) of a cloned polytropic virus, PTV-1. This thymotropic virus is leukaemogenic in CBA/J, but not in NIH/Swiss mice or their inbred counterpart, NFS (ref. 3 and unpublished observations). Its gp70 and genomic RNA are typical of a recombinant virus³. The mice were killed 4-6 weeks after inoculation and the levels of polytropic virus in their thymuses were measured by an infectious centre assay10. Polytropic virus is undetectable in uninoculated animals of the strains used in these experiments³.

CBA/J mice were readily infected by PTV-1, whereas NFS mice had either low or undetectable levels of virus. F₁ mice behaved like CBA/J mice. Susceptibility to PTV-1 is therefore a dominant trait. The upper limit of resistance (100 FFU per 10⁷ thymocytes) was defined by the mean + 2 s.d. of the results in NFS mice and the lower limit that defined susceptibility (440 FFU per 10⁷ thymocytes) was taken as the mean – 2 s.d. of the results in CBA/J mice. The 66 backcrosses to the NFS parent segregated, by these standards, into susceptible and resistant mice in a ratio of 38:28. This ratio is not significantly different from 33:33 ($\chi^2 = 0.88$, P > 0.1). The corresponding ratio in 61 F₂ mice was 44:17, which is not significantly different from 45:15 (3:1) ($\chi^2 = 0.14$, P > 0.1). Exclusion of the eight F_2 mice with intermediate values (between 100 and 440 FFU per 10⁷ thymocytes) does not change the significance of the result $(\chi^2 = 0.53, P > 0.1)$. All backcrosses to the CBA/J parent were susceptible. These results are consistent with the conclusion that a single dominant gene confers susceptibility to infection by PTV-1. We shall refer to this gene as PTV^s .

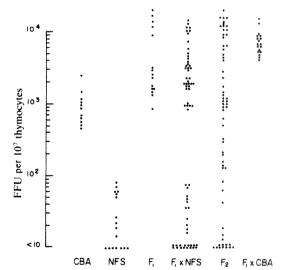


Fig. 1 Results of infection with PTV-1. Mice were injected intraperitoneally with 10^3-10^4 FFU of PTV-1 within 24 h of birth. The animals were killed 4-6 weeks later and cell suspensions prepared from their thymuses. These cells were tested by an infectious centre assay³ on mink lung cells (CCL64). X-C negative viruses capable of infecting both mink and mouse (NIH 3T3) cells were detected in all CBA/J mice injected with PTV-1. Neither mink-tropic nor mouse-tropic viruses can be detected in agematched uninjected CBA/J or NFS mice.

Table 1 Results of infection with ETV-1 (cloned ecotropic retro-

	CBA	NFS
ETV-1	3.3 ± 0.7	3.5 ± 1.2
PTV-1	3.1 ± 0.4	0.9 ± 0.5

Mice were injected intraperitoneally with 10³-10⁴ FFU of ETV-1 within 24 h of birth. The animals were killed 4-6 weeks later and thymocytes were tested in an infectious centre X-C assay¹. Values for ETV-1 are expressed as \log_{10} X-C plaques/ 10^7 thymocytes \pm s.e. Data for PTV-1 are taken from Fig. 1.

The mechanism whereby PTV's confers susceptibility to PTV-1 is unknown. Its action is specific for the polytropic virus, because NFS mice are as susceptible as CBA/J mice to infection by a cloned ecotropic virus (Table 1). The effect of PTV^s does not seem to be immunological because CBA/J and NIH/Swiss

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- 1. Hartley, J. W., Wolford, N. K., Old, L. J. & Rowe, W. P. Proc. natn. Acad. Sci. U.S.A. 74, 789-792 (1977).
- Hiai, H., Morrissey, P., Khiroya, R. & Schwartz, R. S. Nature 279, 247-249 (1977).

- Green, N. et al. J. exp. Med. 152, 249-264 (1980).
 Elder, J. H. et al. Proc nam. Acad. Sci. U. S. A. 74, 4676-4680 (1977).
 Rommelaere, J., Faller, D. V. & Hopkins, N. Proc. nam. Acad. Sci. U.S.A. 74, 495-499
- 6. Elder, J. H., Jensen, F. C., Bryant, K. L. & Lerner, R. A. Nature 267, 23-28 (1977)

mice produced comparable levels of antiviral antibodies after neonatal infection with either PTV-1 or an ecotropic virus (unpublished observation). We favour the view that this gene specifies a receptor on thymocyte surface membranes for the constant region⁶ of recombinant gp70 because that glycoprotein is a major determinant of viral host range¹¹. The specificity of PTVs for the recombinant virus (Fig. 2) supports this interpretation, as the only known difference between viruses of this type and ecotropic viruses is in the gp70 (refs 3,4,6). We cannot exclude an effect that would be analogous to that of Fv-1, which acts after penetration of the virus¹². This possibility seems unlikely, however, because PTV-1 readily infects NIH 3T3 cells in vitro³. Finally, the present results support the view ^{3,8} that the highly selective thymotropism of certain polytropic viruses is an important factor in their leukaemogenicity.

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- 7. Zielinski, C. C., Waksal, S. D., Tempelis, L. D., Khiroya, R. H. & Schwartz, R. S. Nature
- 200, 409-490 (1900).
 Cloyd, M. W., Hartley, J. W. & Rowe, W. P. J. exp. Med. 151, 542-552 (1980).
 Nowinski, R. C. & Hayes, E. F. J. Virol. 27, 13-18 (1978).

- Kawashima, K. et al. Proc. natn. Acad. Sci. U.S.A. 74, 789-792 (1976),
 Stephenson, J. R. in Molecular Biology of RNA Tumor Viruses, 245-297 (Academic, New York, 1980).
- 12. Pincus, T. in Molecular Biology of RNA Tumor Viruses, 77-130 (Academic, New York, 1980).

Sequence and specificity of two antibacterial proteins involved in insect immunity

H. Steiner*, D. Hultmark*, Å. Engström†, H. Bennich† & H. G. Boman*

*Department of Microbiology, University of Stockholm, S-106 91 Stockholm, Sweden [†]The Biomedical Centre, University of Uppsala, S-751 23 Uppsala, Sweden

Immune responses have been described for many different insect species1. However, it is generally acknowledged that insects lack lymphocytes and immunoglobulins and their immune systems must therefore differ from those of vertebrates. An effective humoral immune response has been found in pupae of the cecropia moth, Hyalophora cecropia¹. The expression of this multicomponent system requires de novo synthesis of RNA and proteins² and its broad antibacterial activity is due to at least three independent mechanisms³, the most well known of which is the insect lysozyme⁴⁻⁷. However, this enzyme is bactericidal for only a limited number of Gram-positive bacteria. We recently purified and characterized P9A and P9B, which are two small, basic proteins with potent antibacteral activity against Escherichia coli and several other Gram-negative bacteria⁷. We believe that P9A and P9B play an important part in the humoral immune responses described previously and that the P9 proteins represent a new class of antibacterial agents for which we propose the name cecropins. We describe here the primary structures of cecropins A and B. We also show that cecropin A is specific for bacteria in contrast to melittin, the main lytic component in bee venom9 which lyses both bacteria and eukaryotic cells.

A two-step chromatographic purification of cecropins A and B has been recently described⁷. This purification was improved for sequence work by first using gel filtration on Sephadex G100.

The tentative sequences of both cecropins are given in Fig. 1. Residues 1-33 were obtained by automated Edman sequence analysis and the order of the C-terminal residues 34-37 was deduced from carboxypeptidase Y degradations. This part of the sequence is tentative until confirmed by an independent method. The C-terminal Lys and Ser residues could only be detected after acid hydrolysis of the carboxypeptidase digest; therefore we conclude that the C-terminals are blocked.

The cecropins have very similar overall structures. The Nterminal sequences (residues 1-10) are strongly basic and the central regions (residues 22-30) strongly hydrophobic. The amino acid compositions obtained from the sequences and from automated analyses are compared in Table 1. The agreement between the two sets of data support the structures shown in Fig. 1. Further evidence for the sequences was obtained by partial cleavage of cecropin A at residue 17 using acid hydrosis 10 and isolation of the C-terminal fragment by high-voltage electrophoresis. The amino acid composition of this peptide was in agreement with the structure given in Fig. 1. Cecropin B was cleaved by cyanogen bromide at the single methionine in position 11 and the resulting fragments analysed. This analysis confirmed the order and identity of residues 1-33 but despite several different approaches, we have failed to obtain Edman sequencing data for residues 34-37.

Table 1 Amino acid compositions of cecropins A and B

Amino	Amino Cecropin A		Cecro	pin B
acid	Analysis	Sequence	Analysis	Sequence
Asp	2.01*	1	2.15*	0
Asn		1		2
Thr	1.07	1	0.33	0
Ser	0.17	0	0.53	1
Glu	3.89*	1	1.95*	2
Gln		3		0
Pro	0.95	1	1.04	1
Gly	4.05	4	4.07	4
Ala	4.88	5	4.84	5
Cys	0	0	0	0
Val	3.31	4	2.47	3
Met	0	0	0.80	1
Ile	3.65	5	3.48	5
Leu	1.09	1	1.95	2
Tyr	0	0	0.10	0
Phe	0.98	1	0.87	1
Lys	6.42	7	6.75	7
His	0.06	0	0.29	0
Arg	0.94	1	1.98	2
Trp		1		1
Total residues		37		37

Amino acid composition of the acid hydrolysate (6 M HCl, 100 °C, 24 h) of cecropin A was analysed at the Institute of Biochemistry, Uppsala, Sweden. The results are expressed as mol amino acid per mol protein. These values were calculated with a program that minimizes the deviations from integral numbers.

* Including the corresponding amide.



Fig. 1 The tentative amino acid sequence for cecropins A and B. Replacements of amino acids within the first 33 residues are boxed by solid lines. The C-terminal part boxed by a broken line is tentative. R and R' are unidentified blocking groups. The purified proteins used in sequence determination were obtained by collecting immune haemolymph 7 days after vaccination with live Enterobacter cloacae as previously described. The haemolymph was applied to a Sephadex G-100 column equilibrated with 0.15 M ammonium acetate buffer, pH 5, containing phenylthiourea. The cecropins eluted together with the lysozyme. This material was pooled and further purified by ion-exchange chromatography.

Both forms of cecropin contain one residue each of Trp and Phe. On this basis the theoretical absorbance $(A_{280}, 1 \text{ mg ml}^{-1})$ was calculated to be 1.39 and 1.38 for cecropins A and B, respectively, which agrees well with the respective values of 1.36 and 1.31 determined experimentally, based on the protein concentration obtained from amino acid analysis. This means that an earlier value for cecropin A based on Lowry determination must now be disregarded.

The molecular weights (MWs) calculated from the sequence data (omitting the blocking groups) are 4,005 and 4,036 for cecropins A and B, respectively. Equilibrium centrifugation of cecropin A in 0.19 M ammonium formate, pH 6.5, gave a MW of 4,200±300, which indicates a monomeric form. Furthermore, dimethylsuberimidate did not produce any cross-linking between cecropin molecules in conditions which showed melittin to be a tetramer. In contrast, gel filtration of cecropin A on a calibrated column of Sephacryl S-200 gave a MW of ~14,000. On Sephadex G-100, cecropins A and B eluted together with the lysozyme for which the MW was found to be 15,000 (ref. 7). Thus we cannot determine whether cecropin A is always a monomer.

The following points emerge from the structure of the cecropins. First, the sequences obtained for cecropins A and B show a strong homology; they share 25 amino acid residues and differ in only 12. This suggests that both originate from a gene duplication which may stretch only to residue 32. Assuming that the genetic code is universal, 7 out of 8 amino acid replacements in the first 32 residues can be explained by single shifts in the first base, while the remaining one, a Gln-Arg shift requires a single shift in the second base. More complicated genetic events are needed to explain the amino acid differences in the C-terminal part.

Second, the cecropin sequences have been compared with various other known protein sequences and the greatest

homology was found in residues 3–7 in cerropin A (Lys-Leu-Phe-Lys-Lys)—these are identical to residues 40–44 in the major coat protein of the filamentous phages fd and fl (ref. 11). Homology was also found in residues 25–28 (Ala-Val-Ala-Val) which are also present in the signal peptide of the λ receptor protein 12. It is possible that these two sequences could be involved in the membrane-crossing activities of the respective proteins.

Third, the amino acid composition and size of the cecropins are different from those of the bactericidal and basic proteins in polymorphonuclear leukocytes¹³ and from the antibacterial factors in seminal fluid¹⁴, saliva¹⁵ and milk¹⁶. Thus we conclude that cecropins A and B differ from other known antibacterial proteins. Similar compounds have been found in many other Lepidoptera¹⁷ and we suggest that the name-cecropin should be applied to all antibacterial proteins which show a substantial homology with cecropins A and B.

We have previously shown that cecropins kill and lyse Escherichia coli and some other bacteria⁷. Using other methods we have now compared the biological activity of the cecropins with the bee venom toxin melittin (for a review see ref. 9). The nature of these two molecules is essentially similar; they are both small polypeptides with one part of the chain being strongly basic and one part predominantly hydrophobic. However, the C-terminal in melittin is basic, whereas it is the N-terminal that is basic in the cecropins.

The antibacterial effects of haemolymph, lysozyme, melittin and cecropins A and B were tested on six different bacteria (see Table 2). Activity against all organisms tested was induced in the haemolymph by vaccination with Enterobacter cloacae. Both cecropins had similar antibacterial spectra, although the B-form was significantly more active against Bacillus subtilis. Melittin showed no antibacterial activity against Serratia marcescens and Xenorhabdus nematophilus and for the remaining bacteria it had a lower activity than the cecropins. Differences in activity against the various organisms in Table 2 must be interpreted with care as some of the bacteria can produce proteolytic exoenzymes.

Figure 2 shows the bactericidal and lytic activities of cecropin A and melittin on $E.\ coli$ D31 and on Chang liver cells. Melittin has a high lytic activity against both $E.\ coli$ and the Chang liver cells whereas cecropin A was specific for the bacterial cells. Even at concentrations as high as 20 μ M there was no detectable effect on the Chang liver cells, while a 300 times lower concentration gave 50% lysis of $E.\ coli$. The specificity of the cecropins agrees with earlier findings³ that immune haemolymph has no effect on sheep erythrocytes or insect tissue culture cells (unpublished data).

We have drawn two conclusions from our experiments. First, that the eccropins are the main antibacterial components so far identified in immune haemolymph which act against potential insect pathogens like S. marcescens, Pseudomonas aeruginosa

 Table 2
 Antibacterial activities of lysozyme, cecropins A and B and melittin

Organism		Diame			eter of inhibition	zone (mm)	Water and the second se			
	Strain	NH	IH	Lysozyme	Cecropin A	Cecropin B	Melittin	Blank		
E. coli K12	D31	2.7*	13.2	2.7*	14.0	12.5	7.6	2.7*		
S. marcescens	Db1108	2.7*	6.2	2.7*	7.6	7.2	2.7*	2.7*		
P. aeruginosa	OT97	2.7*	3.9	2.7*	8.6	9.7	4.0	2.7*		
X. nematophilus	Xn21	2.7*	5.0	2.7*	11.0	10.0	2.7*	2.7*		
Bacillus megaterium	Bm11	2.7*	9.5	7.7	10.0	10.0	8.0	2.7*		
E. coli K12	D31		13.0		18.0	17.0				
B. subtilis	Bs11		5.2		2.7*	7.2				

Antibacterial activity was recorded as inhibition zones on thin agar plates of a rich medium plus $\sim 8 \times 10^4$ viable cells per 6 mi. As all strains are streptomycin resistant, all the plates contained streptomycin (100 μ g ml⁻¹). The concentration of cecropins A and B and melittin was 0.13 mM in all cases except the last two, where the cecropin concentration was 0.55 mM. The concentration of the cecropia lysozyme was 0.03 mM. The volume applied to each well was 3 μ l. Normal haemolymph (NH), immune haemolymph (IH) and a blank with 0.4 M ammonium formate buffer were used as references. IH, lysozyme and the cecropins were prepared as described in Fig. 1 legend. The diameter of the inhibition zones is proportional to the log of the concentration of inhibiting agent¹⁷. Well diameter 2.7 mm.

^{*} No inhibition was detected.

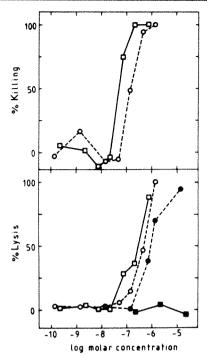


Fig. 2 Comparison of the action of cecropin A (squares) with melittin (circles) on E. coli D31 (open symbols) and Chang liver cells (closed symbols). E. coli (10⁸ viable cells per ml) was incubated with the bacteriolytic agent at 37 °C in 0.1 M phosphate buffer, pH 6.4. After 30 min the tubes were put on ice and samples were withdrawn and diluted for viable count (given as per cent killing in the upper part of the figure). The remaining incubation mixture was centrifuged, and A260 of the supernatant was measured. Lysis is given as per cent of maximum release, corrected for the spontaneous release in buffer alone. Growth of strain D31 and other details were as before⁷. Chang liver cells were cultured in Parker 199 medium containing 5% fetal calf serum (FCS). The cells were washed and labelled with Na₂⁵¹ CrO₄ (0.1 mCi ml⁻¹) at 37 °C for 1 h in a Tris-Hanks buffered solution containing 3% FCS. The monolayer was then trypsinated and washed (R. Alsheikhly, in preparation). The lytic agent was added to 10⁵ cells in 0.1 ml Tris-Hanks medium. The percentage of ⁵¹Cr in the supernatant was measured after a 2-h incubation at 37 °C.

and X. nematophilus. A comparative study 17 which included the greater wax moth, Galleria mellonella, and seven other Lepidoptera showed that cecropin-like substances are widely distributed in this order. As lysozyme is bactericidal for only a limited number of Gram-positive bacteria we believe that its main function is to remove the debris remaining after lysis of bacteria by cecropin.

Second, the cecropins must have a structure that prevents attack on the insect itself. This structural information is presumably absent in melittin; bees avoid self destruction by synthesizing an inactive precursor which is activated by a sequential liberation of dipeptides18. Future studies of the sequences of other cecropins should yield more information about the recognition of self in insects as well as an understanding of the bactericidal mechanism of the cecropins.

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- 1. Boman, H. G. in Microbial Control of Insects, Mites and Plant Diseases, 1970-1980,
- 769-784 (Academic, New York, 1981).

 Boman, H. G., Boman, A. & Pigon, A. Insect Biochem. 11, 33-42 (1981).

 Rasmuson, T. & Boman, H. G. in Developmental Immunobiology, 83-90 (Elsevier,
- A. Powning, R. F. & Davidson, W. J. Comp. Biochem. Pysiol. B55, 221-228 (1976).
 5. Jollès, J., Schoentgen, F., Croizier, G., Croizier, L. & Jollès, P. J. molec. Evol. 14, 267-271
- 6. Jarosz J. Biol. Zbl. 98, 459-471 (1979).

- 7. Hultmark, D., Steiner, H., Rasmuson, T. & Boman, H. G. Eur. J. Biochem. 106, 7-16
- Chadwick, J. M. & Aston, W. P. in Animal Models of Comparative and Developmental Aspects of Immunity and Disease, 1-14 (Pergamon, New York, 1978).
 Mackler, B. F. & Kreil, G. Inflammation 2, 55-65 (1977).
 Inglis, A., McKern, N., Roxburgh, C. & Strike, P. in Methods in Peptide and Protein Sequence
- Inglis, A., McKern, N., ROXDURgh, C. & Strike, P. in Methods in Peptide and Protein Sequence Analysis, 329-343 (Elsevier, Amsterdam, 1980).
 Webster, R. E. & Cashman, J. S. in The Single-Stranded DNA Phages, 557-569 (Cold Spring Harbor Laboratory, New York, 1978).
 Hedgpeth, J., Clément, J. M., Marchal, C., Perrin, D. & Hofnung, M. Proc. natn. Acad. Sci.
- U.S.A. 77, 2621-2625 (1980).
- 13. Spitznagel, J. K. in Salvia and Dental Caries, 391-411 (Information Retrieval, New York,
- Reddy, E. S. P. & Bhargaya, P. M. Nature 279, 725-728 (1979)
- 15. Mandel, I. D. in Salvia and Dental Caries, 473-491 (Information Retrieval, New York,
- 16. Harmon, R. J. in Salvia and Dental Caries, 413-427 (Information Retrieval, New York,
- Hoffmann, D., Hultmark, D. & Boman, H. G. Insect Biochem. (in the press).
- 18. Kreil, G., Haiml, L. & Suchanek, G. Eur. J. Biochem. 111, 49-58 (1980)

Is membrane expansion relevant to anaesthesia?

N. P. Franks

Biophysics Section, Department of Physics, Imperial College of Science and Technology, Prince Consort Road, London SW7 2AZ, UK

W. R. Lieb

Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL, UK

General anaesthesia can be induced by a wide variety of structurally dissimilar molecules. Consequently, the mechanism must involve some rather nonspecific interactions at the target site, generally held to be in nerve membranes. The primary site of action has been postulated to be either lipid or protein or both. Although recent work 1-4 has cast doubt on the lipid hypotheses, protein models still flourish. In particular, Seeman and his co-workers⁵⁻⁸ have shown that biological membranes expand when anaesthetic molecules are added, and that this expansion is far greater than that which occurs with lipid bilayers. It has been suggested that this difference is due to extensive conformational changes in the membrane proteins, and several mechanisms have been proposed^{6,9,10} to explain this large expansion of proteins. We now report the first direct measurements of the volumes occupied by general anaesthetic molecules in both biological membranes and lipid bilayers. We show that, in fact, biological membranes expand much less and lipid bilayers expand more than previously reported. The volume that a general anaesthetic molecule occupies is essentially the same in biological membranes, lipid bilayers and water. Our results lead us to question all generalized membrane expansion hypotheses for the mechanism of general anaesthesia, in favour of hypotheses which include more specialized target sites.

The approach we used consisted of determining membrane densities and membrane/buffer partition coefficients as a function of anaesthetic concentration. A straightforward and extremely accurate method of measuring solution densities, using a vibrating tube, has been developed by Kratky and co-workers¹¹. Commercially available instruments are capable of measuring densities to an accuracy of about 10^{-6} g cm⁻³ measuring the density $\rho_{\rm sus}$ of a membrane suspension and the density ρ_{buf} of the buffer alone, the density of the membrane can be calculated as

$$\rho_{\text{mem}} = \frac{\rho_{\text{sus}} \, \rho_{\text{buf}}}{\rho_{\text{buf}} - R \, (\rho_{\text{sus}} - \rho_{\text{buf}})} \tag{1}$$

where R is the ratio of buffer mass to membrane mass. (This equation can be derived from the simple relationship v_{mem} + $Rv_{\text{buf}} = (1+R)v_{\text{sus}}$, where v stands for specific volume, the reciprocal of density.) Seeman⁸, using such an instrument, has reported large changes in the density of biological membranes but only very small changes in the density of lipid bilayers due to the presence of ethanol. As some of his data have recently been questioned¹², we began by repeating these measurements.

Our results using ethanol are shown in Fig. 1. The curves in Fig. 1a show the progressive decrease in densities of both buffer and a suspension of human red blood cell membranes, as a

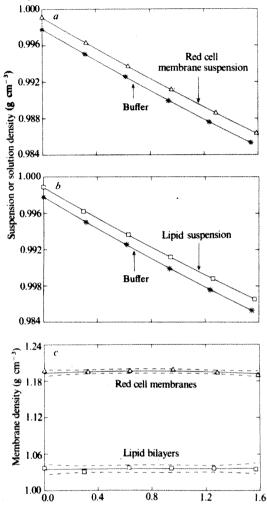


Fig. 1 Membrane density measurements with ethanol. a, Density of human red blood cell membrane suspension (\triangle) and of buffer (*) as a function of the buffer ethanol concentration. b, Density of bovine spinal cord lipid suspension (\square) and of buffer (*) versus buffer ethanol concentration. c, Density of red cell membranes (△) and spinal cord lipid membranes (□) versus buffer ethanol concentration, together with 95% confidence envelopes (dashed lines). Red cell membranes were prepared from transfusion blood (<1 week old) according to the method of Dodge et al. 19. Total spinal cord lipids were especially prepared by Lipid Products Ltd and supplied as a solution in chloroform/methanol. Most of the solvent was removed using a rotary evaporator; remaining traces were removed by pumping under high vacuum for 6 h. The dry lipids were dispersed in buffer by vortex action and brief immersion in a sonicating bath. The buffer used in all our experiments was 5 mM potassium phosphate (pH 8). Precise values for ethanol concentrations were obtained using $c = c_{\rm stk}(M_{\rm stk}/M)(\rho/\rho_{\rm stk})$, where c, M and ρ were the ethanol concentration, the mass and the density, respectively, of the final solution or suspension, and $c_{\rm stk}$, $M_{\rm stk}$ and $\rho_{\rm stk}$ were the corresponding quantities for stock solutions of ethanol in buffer, which were added to membrane suspensions or buffer. For the red cell suspension, a small correction (always <1%) in c was made for partitioning of ethanol into the membranes, using a value of 0.14 moles ethanol per kg membrane/moles ethanol per I buffer. To determine membrane weights, dry weights of the membrane suspensions were measured gravimetrically and corrected for buffer salt weight. The value of R (=buffer weight/membrane weight) for the red cell membrane suspensions was 126.6 ± 2.5 , and 33.33 ± 0.39 for the spinal cord lipid suspension; the errors are for 95% confidence limits. All density measurements were performed using a precision density meter (Anton Paar, Model DMA O2C) at 25 °C. Temperature was maintained constant to within ± 0.01 °C. The curves in a and b were fitted to quadratics using the method of least squares. The curves and data points in c were calculated from the appropriate curves and data points of a and b using equation (1). The 95% confidence envelopes were calculated by least squares fitting of the data points to a quadratic

1.2

Buffer ethanol concentration (M)

04

function of the ethanol concentration in the buffer. Figure 1b gives the analogous results using bovine spinal cord lipids. Finally, Fig. 1c shows red cell membrane and spinal cord lipid densities, together with 95% confidence envelopes, as a function of the buffer ethanol concentration.

The most obvious feature of Fig. 1c is the clear difference in density between red cell and lipid membranes, due, of course, to the fact that red cell membranes contain about 50% protein. More important in the present context is the fact that, as regards the change in density with ethanol concentration, there is no significant difference between red cell membranes and spinal cord lipids. Indeed, there is no significant change in the density of either membrane over the range of ethanol concentrations we used. Our data are in direct disagreement with the experimental results reported by Seeman⁸, which showed a large (5%) change in red cell membrane density over the same range of ethanol concentrations.

It is important to appreciate that there is no simple relationship between changes in membrane density and changes in membrane volume. For example, if an anaesthetic has the same density in a membrane as the membrane itself, large expansions of membrane volume could occur without any change in density. The change in membrane volume can be calculated if one knows the partial molar volume, \bar{V}_{an} , of the anaesthetic as a function of its membrane concentration c_{mem} . \vec{V}_{an} , which has units of volume per mol, represents the change in membrane volume per mol of anaesthetic at a given anaesthetic concentration. The partial molar volume is directly related to changes in membrane density by the expression¹

$$\bar{V}_{\rm an} = \frac{M_{\rm an} - (\partial \rho_{\rm mem} / \partial c_{\rm mem})}{\rho_{\rm mem} - c_{\rm mem} (\partial \rho_{\rm mem} / \partial c_{\rm mem})} \tag{2}$$

where $M_{\rm an}$ is the molecular weight of the anaesthetic, and $\rho_{\rm mem}$ is the membrane density at an anaesthetic molar concentration in the membrane of c_{mem} . If the membrane density is observed to change linearly with concentration, that is, if ρ_{mem} = $\rho_{\text{mem}}^0 + mc_{\text{mem}}$, then equation (2) reduces to

$$\bar{\mathbf{V}}_{\rm an} = (\mathbf{M}_{\rm an} - \mathbf{m})/\rho_{\rm mem}^{0} \tag{3}$$

where m is the slope of the curve of membrane density versus c_{mem} , and ρ_{mem}^0 is the membrane density in the absence of anaesthetic. Using this equation and the red cell membrane data from Fig. 1c, and taking a membrane/buffer partition coefficient of 0.14, $\bar{V}_{\rm an}$ for ethanol lies between 20 and 65 cm³ mol^{-1} , with 95% confidence. Furthermore, the mean \vec{V}_{an} for ethanol in the spinal cord lipids also falls within this range. Now, the partial molar volume of ethanol in buffer, calculated directly from the buffer density data of Fig. 1a,b, varies between 53.5 and 55.1 cm³ mol⁻¹ over the ethanol concentrations used. Thus, there seems to be no dramatic difference in the volume that an ethanol molecule occupies in red cell membranes, lipid bilayers and water.

However, ethanol is not the best choice of anaesthetic molecule if one is interested in measuring partial molar volumes in membranes. This is because, being relatively polar, ethanol has a small membrane/buffer partition coefficient Consequently, only indirect and uncertain estimates of K exist or can be easily obtained. Furthermore, ethanol is not an anaesthetic which is used clinically. We therefore repeated our density measurements using the apolar anaesthetic halothane (Fig. 2) which is both widely used clinically and for which we were able to measure accurate membrane/buffer partition coefficients as a function of anaesthetic concentration. Note that the solution and suspension densities all increase with halothane concentration, whereas the opposite behaviour (decreasing density) was seen with ethanol concentrations (see Fig. 1). This, of course, reflects the fact that liquid halothane ($\rho = 1.87 \, \mathrm{g \ cm^{-3}}$) is much denser than membranes and buffer, whereas ethanol ($\rho = 0.79 \text{ g cm}^{-3}$) is less dense. Figure 2c shows the red cell membrane and spinal cord lipid densities (with 95% confidence envelopes), calculated using the data from Fig. 2a, b and equation (1).

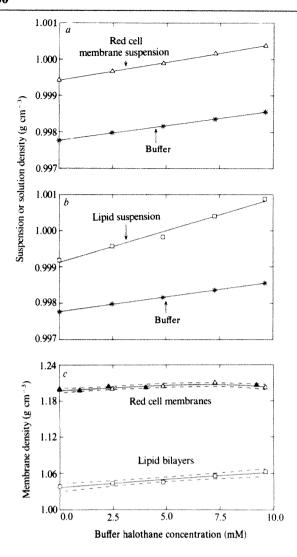


Fig. 2 Membrane density measurements with halothane. a, Density of human red blood cell membrane suspension (\triangle) and of buffer (*) as a function of the buffer halothane concentration. b, Density of bovine spinal cord lipid suspension () and of buffer (*) versus buffer halothane concen tration. c, Density of red cell membranes (\triangle and \blacktriangle) and spinal cord lipid membranes ([]) versus buffer halothane concentration, together with 95% confidence envelopes (dashed lines). The membrane suspensions were prepared and the density measurements performed as described in Fig. 1 legend. Known partial pressures3 of halothane in nitrogen were bubbled through the buffer and then passed over the membrane suspension, which was contained in a small (5 ml) round-bottomed flask. The suspension was stirred, using a glass-encapsulated magnetic 'flea'. This had the important technical advantage that the buffer concentrations of anaesthetic in equilibrium with the membranes was known directly and did not have to be corrected for partitioning into the membranes. The equilibrium concentration of halothane in the buffer and the aqueous phase of the membrane suspension was calculated² using an Ostwald solubility (water/gas partition) coefficient²⁰ of $\lambda = 1.5$ (at 25 °C). To avoid loss of halothane to air, the solutions were directly transferred to the density meter in a syringe containing no air pockets. The value of R (= buffer weight/membrane weight) was corrected for the differential partitioning of halothane between membranes and buffer using $R = R_0(1+M_{\rm an}c_{\rm buf}/\rho_{\rm buf}^0)/(1+M_{\rm an}Kc_{\rm buf}/\rho_{\rm mem}^0)$, where R_0 is the value of R when $c_{\rm buf}=0$. R_0 was $100.0\pm1.9~(\triangle)$, $74.3\pm3.4(\triangle)$ and 26.55±0.24 (□); the errors are for 95% confidence limits. Partition 20.33 ± 0.24 (a), the errors are 10 ± 9.3 confidence limits. Farthful coefficients K (=moles halothane per l membrane divided by moles halothane per l buffer) were determined using 14 C-labelled halothane (NEN) and equilibrium dialysis at 25 °C. The labelled halothane was repeatedly washed with buffer until its halothane/buffer partition coefficient was constant and consistent with its known solubility in water. For the red cell membrane determinations, SDS-polyacrylamide gels run before and after dialysis showed that no significant hydrolysis of membrane proteins had occurred. The measurements were performed as a function of aqueous concentration of halothane from zero to about 8 mM and fitted to least-squares polynominal curves of the statistically appropriate order²¹. The results were $K = 29.5 + 0.84c_{\text{but}}$ for spinal cord lipids and $K = 38.4 - 5.24c_{\text{but}} + 0.770c_{\text{bu}}^2$ for red cell membranes, where $c_{\rm buf}$ has units of moles halothane per l buffer. The density curves in a and b were fitted to straight lines using the method of least squares, and the curves and data points (\triangle, \Box) in c were calculated from the appropriate curves and data points of a and b using equation (1). The additional data points (\triangle) in c are the results from a completely independent red cell membrane experiment and give a measure of the reproducibility of the data. For the red cell membranes two data points are superimposed at zero halothane concentration. The 95% confidence envelopes were calculated by least-squares fitting of the data points.

The data in Fig. 2c clearly show that there is a significant increase in the densities of both red cell membranes and lipid bilayers with increasing halothane concentration (except in the very highest concentration range for red cell membranes—see later). Accurate values of the partial molar volumes $\bar{V}_{\rm an}$ can be obtained using these data if the concentrations of the anaesthetic in the membranes are known. To convert buffer concentrations to membrane concentrations, the partition coefficient K (mols halothane per I membrane divided by mols halothane per I buffer at equilibrium) had to be determined. We measured K as a function of halothane concentration for both red cell and lipid membranes, using equilibrium dialysis and 14C-labelled halothane. Combining these results with the density measurements shown in Fig. 2c, we could calculate membrane density as a function of membrane halothane concentration (Fig. 3). The relationships between membrane density and membrane halothane concentration do not differ significantly from straight lines up to about 300 mM. Therefore, we could calculate $\bar{V}_{\rm an}$ in this range using equation (3), for both red cell and lipid bilayer membranes. Using equation (3) with $M_{an} = 197.4 \text{ g mol}^{-1}$ and taking m and ρ_{mem}^0 as the slopes and intercepts of the least squares lines of Fig. 3, the values ($\pm 95\%$ confidence limits) are $V_{\rm an} = 134 \pm 14 \, {\rm cm}^3 \, {\rm mol}^{-1}$ for human red blood cell membranes and $\vec{V}_{an} = 124 \pm 24 \text{ cm}^3 \text{ mol}^{-1}$ for bovine spinal cord lipids. Thus, there is no significant difference between the partial molar volume of halothane in red cell and lipid membranes. It follows that, at equal membrane concentrations, halothane expands both membranes by the same amount. Furthermore, $ar{V}_{
m an}$ in these membranes is about the same as it is in buffer $(116.7 \pm 3.2 \text{ cm}^3 \text{ mol}^{-1} \text{ at } 95\% \text{ confidence, calculated using the})$ buffer data of Fig. 2).

Our results using halothane thus provide a firm quantitative basis for the conclusions which could be drawn from our experiments with ethanol. We find virtually no difference in the volume that a given anaesthetic molecule occupies in biological membranes, lipid bilayers or water. This was true for both halothane and ethanol, two very different general anaesthetics. We should point out that the range of anaesthetic concentrations used in our experiments included not only general anaesthetic but also nerve blocking levels.

Our measurements throw new light on membrane expansion theories of anaesthesia. These theories have the attraction of providing a conceptually simple framework for understanding the phenomenon of pressure reversal of general anaesthesia. The most popular of these, the critical volume hypothesis, states¹⁴ that "Anaesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of molecules of an inert substance. If the volume of this hydrophobic region can be restored by changes of temperature or pressure, then the anaesthesia will be removed." At concentrations of halothane that produce general anaesthesia in man ($c_{\text{buf}} = 0.27 \text{ mM}$), the expansion $(\bar{V}_{\rm an}c_{\rm mem})$ calculated from the data of Fig. 3 for both membranes and bilayers is only about 0.1%. This observed expansion is very small, in view of recent measurements¹⁵ which show that cholesterol-containing lipid bilayers expand by about 0.1% per °C. Thus, were the critical volume theory to be applied to whole membranes, it would predict that a rise in temperature of only a few degrees should induce general anaesthesia. This is not observed in cold-blooded animals. (A similar line of reasoning^{2-4,16,17} has been used to discredit both lipid phase transition models and bilayer fluidization hypotheses of anaesthesia.)

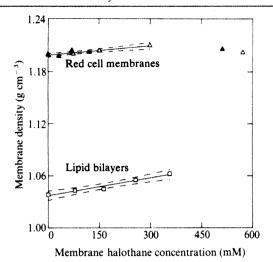


Fig. 3 Membrane density as a function of membrane halothane concentration and the determination of the partial molar volume \vec{V}_{an} for human red cell membranes (upper line) and bovine spinal cord lipids (lower line). The slope and intercept of each least-squares straight line was used to calculate \tilde{V}_{nn} with equation (3); the 95% confidence limits were calculated using these values and the variance—covariance matrix²². The red cell data points above 500 mM, where we suspect that some protein denaturation had occurred²³, were not included in the analysis. For red cell membranes, $134 \pm 14 \text{ cm}^3 \text{ mol}^{-1}$; for lipid bilayers, $\overline{V}_{an} = 124 \pm 24 \text{ cm}^3 \text{ mol}^{-1}$

Furthermore, our observation that the volume occupied by a halothane molecule is essentially the same in membranes, water and liquid halothane shows that although membranes do indeed expand, the expansion is almost wholly accounted for by the volume of the halothane molecules themselves and does not, as previously thought⁸ for biological membranes, involve the creation of a large additional free volume which might then be compressed by the application of high pressures.

Overall, we conclude that the expansion of whole membranes or lipid bilayers is not relevant to the mechanism of anaesthesia. On the other hand, our results are consistent with the idea that general anaesthesia is caused by interactions with specialized regions of nerve membranes, which have properties quite different from those we have measured for whole membranes. Indeed, the partial molar volume of an anaesthetic molecule in these regions may well be much larger than in the rest of the membrane or in water; pressure reversal could then be simply a consequence of forcing the anaesthetic away from its target¹⁸ We have previously shown^{2,3}, using X-ray and neutron diffraction, that lipid bilayer structure is not significantly affected by general anaesthetics at up to 10 times clinical concentrations. Thus, it seems unlikely that general anaesthesia involves any sizeable structural change in the lipids of such specialized regions. This leaves us to suppose that it is the structure of particularly sensitive membrane proteins which are directly affected. For example, it could be that the target site is a multisubunit protein immersed in the membrane and that anaesthetics interfere with its function by interposing themselves between the subunits and disrupting the packing. The fact that general anaesthesia can be induced by a wide variety of structurally dissimilar molecules, whereas most drug/receptor interactions are rather specific, follows directly from such an idea. (A specific key is necessary to unlock a door, but anything can be used to prevent it from closing.)

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- Boggs, J. M., Yoong, T. & Hsia, J. C. Molec. Pharmac. 12, 127-135 (1976).
 Franks, N. P. & Lieb, W. R. Nature 274, 339-342 (1978).
 Franks, N. P. & Lieb, W. R. J. molec. Biol. 133, 469-500 (1979).
 Richards, C. D. et al. Nature 276, 775-779 (1978).

- Seeman, P., Kwant, W. O. & Sauks, T. Biochim. biophys. Acta 183, 499-511 (1969). Seeman, P. Pharmac. Rev. 24, 583-655 (1972).
- Seeman, P. & Roth, S. Biochim. biophys. Acta 255, 171-177 (1972) Seeman, P. Experientia 30, 759-760 (1974).
- Ueda, I., Kamaya, H. & Eyring, H. Proc. natn. Acad. Sci. U.S.A. 73, 481-485 (1976).
 Eyring, H., Woodbury, J. W. & D'Arrigo, J. S. Anesthesiology 38, 415-424 (1973).
- Kratky, O., Leopold, H. & Stabinger, H. Meth. Enzym. 27, 98-110 (1973)
- 12. Richards, C. D. in Topical Reviews in Anaesthesia Vol. 1 eds Norman, J. & Whitwam, J. G.) 1-84 (John Wright, Bristol, 1980).
- 13. Richards, E. G. An Introduction to Physical Properties of Large Molecules in Solution (Cambridge University Press, 1980).
- 14. Miller, K. W. & Smith, E. B. in A Guide to Molecular Pharmacology-Toxicology Vol. 1 (ed. Featherstone, R. M.) 427-475 (Dekker, New York, 1973).

 15. Melchior, D. L., Scavitto, F. J. & Steim, J. M. Biochemistry 19, 4828-4834 (1980)
- Simon, S. A. & Bennett, P. B. in Molecular Mechanisms of Anesthesia Vol. 2 (ed. Fink, B. R.) 305-318 (Raven, New York, 1980).
- 17. Pang, K. Y., Braswell, L. M., Chang, L., Sommer, T. J. & Miller, K. W. Molec. Pharinac. 18, 84-90 (1980)
- Richards, C. D. Int. Rev. Biochem. 19, 157-220 (1978).
 Dodge, J. T., Mitchell, C. & Hanahan, D. J. Archs Biochem. Biophys. 100, 119-130 (1963).
 Regan, M. J. & Eger, E. I. Anesthesiology 28, 689-700 (1967).
- 21. Snedecor, G. W. & Cochran, W. G. Statistical Methods 6th edn (Iowa State University Press,
- Cleland, W. W. Adv. Enzym. 29, 1-32 (1967).
- 23. Metcalfe, J. C., Seeman, P. & Burgen, A. S. V. Molec. Pharmac. 4, 87-95 (1968).

Fast inward and outward current channels in a non-spiking neurone

Maurizio Mirolli*

Békésy Laboratory of Neurobiology, 1993 East-West Road, University of Hawaii, Honolulu, Hawaii 96822, USA

Although the crustacean coxal receptors are non-spiking2.3, indirect pharmacological and electrophysiological evidence suggests that fast sodium channels may be present in their membrane⁴⁻⁶. The properties of these channels are not known, but it has been suggested that they might be "incompletely differentiated", perhaps lacking "appropriate gating mechanisms", and/or "too sparsely distributed". The former hypothesis is not supported by the results of voltage-clamping experiments done on dendritic segments isolated from these mechanoreceptors. Instead, the results reported here provide direct evidence for a voltage-dependent fast inward current, sensitive to tetrodotoxin (TTX) and requiring external sodium (but not calcium). This current is shunted by a transient fast outward current, also voltage dependent, and it is suggested that this shunting may account, at least in part, for the non-spiking behaviour of the coxal receptors.

The largest dendrite (T fibre 1) among those supplying the coxal muscle receptor organ in the crab Portunus sanguinolentus (Herbst) was ligated close to its sensory endings and to its entry into the thoracic ganglion, and then cut distal and proximal to the two ligatures. The input properties of the isolated dendrite were similar to those of intact coxal receptors3. In nine experiments the average and standard deviation of the resting potential were 63.8 ± 5.5 mV and those of the input resistance were $2.2 \pm 1.5 \,\mathrm{M}\Omega$. The current-voltage relationship was characterized by a pronounced outward-going rectification in the depolarizing quadrant. Depolarization by current pulses did not elicit overshooting action potentials (Fig. 1a).

Dendrites could be clamped at holding potentials close to or considerably more negative (up to -80 mV) than their resting potential for several hours without deterioration of their input properties. Depolarizing and hyperpolarizing clamping voltages of small amplitude resulted in symmetrical clamping currents, but at voltages more positive than about -50 mV, a transient inward current (I_N) became evident (Fig. 1b). Both amplitude and time to peak of I_N depended on the magnitude of the test clamping voltage: the amplitude first increased with larger values of the test voltage and then decreased (Fig. 1b), while the time to peak decreased from 3-5 ms to less than 1 ms with increasing intensity of the test voltages. I_N was also dependent on the holding voltage and was completely inactivated in dendrites held at voltages more positive than -55 mV.

 I_N was sensitive to pharmacological agents known specifically to affect voltage-dependent sodium channels8. It was abolished

^{*} Permanent address: Indiana University, School of Medicine, Medica: Sciences Program, Physiology Section, Myers Hall, Bloomington, Indiana 47405, USA

when TTX in concentrations as low as 10^{-8} M was added to the saline (Fig. 1c, d), and when external sodium was substituted by choline, tetramethyl ammonium or tetraethyl ammonium. By contrast, I_N was not affected by agents known to interfere with calcium currents°. Thus, it was not increased when calcium was substituted by either barium or strontium, nor was it abolished or reduced when cadmium (10^{-4} M), manganese or cobalt (both 5×10^{-3} M) were added to the saline.

Even in TTX, clamping currents were not symmetrical in the hyperpolarizing and depolarizing range except, as noted, for small voltage steps. Instead, depolarizing voltages resulted in early fast outward current transients considerably larger than the capacitative transients obtained with hyperpolarizing tests of equal amplitude (Fig. 1c, d legend). For several criteria the fast outward current behaved in a manner similar to the fast inward current; thus, it could not be demonstrated in dendrites held at potentials more positive than about -45 mV, and its time to

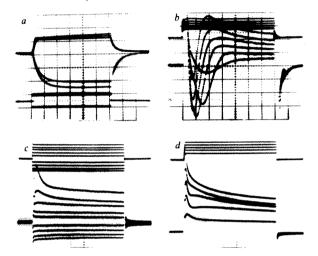


Fig. 1 Example of data obtained by current and voltage clamping in isolated dendritic segments. The preparations, 3-6 mm in length and 80-120 µm in diameter, were pinned to the Sylgard floor of a chamber having an approximate volume of 0.016 ml, and perfused at the rate of about 0.5 ml min⁻¹ with a saline solution of the following molar composition: Na, 0.47; K, 0.01; Ca, 0.026; Mg, 0.052; Cl, 0.513; SO₄, 0.022, buffered to pH 7.6 with 0.005 mol of Na-HEPES. The voltage clamping circuit was modelled after that of Connor and Stevens¹⁰. The current injecting pipette was screened. Position and tip resistance of the two micropipettes used for clamping were critical in obtaining good results. Stable input properties and adequate clamping speed were obtained with pipettes having a resistance of $4-7 \text{ M}\Omega$, inserted in the middle of the dendrites at about 50 µm from each other. Fast clamping was not possible with micropipettes having higher resistance, while the use of micropipettes having lower resistance results in unstable input properties. In each record, voltage is recorded in the top trace and current in the lower one. Calibrations: one major grid division is equal to: a, 20 mV, 50 nA, 50 ms; b, 50 mV, 100 nA, 1 ms; c, 50 mV, 20 nA, 5 ms; d, 100 mV, 50 nA, 10 ms. a, Current clamping of a dendritic segment 5.8 mm long, whose membrane potential (V_m) was -65 mV and input resistance (R_i) 2.2 M Ω . Note lack of spiking activity and pronounced outward-going rectification above -45 mV. b, Voltage clamping of a segment 4.6 mm long $(V_{\rm m}-65~{\rm mV};\,R_{\rm i}\,1.75~{\rm M}\Omega)$. Inward current $(I_{\rm N})$ corresponds to a downward deflection of the current trace. IN reached a maximum value (360 nA) when the segment was depolarized to $-35\,\text{mV}$ from a holding potential of -75 mV. c, d, Outward currents recorded in saline containing tetrodotoxin $(5 \times 10^{-8} \text{ M})$ in a segment 3.6 mm long whose resting potential was -67 mV and input resistance 2.4 MΩ. The preparation was held at -80 mV except during testing. As shown in d, at clamping voltages more positive than -30 mV, the initial outward transient is followed by a second one characterised by a slower rate of decay. This second outward transient is not considered in the text because its time course is too slow to affect the inward current directly. Preliminary results indicate that the slower outward transient can be blocked by external tetraethyl ammonium $(5 \times 10^{-2} \text{ M})$ whereas the initial fast transient is not. Both outward currents are blocked by 4-amino-pyridine $(5 \times 10^{-3} \text{ M})$.

peak decreased with increasing values of the test voltage, from 3-5 ms to <1 ms (Fig. 1c, d). Unlike the inward current, however, the early outward current transient increased monotonically in magnitude with larger values of the clamping voltage (Fig. 1c, d).

The shunting effect of the outward current transients on the inward current flowing through the TTX-sensitive channels can be evaluated from Fig. 2. The difference between clamping currents, recorded in the same preparation in control and in TTX saline, or net inward current, is substantially larger than $I_{\rm N}$ and has a null point at about +50 mV and thus at a voltage close to the reversal potential expected for a sodium current. $I_{\rm N}$, on the other hand, has a null point at about -10 mV, close to the peak values of the partial action potentials which can occasionally be recorded from intact coxal receptors^{3,4}.

The net inward current density, calculated from data such as those presented in Fig. 2, on the assumption that the dendritic segments were properly space clamped, is about $20 \,\mu\text{A} \,\text{cm}^{-2}$ and thus much lower than that measured in excitable membranes. It should be emphasised, however, that the validity of this assumption (as well as other assumptions involved in the

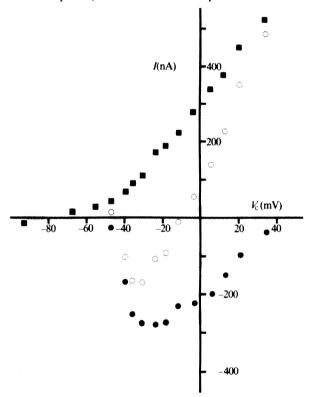


Fig. 2 Plot of clamping currents obtained from the same preparation in control saline (O) and in saline containing TTX () in response to stimuli of equal amplitude (V_c) . Currents were measured at times corresponding to peak amplitude of the inward current in control saline. Their difference, net peak inward current, is plotted (). Clamping was not sufficiently fast at voltages larger than about +15 mV, and therefore no reliable results were obtained close to the null potential of the inward current. By extrapolation, this potential can be seen to be about +50 mV. This dendritic segment was 6 mm long, and its surface was 1.5× 10⁻² cm². As the length constant of the coxal receptor dendrites is at least 3 cm (refs 3, $\overline{7}$) the preparations should have been properly space clamped if the ligatures at their endings were to provide a complete seal¹⁸. On this assumption, peak net inward current density could be calculated to be, in this example, 19 µA cm⁻ Two other experiments yielded values of 18 and 48 µA cm the null potentials were, respectively, +25 and +43 mV. Assuming further that the effective membrane capacitance of the dendrites was about 1 µF cm⁻², the current density thus calculated would be about 10 times lower than that required to charge the membrane to the null potential of the inward current. If the endings were leaky, the amount of membrane under clamp control would have been less, and hence current density would be underestimated by an unknown amount.

calculations) cannot be properly evaluated on the basis of the available data (see Fig. 2 legend). Thus, it seems reasonable to conclude that the inability of the coxal receptor dendrites to develop full action potentials does not depend on a lack of functional sodium channels but rather on the shunting of the sodium current by the transient opening of a fast outward conductance and, possibly, on an abnormally low density of the sodium channels themselves. These two factors are not mutually exclusive; on the contrary, both can be easily interpreted as an expression of the specialization of the coxal receptors for transmitting information with analogue signals^{2,3}

Results consistent with those presented in Figs 1 and 2 were also obtained in the S dendrite of Portunus, as well as in the S and T dendrites of other swimming crabs. Moreover, a fast TTXsensitive and sodium-dependent inward current and a fast initial outward transient could also be demonstrated when clamping intact Portunus coxal receptors. In the intact cells no fast clamping could be obtained at voltages more positive than about -20 mV, and therefore a satisfactory study of the fast currents has not been possible.

The coxal receptors are not unique in exhibiting transiently activated outward currents 10-17. There is convincing evidence that currents of this type are important in regulating repetitive firing of normally spiking neurones 10,14. The early fast outward channels of the coxal receptors, characterised by activation and inactivation rates comparable with those of the inward channels, seem to be specialised for suppressing spiking. Whether this specialisation represents an extreme case of adaptation of membrane components also found in other cells, or whether it reflects unique ionic and pharmacological properties, remains to be determined.

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- Alexandrowicz, J. S. & Whitear, M. J. J. mar. biol. Ass. U.K. 36, 603-628 (1957). Ripley, S. H., Bush. B. M. H. & Roberts, A. Nature 218, 1170-1171 (1968). Mirolli, M. J. exp. Biol. 78, 1-27 (1979). Roberts, A. & Bush, B. M. H. J. exp. Biol. 54, 515-524 (1971).

- Lowe, D. A., Bush, B. M. H. & Ripley, S. H. Nature 274, 289–290 (1978). Bush, B. M. H., DiCaprio, R. A. & Taylor, P. S. J. Physiol., Lond. 303, 20–21P (1980). Bush, B. M. H. in Neurones Without Impulses (eds Roberts, A. & Bush, B. M. H.) 147-176 (Cambridge University Press, 1981)

- (Cambridge University Press, 1981).
 Narahashi, T. Physiol. Rev. 54, 813-889 (1974).
 Akaike, N., Lee, K. S. & Brown, A. M. J. gen. Physiol. 71, 509-531 (1978).
 Connor, J. A. & Stevens, C. F. J. Physiol. Lond. 213, 1-19, 21-30 (1971).
 Neher, E. J. gen. Physiol. 58, 36-53 (1971).
 Nakajima, S. & Onodera, S. J. Physiol., Lond. 200, 161-185 (1969).
 Thompson, S. H. J. Physiol., Lond. 265, 465-488 (1977).

- Aldrich, R. W., Getting, P. A. & Thompson, S. H. J. Physiol., Lond. 291, 507-530 (1979). Nakajima, S. & Kusano, K. J. gen. Physiol. 49, 613-628 (1966).

- Frankenheuser, B. & Waltman, B. J. Physiol., Lond. 148, 677-682 (1959). Miyazaki, S. I., Ohmori, H. & Sasaki, S. J. Physiol., Lond. 246, 55-78 (1975).
- 18. Norman, R. S. Biophys. J. 12, 22-45 (1972).

Inhibition of smooth muscle tension by cyclic AMP-dependent protein kinase

W. Glenn L. Kerrick* & Phyllis E. Hoar

Department of Physiology and Biophysics, SJ-40, University of Washington, Seattle, Washington 98195, USA

β-adrenergic relaxation of smooth muscle by catecholamines has been associated with elevated levels of cyclic AMP^{1,2}. The question arises whether subsequent activation of cyclic AMPdependent protein kinase^{3,4} has a role in the regulation of smooth muscle contraction. There is substantial evidence that a Ca²⁺-activated myosin light chain kinase/phosphatase system regulates smooth muscle contraction⁵⁻¹⁵ ', and Adelstein *et* al. 16,17 have shown that the catalytic subunit of cyclic AMPdependent protein kinase^{3,4} plays a part in this regulation, by phosphorylation of the high molecular weight subunit of the light chain kinase, which results in a decrease in the activity of the kinase. Here we have shown for the first time that the catalytic subunit of the protein kinase inhibits Ca2+-activated tension in skinned smooth muscle fibre preparations.

Figure 1a shows that after a submaximal (pCa = 5.0) and a maximal (pCa = 3.6) contraction, addition of 10^{-6} M catalytic subunit of cyclic AMP-dependent protein kinase results in a slow inactivation of the fibres. After transfer of the fibre bundle from this high [Ca²⁺] catalytic subunit solution to a relaxing solution (pCa = 8.0) the fibres relaxed. When they were reimmersed in the same solution (pCa = 5.0) in which they initially gave ~90% maximum tension, they generated only 14% of the subsequent tension in pCa 3.6 solution. This shows that pretreatment with the catalytic subunit of protein kinase resulted in a decrease in the Ca2+-sensitive threshold for contraction of the muscle fibre preparation. Transfer of the fibre preparation from high $[Ca^{2+}]$ solution (pCa = 3.6) to an identical solution containing 5 µM calmodulin resulted in a further increase in tension.

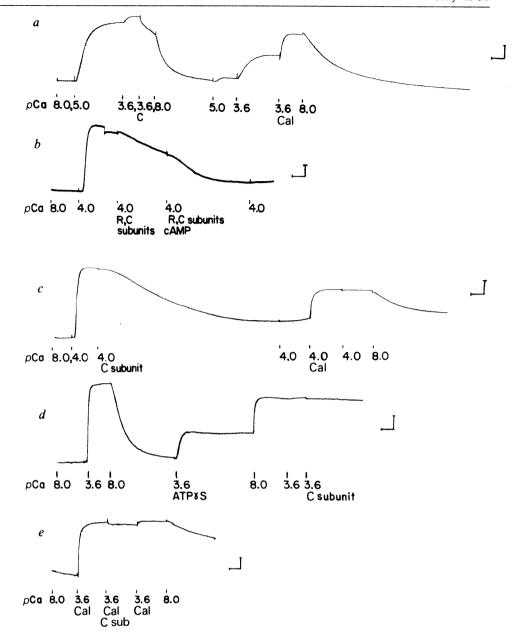
Figure 1b shows that after a maximal Ca2+-activated contraction, addition of both the regulatory and catalytic subunits of protein kinase results in a slow inactivation of the fibres and that addition of cyclic AMP (10⁻⁴ M) produces faster relaxation. Cyclic AMP is known to activate protein kinase by dissociation of the catalytic subunit from the regulatory subunit⁴; the free catalytic subunit is then able to inhibit the fibres. Initial slow inactivation of the fibre bundle is presumably due to either the production by the fibres of small amounts of cyclic AMP from ATP, or incomplete association of mixed catalytic and regulatory subunits, or both. A higher than physiological concentration of cyclic AMP was used to avoid diffusional problems associated with cyclic AMP binding sites in the fibres. After maximal activation of the fibre bundle (pCa = 4.0, Fig. 1c) and inhibition by the catalytic subunit, immersion of the fibres in a solution containing high [Ca2+] but no catalytic subunit resulted in little detectable activation of the fibres over the time period observed. However, in the presence of high [Ca²⁺] plus calmodulin, the fibres contracted immediately, giving partial recovery

Figure 1d shows that after a skinned fibre bundle had been pretreated with high [Ca²⁺] and adenosine 5'-O-(3-thiotriphosphate) (ATPyS) to irreversibly thiophosphorylate the myosin LC₂₀ light chains, the fibre bundle became fully activated when immersed in a solution containing ATP and no calcium (pCa = 8.0) and showed no further increase in tension in high $[Ca^{2+}]$ solution (pCa = 3.6). Following this irreversible thiophosphorylation and tension activation, addition of the catalytic subunit was unable to inactivate the fibre bundle. Thus when the light chains are irreversibly thiophosphorylated 10-13, the catalytic subunit of protein kinase can no longer inhibit the contraction. In the presence of 5 µM calmodulin (Fig. 1e), 1 µM catalytic subunit produced very little change in Ca²⁺-activated tension generation, in contrast to its action in the absence of calmodulin. The results shown in Fig. 1 are typical of results obtained with either the skeletal or cardiac catalytic subunit of cyclic AMP-dependent protein kinase.

Our data thus agree with the light chain kinase/phosphatase mechanism for Ca²⁺ activation of smooth muscle and the proposed inhibition of this mechanism by the catalytic subunit of the cyclic AMP-dependent protein kinase 16-18. The model proposed by Adelstein et al. 16.17, in which the phosphorylation of the high molecular weight subunit of light chain kinase by the catalytic subunit of the cyclic AMP-dependent protein kinase decreases the ability of the Ca²⁺-binding subunit of calmodulin to interact with it, predicts that tension would be inhibited by this catalytic subunit and the inhibited fibres would appear less sensitive to calcium (Fig. 1a). Once the fibres have been inhibited partially (Fig. 1a) or completely (Fig. 1c), addition of calmodulin should increase the Ca2+ sensitivity of the muscle according to the law of mass action. Also, once the LC₂₀ light chains have been irreversibly thiophosphorylated by the use of the ATP analogue ATP_YS¹⁰⁻¹³, inhibition of the light chain kinase by the catalytic subunit should have no effect on tension development (Fig. 1d). As the only site of irreversible thiophosphorylation is myosin LC₂₀ (ref. 11), the site of action of cyclic AMP-dependent protein kinase cannot be the thin

^{*} Present address: Department of Physiology and Biophysics, University of Miami, PO Box 016430, Miami, Florida 33101, USA.

Fig. 1 Tension-time records of chicken gizzard skinned fibres. All solutions contained ATP unless indicated by ATPyS. Additions to the solutions are indicated below the pCa value and refer only to that solution, not to subsequent ones. a, Effect of 1 µM cardiac catalytic (C) subunit of cyclic AMP-dependent protein kinase on submaximal Ca2+ activated tension. Recovery of maximum tension with $5\,\mu M$ calmodulin (Cal) is shown. Vertical calibration bar, 8.3 mg. b, Inhibition of Ca2+-activated tension by 1 μM catalytic (C) subunit plus 2 μM cyclic AMP-free regulatory (R) subunit of skeletal cyclic AMP-dependent protein kinase, followed by addition of cyclic AMP (cAMP, 10^{-4} M). Vertical calibration bar, 1.9 mg. The mechanical artefact seen during the initial pCa = 4.0 concentration was due to a small knock to the table. c. Complete inhibition of Ca2+ activated tension by 1 µM skeletal catalytic (C) subunit of cyclic AMP-dependent protein kinase followed by partial recovery with 5 μM calmodulin (Cal). Vertical calibration bar, $11.1\,\text{mg}$. d, Lack of tension response to $1\,\mu\text{M}$ catalytic (C) subunit of cardiac cyclic AMP-dependent protein kinase after pretreatment (irrethiophosphorylation of 20,000-MW myosin light chains) of the fibre bundle with high $[Ca^{2+}]$ (pCa = 3.6) and ATPyS. Vertical calibration bar, 10.0 mg. e, Lack of inhibition by 1 µM cardiac catalytic subunit (C sub) in the presence of 5 uM calmodulin (Cal). Vertical calibration bar, 6.1 mg. The horizontal calibration bar represents 5 min in each case. Sections of muscle (~1-2 mm³) were cut from fresh chicken gizzards and lightly homogenized by a method described elsewhere for striated muscle²⁰ and previously used in this laboratory for gizzard skinned fibre pre-parations¹¹. Small bundles of fibres were visualized by background light using a binocular microscope and the two ends of a fibre bundle were clamped in small stainless steel forceps of a tension transducer apparatus similar to that of Hellam and Podolsky²¹. The fibres were activated and relaxed by transferring them to different test bathing solutions containing various amounts of Ca2+ and protein concentrations. The solutions contained 70 mM K⁺ + Na⁺, 1 mM Mg²⁺, 2 mM Mg-ATP2-, 15 mM creatine phosphate, 15



units of creatine phosphokinase (CPK) per ml solution, 82-84 mM imidazole, 7 mM EGTA total, varying amounts of Ca^{2+} (pCa = 8-3.6) and propionate as the major anion. The pH was kept constant at 7.00 (± 0.02) and the ionic strength kept at 0.15 by varying the amount of imidazole propionate. However, the solution in which ATP γ S was substituted for ATP contained 85 mM K⁺ + Na⁺ and no creatine phosphate/CPK regenerating system, but was otherwise identical to the solutions described above. The total concentrations of magnesium, potassium and calcium propionate, disodium ATP, disodium creatine phosphate and imidazole required were determined by a computer program using binding constants obtained from the literature²².

filament, proposed by Nonomura and Ebashi¹⁹ to regulate smooth muscle by a leiotonin system. Furthermore, the presence of calmodulin prevents the inhibition of tension by the catalytic subunit (Fig. 1e). If phosphorylation of the 125,000-molecular weight (MW) light chain kinase decreases its interaction with calmodulin¹⁷, the presence of added calmodulin should overcome this by the laws of mass action. We will present elsewhere a detailed study of the relationship between increased phosphorylation of myosin light chain kinase, decreased phosphorylation of LC₂₀ light chain and inhibition of tension generation.

We have treated two other smooth muscle types, skinned using Triton X-100, with the catalytic subunit of protein kinase and obtained the same results as for chicken gizzard. These muscles were rabbit pulmonary artery and the aorta from the Pacific Coast dogfish which evolved 400 Myr ago. Therefore, the mechanism for this inhibition of tension in smooth muscle by cyclic AMP-dependent protein kinase seems to have evolved before much of the animal kingdom as we know it today.

These data show for the first time that the catalytic subunit of protein kinase can inhibit the physiological variable, Ca²⁺-

activated tension, in skinned smooth muscle fibre preparations. Pretreatment of the smooth muscle fibres with this catalytic subunit causes a decrease in the apparent Ca^{2+} sensitivity of the muscles, an effect which can be reversed by the addition of calmodulin. In the presence of both the catalytic and regulatory subunits of protein kinase, addition of cyclic AMP increased the rate of inhibition of tension. These results agree with the mechanism described elsewhere ^{16,17}, which involves this protein kinase system in the β -adrenergic relaxation of smooth muscle, but do not support the thin filament control mechanism proposed by Nonomura and Ebashi¹⁹.

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- 1. Robison, G. A., Butcher, R. W. & Sutherland, E. W. Cyclic AMP 204-210 (Academic, New York, 1971).
- 2. Kramer, G. L. & Hardman, J. G. Handbook of Physiology Sect. 2, Vol. 2 (eds Bohr, D. F., Somlyo, A. P. & Sparks, H. V.) 179-199 (American Physiological Society, Bethesda,
- Greengard, P. Science 199, 146-152 (1978).
- Krebs, E. G. & Beavo, J. A. A. Rev. Biochem. 48, 923-959 (1979).
 Aksoy, M. O., Williams, D., Sharkey, E. M. & Hartshorne, D. J. Biochem. biophys. Res.
- 6. Chacko, S., Conti, M. A. & Adelstein, R. S. Proc. natn. Acad. Sci. U.S.A. 74, 129-133
- Sobieszek A. Eur. I. Biochem. 73, 477-483 (1977).
- Sobieszek, A., Eur. J. Biochem. 73, 417-483 (1917).
 DiSalvo, J., Gruenstein, E. & Silver, P. Proc. Soc. exp. Biol. Med. 158, 410-414 (1978).
 Sherry, J. M. F., Górecka, A., Aksoy, M. O., Dabrowska, R. & Hartshorne, D. J. Biochemistry 17, 4411-4418 (1978).
 Cassidy, P., Hoar, P. E. & Kerrick, W. G. L. J. biol. Chem. 254, 11148-11153 (1979).
- Hoar, P. E., Kerrick, W. G. L. & Cassidy, P. S. Science 204, 503-506 (1979).
 Cassidy, P., Hoar, P. E. & Kerrick, W. G. L. Pflügers Arch. ges. Physiol. 387, 115-120
- 13. Kerrick, W. G. L., Hoar, P. E. & Cassidy, P. S. Fedn Proc. 39, 1558-1563 (1980) 14. Kerrick, W. G. L., Hoar, P. E., Cassidy, P. S. & Malencik, D. A. in Regulation of Contraction: Excitation-Contraction Coupling (eds Grinnell, A. D. & Brazier, M. A. B.) (Academic, New York, in the press)
- 15. Kerrick, W. G. L. in Calcium and Cell Function Vol. 2 (ed. Cheung, W. Y.) (Academic, New
- 16. Adelstein, R. S., Conti, M. A., Hathaway, D. R. & Klee, C. B. J. biol. Chem. 253, 8347-8350 (1978)
- 17. Conti. M. A. & Adelstein, R. S. Fedn Proc. 39, 1569-1573 (1980).
- Silver, P. J. & DiSalvo, J. J. biol. Chem. 254, 9951-9954 (1979)
- Nonomura, Y. & Ebashi, S. Biomed. Res. 1, 1-14 (1980)
- Kerrick, W. G. L. & Krasner, B. J. appl. Physiol. 39, 1052-1055 (1975). Hellam, D. C. & Podolsky, R. J. J. Physiol., Lond. 200, 807-819 (1969)
- Donaldson, S. K. B. & Kerrick, W. G. L. J. gen. Physiol. 66, 427-444 (1975).

Progesterone inhibits membrane-bound adenvlate cyclase in Xenopus laevis oocytes

Joëlle Finidori-Lepicard*, Sabine Schorderet-Slatkine†, Jacques Hanoune & Etienne-Emile Baulieu*§

- * U 33 Inserm and Faculté de Médecine, 78 rue du Général Leclerc, 94270 Bicêtre, France
- † Département de Gynécologie et d'Obstétrique, Hôpital Cantonal, Genève, Switzerland
- ‡ U 99 Inserm, 51 avenue du Maréchal de Lattre de Tassigny, 94000 Créteil, France

Recent experimental evidence indicates that progesterone acts at the cell surface to trigger protein synthesis and to reinitiate the first meiotic division in Xenopus laevis oocytes1,2. The steroid hormone is physiologically released by follicle cells surrounding oocytes in the ovaries, and this naturally occurring event can be reproduced in vitro by adding progesterone to the incubation medium. Recently, cyclic AMP has been implicated in the mechanism of progesterone action in oocytes3,4; there was an almost immediate decrease in cyclic AMP concentration in oocytes after addition of progesterone in vitro, whether or not the oocytes were pretreated with cholera toxin5,6. Adenylate cyclase in X. laevis oocytes is compartmentalized, >50% soluble and ~30% is found in the plasma membrane-containing fraction⁷. We report here that physiological concentrations of progesterone selectively inhibit membrane-bound adenylate cyclase activity, after addition to intact oocytes or in cell-free experiments; this specificity confirms the proposed membrane site of action for the hormone when reinitiating meiosis and is the first example of a 'direct' enzymatic action of a steroid (not by protein synthesis) related to a physiological function.

Adenylate cyclase activity in oocytes was measured in four subcellular fractions isolated by differential centrifugation (Fig. 1a), and was largely found in two compartments, a 10,000g pellet (P-10,000; 20-30%) and the cytosol (50-65%), a distribution similar to that observed in the early stages of male germ-cell development⁸. Both particulate and soluble forms of the enzyme appeared equally active in the presence of either Mg²⁺ or Mn²⁺, but only the particulate activity was stimulated (two- to threefold) by 10 mM sodium fluoride. Electron microscopic examination of P-10,000 showed a uniform vesiculated membrane structure and enzyme patterns suggested an enrichment in plasma membrane. Incubation of oocytes with progesterone produced a rapid inhibition of the P-10,000associated cyclase. Its specific activity was reduced by 65% while the soluble activity was unchanged. Cycloheximide had no effect on particulate cyclase inhibition. The facts that only the membrane-bound cyclase was inhibited by progesterone, and that more than half of the oocyte enzymatic activity is soluble, explain the limited decrease in total cyclic AMP observed in intact oocytes. Therefore, cholera toxin was used to magnify the progesterone-induced decrease of cyclic AMP in oocytes; after cholera toxin has been added to increase cyclic AMP levels (to 150-300% of control), the addition of progesterone produces an immediate decrease to basal level⁶. Adenylate cyclase determinations confirmed the dual nature of the enzyme in oocytes, as only the P-10,000 cyclase was stimulated by cholera toxin (5-10-fold), while this stimulation was reversed by addition of progesterone to the oocyte incubation medium just before cell fractionation (Fig. 1b).

To demonstrate that progesterone interacts directly with the plasma membrane cyclase activity, without an intermediary

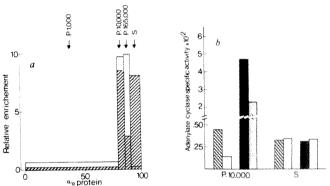


Fig. 1 Adenylate cyclase activity in subcellular fractions of untreated and progesterone- and/or cholera toxin-treated X. laevis oocytes. Fully grown oocytes, obtained as described elsewhere4, were washed and homogenized in 10 vol of 1 mM NaHCO3, 3 mM EDTA, at 4 °C, with a Dounce apparatus (pestle A, 10 strokes). The homogenate was centrifuged at 1,000g for 15 min; the pellet (P-1,000) contained most of the vitellus and melanosomes. The supernatant was centrifuged at 10,000g for 20 min and the P-10,000 pellet separated. The supernatant was again centrifuged at 165,000g for 15 min, and both the supernatant (S) and the pellet (P-165,000) were retained. All pellets were resuspended in the same buffer and enzymatic assays were performed immediately. Adenylate cyclase assays were done in 60 μ l containing 1 mM [α - 32 P]ATP (10^6 c.p.m.), 3 mM MgCl₂, 1 mM EDTA, 1 mM cyclic AMP, 50 mM Tris-HCl pH 7.6, plus an ATPregenerating system consisting of 25 mM phosphocreatine and 1 mg ml creatinine phosphokinase. The reaction was terminated by a modification ¹⁴ of the procedure of White ¹⁵. In these conditions, the degradation of ATP was negligible and the enzymatic activity was linear with time up to 60 min and linear with protein up to 60 µg per assay; this was also the case for inhibition experiments. Labelled cyclic AMP was isolated according to Rachamadran 16. The yield was calculated by previous addition of cyclic [8-3H]AMP; it was unchanged when the experiment was performed in the presence of steroids, eliminating a significant stimulation of the phosphodiesterase(s) by the steroids. 5'-nucleotidase activity was estimated as described elsewhere¹⁷. The distribution pattern of adenylate cyclase (22) in oocytes is shown in a, superimposed on that of 5'-nucleotidase (

). Enzyme activities and protein concentrations were measured in the whole homogenate and in the separated fractions. Relative enrichment for adenylate cyclase is the ratio of specific activity in each fraction divided by specific activity of the whole homogenate. This parameter is plotted against the percentage of the whole homogenate protein recovered in each fraction. The area of the bars is proportional to the recovery of the enzyme in the corresponding fractions. The ordinate measures the degree of purification achieved over the whole homogenate value. The same representation is used for 5'-nucleotidase. b, Adenylate cyclase specific activities in P-10,000 and S fractions of untreated occytes (cross-hatched bars), occytes preincubated with 30 µM progesterone for 90 s (open bars), oocytes preincubated with 50 pmol cholera toxin for 6 h (solid bars), and oocytes incubated with 30 µM progesterone for 90 s at the end of the preincubation with cholera toxin (stippled bars). Cyclase activity is expressed as pmol of cyclic AMP formed in 15 min per mg protein at 32 °C. Protein and 5'-nucleotidase values were identical in control and progesterone/cholera toxin-treated oocytes (data not shown).

§ To whom reprint requests should be addressed

state involving a metabolic change, we performed cell-free experiments. We found that P-10,000 adenylate cyclase from control oocytes was inhibited by addition of progesterone in the enzymatic assay at 20 °C—the physiological temperature for the maturation process—as well as at 32 °C, the optimal temperature for the cyclase assay. There was no lag phase and the decrease in cyclase activity persisted for at least 60 min (in agreement with the prolonged reduction in cyclic AMP level observed in intact oocytes⁶). Progesterone-induced inhibition of cyclase activity was dose dependent: $K_i \sim 0.1 \,\mu\text{M}$ (that is about one order of magnitude lower than the half-maximal effective dose of progesterone when initiating meiosis, as if some trapping or inactivation of the hormone in intact oocytes may be eliminated in the cell-free system). In most cases, maximum inhibition (at 10 µM) reached 80% of control activity. Progesterone reduced the $V_{\rm max}$ of the enzyme without changing the apparent affinities for MgATP and MnATP substrates, and for the activators free Mg²⁺ and free Mn²⁺ (data not shown). Finally, cyclase activity was entirely recovered after extensive membrane washing, which demonstrates the reversibility of progesterone inhibition. Adenylate cyclase activities stimulated by NaF or guanyl-5'-yl imidodiphosphate (Gpp(NH)p, a GTP analogue not hydrolysed to GDP by GTPases in membranes) were also inhibited by progesterone, which also reversed enzyme stimulation in P-10,000 obtained from oocytes preincubated with cholera toxin (Fig. 2). In all these assay conditions, inhibition by progesterone was dose dependent with an apparent K_i similar to that found in basal conditions. This effect of progesterone seemed to be different from the GTP-dependent inhibitions so far described, which are prevented by stable GTP analogues and generally abolished by cholera toxin pretreatment9. Progesterone inhibition did not seem to be mediated by an adenosine effect as it was not suppressed by adenosine desaminase in the assay; adenosine and progesterone inhibitions of cyclase in oocyte membranes were additive⁷

Furthermore, progesterone-induced inhibition of adenylate cyclase does not seem dependent on Ca^{2+} concentrations. In intact oocytes, progesterone effect was observed in Barth's medium containing 1 mM CaCl₂, while in cell-free experiments steroid inhibition occurred in the presence of 1 mM EDTA. In vitro, Ca^{2+} -induced inhibition of cyclase $(K_i = 0.5 \text{ mM})$ and progesterone inhibition were additive, which suggests that these

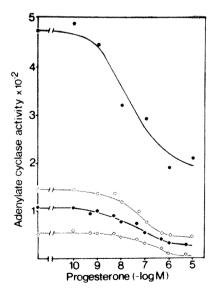


Fig. 2 Inhibition by progesterone of membrane adenylate cyclase activity in X. laevis oocytes treated with cholera toxin or untreated. The P-10,000 fraction was obtained from untreated X. laevis oocytes $(\diamondsuit, \blacklozenge, \bigcirc)$ or from oocytes preincubated for 6 h with 50 pM cholera toxin (\clubsuit) . Progesterone was added in the enzymatic assay, with no other addition $(\diamondsuit, \spadesuit)$, or with 10 mM sodium fluoride (\diamondsuit) or $1 \mu M$ Gpp(NH)p (\bigcirc) . The reaction was initiated by addition of P-10,000 (50 μg protein per assay) to the enzymatic assay medium. Adenylate cyclase activity is expressed as pmol of cyclic AMP formed in 15 min per mg protein.

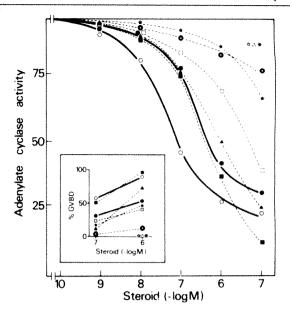


Fig. 3 Membrane adenylate cyclase of X. laevis oocytes: specificity of steroid inhibition. The P-10,000 fraction was incubated with 1 μM Gpp (NH)p and steroids. Adenylate cyclase activity at each concentration of steroid tested is expressed as per cent of the activity measured in the absence of hormone. Percentage of germinal vesicle breakdown (GVBD) is indicated in the inset. It was routinely determined on 30-50 oocytes after 8 h incubation by scoring the appearance of the 'white spot' at the animal pole. Experiments were done exactly as described elsewhere 4. The steroids tested were: progesterone (①); progesterone +10 μM 17α-ethynyloestradiol (①); testosterone (③); cortisone (▲); dehydroepiandrosterone (□); hydrocortisone (★): 19-nortestosterone (Φ); oestrone (★).

two inhibitors use different mechanisms. However, we cannot exclude the possibiltiy that changes in Ca²⁺ contribute to the inhibition of adenylate cyclase activity in intact oocytes.

The inhibitory steroid effect showed the same hormonal specificity as that reported for maturation in intact oocytes. In the P-10,000 cell-free system, testosterone and cortisone, which are almost as efficient in promoting meiosis as progesterone, were also the most active inhibitors of adenylate cyclase ($K_i = 0.1 \,\mu\text{M}$). Dehydroepiandrosterone, cortisol and 19-nortestosterone only reached 2/3-1/3 of the progesterone inhibitory activity, with an apparent $K_i \sim 1 \,\mu\text{M}$; oestrone, 17 α -ethynyloestradiol and progestrone (1,3,5-pregnatrien-3-ol, 20-one) were not active. Moreover, we found that 17 α -ethynyloestradiol, which antagonizes progesterone-induced meiosis apparently in a competitive manner 10, displaced to the right by one order of magnitude the dose-dependent curve of cyclase inhibition by progesterone (Fig. 3).

These results provide the first example of a direct enzymatic action of steroids in a physiological mechanism, the enzyme being a typical plasma membrane component. Intriguing studies with glutamate deshydrogenase demonstrated that steroids can have allosteric effects on the enzyme¹¹, and it has been suggested that this could be an important mechanism for hormone action¹². However, the lack of hormonal specificity made this model system irrelevant to a physiological system. The remarkable correlation between steroid action on meiosis and on membrane adenylate cyclase inhibition strongly suggests that we are dealing with a biochemical event relevant to the biological phenomenon. Experiments are now being done to determine whether other steroid systems show characteristics similar to those observed in oocytes¹³.

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- I. Smith, L. D. & Ecker, R. E. Devl Biol. 25, 233-247 (1971).
- Godeau, J. F., Schorderet-Slatkine, S., Hubert, P. & Baulieu, E. E. Proc. natn. Acad. Sci. U.S.A. 75, 2353-2357 (1978).
- U.S.A. 75, 2333-2337 (1976).

 3. Maller, J. L. & Krebs, E. G. J. biol. Chem. 252, 1712-1718 (1977).

- 4. Schorderet-Slatkine, S., Schorderet, M., Boquet, P., Godeau, F. & Baulieu, E. E. Cell 15,
- Maller, J. L., Butcher, F. R. & Krebs, E. G. J. biol. Chem. 254, 579-582 (1979)
- Malier, J. L., Butter, F. L. & Recos, E. C. J. Eth. M. & Schorderet, M. in International Cell Biology 1980-1981 (Schweiger, H. G. ed.) 860-871 (Springer, Berlin, 1981).
- 7. Finidori-Lepicard, J., Schorderet-Slatkine, S., Hanoune, J. & Baulieu, E. E. (in preparation).
- Braun, T. & Dods, R. F. Proc. natn. Acad. Sci. U.S.A. 72, 1097-1101 (1975).
 Jakobs, K. H., Aktories, K., Nasch, P., Saur, W. & Schultz, G. in Hormones and Cell Regulation Vol. 4 (eds Dumont, J. & Nunez, J.) 89-106 (Elsevier/North-Holland, Amsterdam, 1980).
- 10. Baulieu, E. E., Godeau, F., Schorderet, M. & Schorderet-Slatkine, S. Nature 275, 593-598
- Tomkins, G. M. & Maxwell, E. S. A. Rev. Biochem. 32, 677-708 (1963). Monod, J. Endocrinology 78, 412-425 (1966).
- Baulieu, E. E. in Central Regulation of the Endocrine System (eds Fuxe, K., Hökfelt, T. & Luft, R.) 239-260 (Plenum, New York, 1978).
- , Stengel, D., Lacombe, M. C., Feldmann, G. & Coudrier, E. J. biol. Chem. 252, 2039-2045 (1977).
- White, A. A. Meth. Enzym. 386, 41-46 (1974)
- Ramachandran, J. Analyt. Biochem. 43, 227-239 (1971).
- 17. Pecker, F., Duvaldestin, P., Berthelot, P. & Hanoune, J. Clin. Sci. 57, 313-325 (1979).

Differences between oestrogen receptor activation by oestrogen and antioestrogen

Henri Rochefort & Jean-Louis Borgna

Unité d'Endocrinologie Cellulaire et Moléculaire, U 148 INSERM, 60, rue de Navacelles, 34100 Montpellier, France

Triphenylethylene antioestrogens such as tamoxifen, nafoxidine and Ci 628 specifically inhibit oestrogen action at the target cell level¹, probably by interacting with the oestrogen receptor (ER) and competitively displacing oestrogens from their binding sites. It is not clear, however, why these ligands are less biologically active than oestrogens when they bind to the ER, as no reliable difference has been found either in the binding affinity of these two series of ligands to the ER or in their ability to translocate the ER to the nucleus1,2. In fact, these antioestrogens are transformed in vivo into hydroxylated metabolites3-5 which display a better antioestrogenic activity than the injected compound and at least the same high affinity as oestradiol for the ER6. With the aim of finding an in vitro criterion to predict the agonistic or antagonistic properties of ER ligands, we have stabilized the ER in its 'native' or non-activated form by the use of molybdate7,8 and have compared the binding of oestradiol (E2) and of 4-hydroxytamoxifen (OHT), an active metabolite of tamoxifen, to the molybdate-treated and to the activated ER. We report here that molybdate prevented the DNA binding and the 4S to 5S transformation of the ER bound to both ligands, and that it increased the dissociation rate of oestrogens but not that of antioestrogens. Moreover, in the absence of molybdate, receptor activation by heating decreased the dissociation rate of E2 but not that of OHT. We conclude that a difference exists between the ER activation triggered by oestrogens and antioestrogens and propose that antioestrogens are acting as allosteric ligands of the ER.

We have looked at three series of in vitro criteria for receptor activation. The first classical criterion is the increased affinity of the receptor for DNA and nuclei. The ability of lamb uterine ER to interact in vitro with calf thymus DNA was evaluated by ultracentrifugation in sucrose⁶ and metrizamide⁹ gradients. In the absence of molybdate, both the ER-E2 and the ER-OHT complexes bound to DNA, confirming that OHT also activate the ER for binding to DNA in vitro⁶ and for translocating the ER into the nucleus1. With molybdate, the DNA binding of the ER was totally inhibited whether ER was bound to an antioestrogen (OHT) or to an oestrogen (E2). A second in vitro test for ER activation is the E2-induced 4S to 5S transformation 10 which is also prevented by molybdate. However, both OHT and E₂ were able to transform the cytosol 4S ER into a 5S ER in the immature rat uterus and both processes were prevented by molybdate.

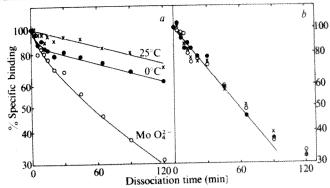


Fig. 1 Dissociation rate of oestradiol (a) and 4-hydroxytamoxifen (b) from the oestrogen receptor before and after heat activation. Lamb uterine cytosol (2 mg protein per ml) prepared in TE buffer (Tris-HCl 10 mM, EDTA 1.5 mM, pH 7.4) in the presence (O) or absence (\bullet , ×) of 10 mM MoO₄² was incubated for 2 h with 5 nM ³H-oestradiol (E_2) (CEA: Specific activity 55 Ci mmol⁻¹) or ³H-4-hydroxy-trans-tamoxifen (OHT) (specific activity 15.8 Ci mmol⁻¹) prepared biologically from ³Htamoxifen or provided by ICI. One part of the cytosol without MoO₄²⁻ was activated by a 30-min treatment at 25 °C, the other part was maintained at 0 °C, the dissociation rate was then determined at 25 °C as described elsewhere⁶, following the addition of $1 \mu M$ unlabelled E_2 at time 0. At the indicated times, aliquots were removed and cooled at 0 °C. The specific binding to the ER was determined after 90 min incubation with charcoal suspension⁵; the non-specific binding was evaluated in a parallel experiment performed with 1 µM unlabelled E2. Results were normalized by taking the pre-dissociation value as 100%. The complexes were stable during the course of the assay. × (25 °C), preactivated ER; (0 °C), non-preactivated ER; ○ (MoO₄²), mollybdate-treated ER.

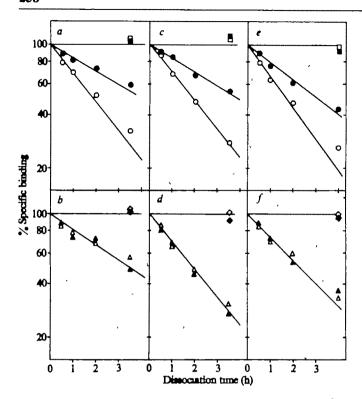
Table 1 Effect of molybdate on the dissociation rate (k^{-}) of various oestrogens and antioestrogens from the oestrogen receptor

a, Species specificity ($(k^-, \times 10^-)$	-4 s ⁻¹)				
	Calf	Lamb	Rat	Mouse	Chicken	MCF ₇
Temperature (°C)	20	20	20	20	15	20
E_2						
Control	0.47	0.14	0.64	1.27	0.68	0.51
+Molybdate	1.10	0.28	2.40	4.01	1.75	1.04
% Increase	135	100	275	215	155	105
OHT						
Control +Molybdate	0.56	0.47	0.69	0.88	0.20	1.92
% Increase	0 ± 10	~0	~0	~0	~0	~0

b, Ligand specificity $(k^-, \times 10^{-4} \text{ s}^{-1})$

	Temperature (°C)	Control	+Molybdate	% Increase
E ₂	20	0.14	0.28	100
E ₁	20	6.60	11.91	80
E ₃	20	0.31	0.58	120
5-Adiol	0	0.63	1.13	80
OHT	20	0.47	0.47	∞=()
T	0	2.30	2.30	= 0
Ci 628	0	0.85	0.85	≈0

a, Species specificity: the dissociation rate constants of E2 and OHT from the cytosol ER of calf, lamb, rat and mouse uterus, of chicken oviduct and of the MCF7 human breast cancer cell line were determined at 20 °C or 15 °C (chicken), following heat 'activation' in the presence or absence of 10 mM sodium molybdate as described in Fig. 2 legend. The values calculated after correction for nonspecific binding and their percentage increase due to molybdate are presented. For OHT studies, the difference between the two linear regressions drawn through the points obtained with and without molybdate was zero or less than 10%. We have therefore represented only the mean values. b, Ligand specificity: the k from the cytosol ER of lamb uterus has been determined at 20 or 0 °C as described in Fig. 2 legend for various oestrogens (oestradiol = E_2 ; oestrone = E_1 ; oestriol = E_3 ; and osta-5-ene-3 β , 17 β diol = 5-Adiol) and antioestrogens (CI 628; tamoxifen = T; 4hydroxytamoxifen = OHT).



Recently, a third criterion has been proposed^{6,11,12}, the slower dissociation rate of oestrogens from the activated ER than from the 'native' non-activated ER. We therefore compared the dissociation rate at 25 °C for ³H-E₂ and ³H-OHT (Fig. 1). As already described², the dissociation rate from the cytosol ER varied according to whether the cytosol was treated at 0 °C or was preheated at 25 °C to activate the complex. In the initially non-activated ER, the dissociation of ³H-E₂ was biphasic. The first slope was more rapid than the second which corresponded to the activated complex formed during dissociation. When the cytosol ER had been preactivated, the dissociation was monophasic and parallel to the second slope. Conversely, with ³H-OHT, no difference of dissociation could be seen whether or not the cytosol was preheated at 25 °C. This suggested a difference of activation between the ER-E₂ and ER-OHT complexes. Because the measurement of the dissociation rate for these high-affinity ligands required elevated temperature which also activated the receptor, we then tried to stabilize the native non-activated receptor by using molybdate.

As seen in Fig. 1, the ER-E2 complex continued to dissociate at the initial rate in the presence of molybdate, which apparently prevents the activation process. Conversely, we found no difference for the dissociation of ³H-OHT from the ER. This seems to be a general phenomenon because, in all species tested, the dissociation of E2 was more rapid from the molybdatestabilized ER, whereas that of OHT was generally not modified by molybdate (Fig. 2, Table 1). In the rat and mouse uterus where antioestrogens are partial agonists 13, the dissociation rate of OHT was also unchanged by molybdate. However, in these species, the metabolite(s) responsible for the agonist activity are unknown. Other oestrogens—oestrone, oestriol and androsta-5-ene, 3B, 17B-diol (5-Adiol)—which bind to the ER with a K_D of 6 nM (ref. 14), also dissociated more rapidly from the ER stabilized with molybdate than from the untreated ER (Table 1b). Conversely, the dissociation rate of the other anticestrogens, tamoxifen and Ci 628, was not altered by molybdate in lamb uterus.

These kinetic experiments indicate an increased affinity of E_2 for the ER during its activation, in contrast to a relatively constant affinity of anticestrogens for the ER. Thus, when anticestrogens compete with E_2 for binding to the ER, the competition should be more effective initially and should diminish progressively with time, E_2 displacing the antices-

Fig. 2 Effect of molybdate on the dissociation rate of oestrogens and anticestrogens from the cestrogen receptor. a, b, Calf uterine cytosol prepared with (\bigcirc, \triangle) or without (\bigcirc, \triangle) 10 mM MoO₄ was incubated for 2 h with 5 nM 3 H-E₂ (\bigcirc, \bigcirc) or 3 H-OHT (\triangle, \triangle) . The cytosol ER was then heated for 30 min at 25 °C and the dissociation rate at 20 °C was evaluated as described previously⁶. Each value was corrected for nonspecific binding. The stability of the complexes was assayed separately $(\Box, \mathbf{m}, \diamondsuit, \spadesuit)$. Results were normalized by taking the pre-dissociation value as 100%. c, d, Same experiment with cytosol of the MCF7 human breast cancer cells. The ER was activated at 20 °C. e, f, Dissociation rate at 0 °C from lamb uterine cytosol incubated with 20 mM ³H-androsta-5ene, 3β,17β-diol (O, ●) (specific activity 58.6 Ci mmol⁻¹) or Cl 628 (Δ, \blacktriangle) (22.5 Cl mmol⁻¹), both from the Radiochemical Centre), in the presence or absence of 2 µM diethylstilboestrol to assay nonspecific binding. After heating for 30 min at 25 °C, the dissociation of the ligands at 0 °C was assayed by adsorption with charcoal for 30 min at 0 °C.

trogen from the ER. We have previously observed such a displacement 15. To specify whether this displacement was due to the activation of the ER or simply to a lack of equilibrium between the two ligands and the ER 16, we have tested the effect of molybdate during competition of 3H-E₂ binding by tamoxifen. Figure 3 shows that the competitive efficiency of tamoxifen was increased by the addition of molybdate. However, the time-dependent increase of E₂ binding was still observed, suggesting that it was due partly to a lack of equilibrium and partly to receptor activation.

The action of non-steroidal antioestrogens is mediated either by the ER as classically proposed or by other receptor entities, such as the recently described¹⁷ protein binding specifically anti-oestrogens. In support of the latter hypothesis, we have found in MCF7 breast cancer cells, which do not metabolize tamoxifen or OHT, that OHT is more active than tamoxifen in inhibiting the induction by E₂ of a 46,000 molecular weight protein¹⁶ and in preventing cell growth¹⁹. However, the finding that the effect of antioestrogens in MCF7 cells 20 and in chick liver is fully and rapidly reversed by the addition of oestrogens. strongly suggests that these antioestrogens are simply acting by competing with oestrogens on the ER. Nevertheless, it is not clear why the ER-antioestrogen complex is not fully active or is in some cases totally unable to induce oestrogen-specific proteins. The simplest explanation is that the E₂-induced activation of the ER is different from that triggered by an antioestrogen. This altered activation of the ER by antioestrogen might therefore be detectable before the nuclear translocation step, even though its consequence will be located at sites of interaction with chromatin. An alternative hypothesis is an altered interaction with chromatin of a normally activated ER-antiquestrogen

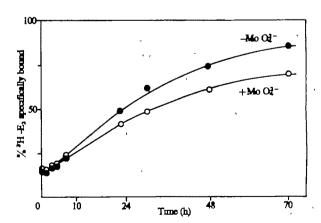


Fig. 3 Time-dependent variation of competitive efficiency of tamoxifen and effect of molybdate. Lamb uterine cytosol was incubated in the presence (○) or absence (●) of molybdate with 2 nM ³H-E₂±0.5 μM tamoxifen for the indicated time periods. The ³H-E₂-specific binding was determined by charcoal assay ⁶ and the binding obtained in the presence of tamoxifen was expressed as a percentage of the non-inhibited binding at each point.

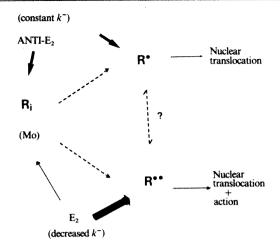


Fig. 4 A model for two types of oestrogen receptor activation induced by oestrogens or antioestrogens. The native ER (Ri) is in the cytosol. After interaction with antioestrogens (anti-E2), it is activated for nuclear translocation (R*) but not for a complete action. After interaction with oestrogens (E2), the ER is fully activated for nuclear translocation and for action (R**). We propose to differentiate between the two types of activation by measuring the in vitro dissociation rate of ligands from the molybdate-stabilized Ri and the activated R. Whereas oestrogens dissociate at a slower rate from R** than from R_i, antioestrogens dissociate at the same rate from both R* and R_i. The width of the full arrows indicates the relative affinity of the ligands for each receptor state.

complex. This could be envisaged, for instance, as a direct interaction of the free drug with DNA or protein in chromatin. Our results strongly support the first possibility. This difference of activation by antioestrogens is detected before the translocation step by simply analysing the dissociation rate of the antioestrogen from the ER with and without molybdate. The consequence of this impeded activation is probably located at the chromatin level, where the ER is thought to trigger the first biochemical responses.

As the oestrogens but not the antioestrogens are able to transform the ER-ligand complex into a more slowly dissociating form, although both ligands can induce the ER nuclear translocation, we propose two kinds of ER activation (Fig. 4). The first (R*) would be obtained after binding of any ligand, oestrogen or antioestrogen, and would trigger a covalent or conformational change of ER allowing its nuclear localization. The second type of activation (R") would be obtained only after binding to oestrogens and would induce a more complete modification of the ER, giving a full metabolic response. A practical in vitro criterion for a full biological activation of the receptor would therefore be a modification of the ER binding site such that the dissociation rate of agonists, but not antagonists, is reduced. It is thus possible to discriminate between oestrogen agonists and antagonists by simply comparing the dissociation rate constants of the ligands from the molybdate-stabilized (non-activated) and from the activated forms of the ER. Note that it is not the actual value of the k^- , but rather its variation during ER activation, which characterizes an active oestrogen. In this model, the affinity of the antioestrogen for the activated and non-activated ER would be similar, whereas the affinity of oestrogen would be higher for the activated form of the ER. We propose that antioestrogens act as allosteric²² ligands which stabilize the ER in inactive (R_i) or partially activated form (R*), whereas oestrogens stabilize the receptor in a fully activated form (R**). These results further support the generally accepted idea that antioestrogens act via the ER and not via a distinct receptor molecule specific for antioestrogens.

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- Clark, J. H. et al. (eds) Dahlem Konf., Life Sci. Res. Rep. 3, 147-169 (1976).
- Rochefort, H., Garcia, M. & Borgna, J. L. Biochem. biophys. Res. Commun. 88, 351-357
- Katzenellenbogen, B. S. Katzenellenbogen, J. A., Ferguson, E. R. & Krauthammer, N. J. biol. Chem. 253, 697-707 (1978).
 Borgna, J. L. & Rochefort, H. J. biol. Chem. 256, 859-868 (1981).
- Jordan, V. C., Collins, M. M., Rowsby, L. & Prestwich, G. J. Endocrinology 75, 305-357
- Borgna, J. L. & Rochefort, H. Molec. cell. Endocr. 20, 71-86 (1980)
- Mauck, L. A. & Notides, A. C. 62nd A. Meet. Endocr. Soc., Abstr. 209 (1980).
- Shyamala, G. & Leonard, L. J. biol. Chem. 255, 6028-6031 (1980) Baskevitch, P. P. & Rochefort, H. Molec. cell. Endocr. 22, 195-210 (1981)
- Notides, A. C. & Nielsen, S. J. J. biol. Chem. 249, 1866-1873 (1974).
 Raynaud, J. P., Bouton, M. M. & Oiasoo, T. Trends pharmac. Sci. 324-327 (1980).

- Raynaud, J. P., Bouton, R. M. & Ossoo, I. Trends printing. 3ct. 324-32. Weichman, B. M. & Notides, A. C. Endocrinology 106, 434-439 (1980). Black, L. J. & Goode, R. L. Life Sci. 26, 1453-1458 (1980).
- Garcia, M. & Rochefort, H. Endocrinology 104, 1797-1804 (1979)

- Rochefort, H. & Capony, F. Biochem. biophys. Res. Commun. 75, 277-285 (1977).

 Aranyi, P. Biochim. biophys. Acta 628, 220-227 (1980).

 Sutherland, R. L., Murphy, L. C., San Foo, M., Green, M. D. & Whybourne, A. M. Nature
- Westley, B. R. & Rochefort, H. Cell 20, 353-362 (1980).
- Rochefort, H., Borgna, J. L., Coezy, E., Vignon, F. & Westley, B. in Non-Steroidal Antiestrogens (eds Sutherland, R. L. & Jordan, V. C.) (Academic, Sydney, 1980).
- Lippman, M., Bolan, G. & Huff, K. Cancer Res. 36, 4595-4501 (1976).
- Capony, F. & Williams, D. L. Endocrinology 19, 2219–2226 (1980).
 Bullock, L. P., Bardin, C. Q. & Sherman, M. Endocrinology 103, 1768–1782 (1978).

Human transforming growth factors induce tyrosine phosphorylation of EGF receptors

Fred H. Reynolds Jr*, George J. Todaro†, Charlotte Fryling† & John R. Stephenson†

* Carcinogenesis Intramural Research Program, Frederick Cancer Research Center, Frederick, Maryland 21701, USA

† Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701, USA

Cultured cell lines of human tumour origin as well as cells transformed by various RNA tumour viruses secrete low molecular weight polypeptide transforming growth factors (TGFs)1.2. In addition to competing with epidermal growth factor (EGF) for binding to its cellular receptor, TGFs can transform morphologically fibroblast and epithelial cells in culture 1-3. In view of accumulating evidence that tyrosine phosphorylation activity is associated with the transforming genes of various tumour viruses⁴⁻¹³, we determined whether phosphotyrosine levels were elevated in these human tumour cells. We show here that TGFs produced by human tumour cells induce phosphorylation of specific tyrosine acceptor sites in the 160,000molecular weight (160 K) EGF receptor.

The demonstration of increased levels of phosphotyrosinecontaining proteins in cells transformed by avian³⁻⁸ and mam-malian⁹⁻¹³ RNA tumour viruses raises the question of whether RNA tumour viruses raises the question of whether regulatory pathways which involve tyrosine phosphorylation may also have significance in the aetiology of spontaneous and chemically induced tumours. We therefore examined phosphotyrosine levels in several established human tumour cell lines. Of six independently derived lines analysed, none showed overall phosphotyrosine concentrations that were significantly greater than those of untransformed control Fisher rat embryo cells (Table 1). Similarly, we (data not shown) and Sefton et al.⁵ have analysed many non-virally transformed mammalian cells of other than human origin and found that the phosphotyrosinecontaining protein levels are comparable with those of untransformed cells. In analogous assay conditions, as reported elsewhere 5.9.12.13, cells transformed by either Gardner feline sarcoma virus (FeSV) or Abelson murine leukaemia virus (AbLV) show 8-10-fold increases in phosphotyrosine levels,

Table 1 Phosphotyrosine levels in human tumour and Fisher rat cells after exposure to either EGF or human TGF

	Phosphotyrosine	(% of total acids) EGF-	phosphoamino TGF-
Cell line	Untreated	stimulated	stimulated
Human tumour:			
A431 carcinoma	< 0.2	2.6	2.4
A673 rhabdomyosarcoma	< 0.2	< 0.2	< 0.2
A2058 melanoma	0.4	0.4	< 0.2
A875 melanoma	< 0.2	0.3	NT
JARC2 carcinoma	< 0.2	< 0.2	NT
A204 rhabdomyosarcoma	< 0.2	< 0.2	NT
Fisher rat:			
Control	< 0.2	< 0.2	< 0.2
AbLV-transformed	1.9	2.0	NT
G-FeSV-transformed	1.7	1.9	1.8
M-MSV-transformed	< 0.2	< 0.2	NT

Cell lines were cultured in Dulbecco's modification of Eagle's medium as described previously¹. Phosphotyrosine determinations were performed by incubation of cells in medium containing ^{32}P -orthophosphate $(1.0~\text{mCi ml}^{-1})$ and as indicated above, either EGF $(0.1~\text{µg ml}^{-1})$ or transforming growth factor (TGF $10~\text{µg ml}^{-1})$, partially purified from medium of A673 cultures. ^{32}P -labelled proteins were prepared, hydrolysed and phosphoamino acids subjected to two-dimensional separation on cellulose TLC plates as previously described¹³. Individual ^{32}P -labelled phosphoamino acids, including phosphoserine, phosphothreonine and phosphotyrosine, were scraped off TLC plates and radioactivity quantified by liquid scintillation counting. Results are expressed as ^{32}P label in phosphotyrosine as a percentage of label in total phosphoamino acids. NT, not tested.

while the levels of phosphotyrosine in Moloney sarcoma virus (MSV)-transformed cells are similar to those in control cell lines.

In view of the phenotypic response of embryo fibroblasts to TGFs and EGF-stimulated tyrosine-specific protein kinase activity associated with the EGF membrane receptors14 examined phosphotyrosine levels in human TGF- and mouse EGF-treated cells. As shown in Table 1, the human tumour line with the greatest concentration of available EGF receptors, A431 (refs 15, 16), showed a pronounced increase in total phosphotyrosine in response to either EGF or TGF, isolated from the culture fluids of the A673 rhabdomyosarcoma cell line¹. The overall extents of tyrosine phosphorylation in these growth factor-treated cells were comparable with those found in RNA tumour virus-transformed cells. Concentrations of phosphotyrosine in other cell lines, including A673 and A2058, which themselves are high-level producers of TGFs¹, remained unaltered after exposure to either human TGF or mouse EGF. Finally, the already elevated levels of phosphotyrosine in Gardner FeSV- and AbLV-transformed rat cells showed no further increase in response to either growth factor.

The A431 cell substrate(s) phosphorylated in response to TGFs were identified by an in vitro extract labelling procedure, which has been previously shown to result in preferential phosphorylation of tyrosine acceptor sites¹⁷. Figure 1 shows that phosphorylation of a 160,000-M_r major protein was observed after exposure of A431 cells to EGF for 1 min (lane a), but was not seen in control cells (lane c). ³²P-labelling of this substrate, designated P160, was observed at EGF concentrations as low as $0.01 \,\mu \text{g ml}^{-1}$ (Fig. 1, lane k), and was maximal at $1.0 \,\mu \text{g ml}^{-1}$ (Fig. 1, lane m). On the basis of both M_r and immunoprecipitation by an antiserum which specifically blocks binding of labelled EGF to the previously described 160 K EGF membrane receptor, this phosphorylated cellular substrate was thought to be the EGF receptor itself. Exposure of A431 cells for 1 min to 100 µg ml⁻¹ of either A673 human tumour-derived TGF (lane e) or sarcoma growth factor (SGF) isolated from supernatant fluids of MSV-transformed cells² (lane g) resulted in P160 phosphorylation comparable with that observed for higher levels of EGF (1.0 µg ml⁻¹). The higher specific activity of EGF as measured by stimulation of P160 phosphorylation or inhibition of 125I-labelled EGF binding reflects the fact that it was fully purified whereas the TGF and SGF used here were only 1% pure. In experiments in which the length of exposure to EGF or TGF was increased to 10 min before extract preparation, the extent of 32 P-labelling of P160 was considerably reduced and a second 32 P-labelled substrate of $\sim 35,000\,M_{\rm r}$ was observed. Analysis of Fisher rat cells for growth factor-induced phosphorylation of P160 showed no change in response to either EGF or TGF (Fig. 1, lanes f,h). Several lower molecular weight (20,000–40,000) proteins were phosphorylated in extracts of TGF- and SGF-treated rat cells but their significance is unknown. Other growth stimulatory factors including phorbol ester derivatives and insulin, when tested for stimulation of EGF receptor phosphorylation, had no effect in these assay conditions. Moreover, pretreatment of cells with phorbol ester derivatives in conditions previously shown to modulate EGF binding 18 by converting available sites from high to low affinity 19,20 , did not affect phosphorylation of P160 in response to either EGF or TGF.

Both the human and MSV-transformed mouse cell-derived factors have been further purified by HPLC²¹. Individual column fractions were assayed for inhibition of EGF binding, transformation of cultured rat embryo fibroblasts and stimulation of EGF receptor phosphorylation. All three activities copurified in a region of a column representing <2.0% of the total input protein which argues strongly that EGF receptor-induced phosphorylation was due to the respective TGFs. In other studies, heating these factors in conditions (100 °C, 5 min) which inactivate protein kinases neither destroyed the ability of TGFs to bind to EGF receptors nor altered P160-induced phosphorylation. Thus, the EGF receptor phosphorylation requires a kinase associated with the receptor. Binding of EGF, SGF, or TGF to this receptor alters its properties to give phosphorylation of the receptor protein.

These findings identify a substrate, P160, which is labelled in the presence of $[\gamma^{-32}P]$ ATP in extracts of growth factor-treated but not untreated control cells. To test whether these extracts represented tyrosine acceptor sites, the 160 K EGF receptor, phosphorylated in response to EGF or to TGF, using *in vitro* extract labelling conditions, was eluted from SDS-polyacrylamide gel electrophoresis gels by trypsinization, and subjected to phosphoamino acid analysis. Figure 2 shows that

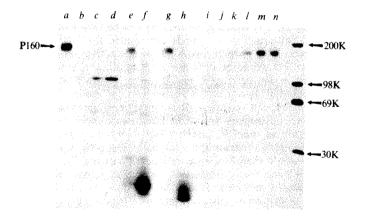


Fig. 1 SDS polyacrylamide gel electrophoresis (SDS-PAGE) analysis of substrates phosphorylated in A431 cell extracts in response to treatment with either mouse EGF or human TGF. A431 human tumour cells (a.c.e. g.in) and Fisher rat embryo cells (b,d,f,h) were grown to confluency in single wells (1 cm diameter) of 24-well cluster plates (Costar). Culture fluids were aspirated, and cells rinsed with 10 mM sodium phosphate pH 7.2, 100 mM NaCl and 5 mM MgCl₂. A total of 0.05 ml of the same buffer containing 0.001(j), 0.01(k), 0.1(l), 1.0(m) or 10(a,b,n) µg EGF, or partially purified 20,000-M, preparations from P-100 columns of either TGF (1 mg; e, f) or SGF (1 mg; g,h), were added. Cultures were kept at 25 °C for 1 min, then disrupted by incubation for 1 min at 4 °C in 0.05 ml 10 mM sodium phosphate pH 7.2, 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate and 5 mM MgCl $_2$ buffer. [γ - 32 P]ATP (50 μ Ci) was added and incubation continued for a further 1 min. Reactions were stopped by addition of 0.05 ml of 0.65 M Tris-HCl pH 6.7, 1.0% SDS, 10% glycerol, 2.5% 2-mercaptoethanol and 0.1% bromphenol blue, heated for 2 min at 90°C and analysed by SDS-PAGE as described elsewhere9. Molecular weight standards include ⁴C-labelled myosin (M_r 200,000), phosphorylase b (98,000), bovine serum albumin (69,000) and carbonic anhydrase (30,000).

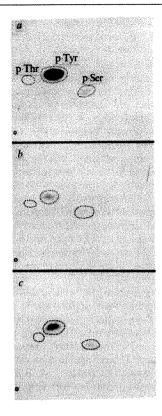


Fig. 2 Phosphoamino acid analysis of the 160 K EGF membrane receptors of A431 cells ³²P-labelled in vitro after exposure of cells to either mouse EGF or human TGF. A431 cells were incubated in medium containing mouse EGF (1 μg ml⁻¹; a) or human TGF (100 μg ml⁻¹; b), extracts prepared, and in vitro phosphorylation reactions performed as described in Fig. 1 legend. Snyder-Theilen FeSV P115 was immunoprecipitated by anti-FeLV from an extract of nonproductively transformed Fisher rat cells after labelling with [γ-³²P]ATP (c). γ-³²P-labelled proteins were recovered from gel slices by incubation in TPCK-trypsin (50 μg ml⁻¹ in 0.05 M ammonium bicarbonate, pH 8.0; Worthington) for 6 h at 37 °C. Supernatant fluids were filtered, lyophilized, washed by resuspension in H₂O and relyophilized. Tryptic peptides were hydrolysed in 6.0 M HCl at 100 °C for 1 h and two-dimensional phosphoamino acid determination performed as described elsewhere 13. The positions of unlabelled standards including phosphothreonine (p-Thr), phosphotyrosine (p-Tyr) and phosphoserine (p-Ser) are indicated by dotted circles.

 32 P-labelled P160 in extracts of either TGF(a)- or EGF(b)-treated cells contained phosphotyrosine as its major labelled phosphoamino acid, with smaller amounts of phosphothreonine and phosphoserine. Similar results were obtained for P160 isolated from extracts of SGF-treated cells. The relative proportions of 32 P-labelled phosphoamino acids in P160 after growth factor-activated phosphorylation were comparable with those found in Gardner FeSV P115 (Fig. 2c), a representative type C virus-encoded transforming protein with tyrosine-specific protein kinase activity 9 .

Although each of the growth factors examined here compete with EGF for binding to its receptor, only SGF and TGF phenotypically transform cells in culture. Analysis of the 160K EGF receptor in A431 cells by tryptic peptide mapping after either EGF- or human TGF-induced phosphorylation, revealed one major (no. 6) and seven minor phosphorylated peptides with corresponding positions, whether the tyrosine kinase was activated by EGF or by TGF (Fig. 3). On phosphoamino acid analysis of individual peptides, each was shown to contain tyrosine as its major phosphorylated species.

Cells transformed by several different RNA tumour viruses show an increase in the overall phosphotyrosine content of their proteins while those transformed by other RNA tumour viruses (mouse sarcoma, rat sarcoma)^{5,7-11,13,22} resemble the human tumour cell lines examined here in that their phosphotyrosine levels are not significantly greater than in untransformed cells. These differences may reflect the fact that viral transforming proteins encoded by avian sarcoma viruses⁵⁻⁸, Gardner and

Snyder-Theilen FeSV $^{9-11}$ and AbLV $^{12.13}$ are expressed at relatively high levels and all possess intrinsic acceptor sites for tyrosine-specific protein kinase activities. Similarly, the increase in total cellular phosphotyrosine in A431 cells in response to EGF or TGF stimulation may be due to the exceptionally large number of EGF receptors $(2-3\times10^6~{\rm per~cell})$ in the A431 cell line. Thus, the inability to detect overall increases in phosphotyrosine in most spontaneously and chemically transformed cells does not argue against a pivotal role of tyrosine phosphorylation in the maintenance of transformation of such cells.

The phenotypic response of cells to both human and mouse cell-derived TGFs is strikingly different from their response to EGF, whereas A431 cells show similar phosphorylation responses to these two classes of growth factor. It is possible that, in addition to their binding to, and phosphorylation of, the 160 K major EGF membrane receptor, TGFs interact with an unidentified TGF-specific cell receptor. Alternatively, interaction of growth factors with, and resulting tyrosine phos-

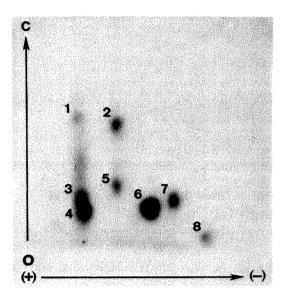


Fig. 3 Two-dimensional tryptic analysis of phosphopeptides derived from the 160 K EGF membrane receptor of A431 cells ^{32}P -labelled in vitro in the presence of human TGF. Extracts were prepared and in vitro phosphorylaton reactions performed as described in Fig. 1 legend. ^{32}P -labelled proteins were recovered from gel slices by incubation in TPCK-trypsin as described in Fig. 2 legend, then incubated for 2 h at 4 °C in 0.1 ml of chilled performic acid (30% $\rm H_2O_2$ and 90% formic acid (1:9) preincubated for 2 h at room temperature), diluted with 2 ml $\rm H_2O$, lyophilized, resuspended in electrophoresis buffer (acetic acid/formic acid/water, 15:5:80, pH 1.9) and spotted on to 10×10 cm TLC glass plates. Electrophoresis was for 30 min at 500 V and chromatography was done in the second dimension in buffer containing butanol, pyridine, acetic acid and water (32:25:5:20). Radiolabelled tryptic peptides were visualized by autoradiography.

phorylation of, the 160 K EGF receptor could be part of the pathway leading to the transformed phenotype. Minor differences in the tryptic phosphopeptide maps of the P160 receptor labelled in response to EGF- and TGF-induced phosphorylation might reflect differences in the interaction of P160 with these growth factors.

The significance of protein kinases which have specificity for tyrosine acceptor sites both in RNA tumour virus-transformed cells and in response to growth regulatory factors is not fully understood. Recent reports of a series of cellular protein kinases, which are activated by phosphorylation of tyrosine acceptor sites and in turn have specificity for tyrosine residues, may be relevant²³. Activation of this pathway leads to phosphorylation of a tyrosine regulatory site(s) on the cellular (Na⁺ + K⁺)ATPase and seems to involve the cellular analogues of certain RNA tumour viruses²⁴. It remains to be determined whether phosphorylation of cellular receptors in response to TGFs directly leads to cell transformation by activation of a phosphorylation cascade.

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- 1. Todaro, G. J., Fryling, C. & De Larco, J. E. Proc. natn. Acad. Sci. U.S.A. 77, 5258-5262
- De Larco, J. E. & Todaro, G. J. Proc. natn. Acad. Sci. U.S.A. 75, 4001-4005 (1978). Todaro, G. J. & De Larco, J. E. in Control Mechanisms in Animal Cells: Specific Growth Factors Vol. 1 (eds Jimenez de Asua, L., Levi-Montalcini, R., Shields, R. & Iacobelli, S.) 223-243 (Raven, New York, 1980).
- Eckhart, W., Hutchinson, M. A. & Hunter, T. Cell 18, 925-933 (1979). Sefton, B. M., Hunter, T., Beemon, K. & Eckhart, W. Cell 20, 807-816 (1980).
- Collett, M. S., Purchio, A. F. & Erikson, R. L. Nature 285, 167-169 (1980)

- Collett, M. S., Tuchio, A. T. & Erisson, R. L. Nature 263, 167-169 (1980). Feldman, R. A., Hanafusa, T. & Hanafusa, H. Cell 22, 757-765 (1980). Pawson, T. et al. Cell 22, 767-775 (1980). Reynolds, F. H. Jr, Van de Ven, W. J. M. & Stephenson, J. R. J. biol. Chem. 255, 11040-11047 (1980)
- 10. Barbacid, M., Beemon, K. & Devare, S. G. Proc. natn. Acad. Aci. U.S.A. 77, 5158-5162
- 11. Reynolds, F. H. Jr, Van de Ven, W. J. M., Blomberg, J. & Stephenson, J. R. J. Virol. 37, 643-653 (1981). Witte, O. N., Dasgupta, A. & Baltimore, D. *Nature* **283**, 826-831 (1980)
- Blomberg, J., Reynolds, F. H. Jr, Van de Ven, W. J. M. & Stephenson, J. R. Nature 286, 504-507 (1980).
- Ushiro, H. & Cohen, S. J. biol. Chem. 255, 8363-8365 (1980).
- 15. Fabricant, R. N., De Larco, J. E. & Todaro, G. J. Proc. natn. Acad. Sci. U.S. A. 74, 565-569
- 16. Haigler, H., Ash, J. F., Singer, S. J. & Cohen, S. Proc. natn. Acad. Sci. U.S.A. 75, 3317-3321 (1978)
- 17. Blomberg, J., Van de Ven, W. J. M., Reynolds, F. H. Jr, Nalewaik, R. P. & Stephenson, J. R. J. Virol. 38, 886-894 (1981). Lee, L.-S. & Weinstein, I. B. Science 202, 313-315 (1978)

- Shoyab, M., De Larco, J. E. & Todaro, G. J. Nature 279, 387-391 (1979).
 Brown, K. D., Dicker, P. & Rozengurt, E. Biochem. biophys. Res. Commun. 86, 1037
- 21. Marquardt, H., DeLarco, J. E. & Todaro, G. J. (in preparation)
- Reynolds, F. H. Jr, Van de Ven, W. J. M. & Stephenson, J. R. J. Virol. 36, 374–386 (1980). Spector, M., O'Neal, S. & Racker, E. J. biol. chem. 256, 4219–4227 (1981).
- 24. Spector, M., Pepinsky, R. B., Vogt, V. M. & Racker, E. Cell (in the press).

Somatostatin modulates effects of angiotensin II in adrenal glomerulosa zone

Greti Aguilera, James P. Harwood* & Kevin J. Catt

Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development. National Institutes of Health, Bethesda, Maryland 20205, USA *Food and Drug Administration, Washington, DC 20204, USA

The octapeptide angiotensin II is a major regulator of the adrenal glomerulosa zone, acting both as an acute stimulus of aldosterone secretion and as a trophic hormone which increases steroidogenic enzymes and angiotensin II receptors in glomerulosa cells^{1,2}. Angiotensin II also mediates the adrenal effects of altered sodium balance, and is essential for the aldosterone response to sodium restriction3.4. However, the adrenal effects of angiotensin II are attenuated during sodium loading, suggesting that other local or humoral factors modulate its actions on adrenal glomerulosa function^{5,6}. Somatostatin, the somatotropin release inhibiting factor of the hypothalamus, has been shown to inhibit the secretion and action of several pituitary7. and non-pituitary hormones⁹⁻¹³. Because somatostatin has been found in several non-neural tissues, and seems to act as a local regulator of endocrine function, we have now examined the possibility that it may also modulate the effects of angiotensin II in the adrenal glomerulosa cell. Our studies have shown that low concentrations of somatostatin specifically inhibit the production of angiotensin II-stimulated aldosterone, and that this action is mediated by specific, high-affinity receptors for somatostatin in the zona glomerulosa.

When incubated with collagenase-dispersed rat adrenal glomerulosa cells14, somatostatin markedly inhibited the ability of angiotensin II to stimulate aldosterone formation. As shown in Fig. 1, the maximal aldosterone response to angiotensin II was reduced by 43% in the presence of 1 nM somatostatin, and was abolished by 1 µM somatostatin. In contrast, the aldosterone

responses to other stimuli, including potassium, ACTH and 8-bromo-cyclic AMP, were completely unaffected by somatostatin at concentrations of up to 1 µM. Although low concentrations of somatostatin (10⁻¹⁰-10⁻⁹ M) had no effect on basal aldosterone production, higher concentrations (>10⁻⁸ M) had a minor stimulatory effect on aldosterone production. The latter effect was inconstant and varied with the source and batch of the peptide, whereas the inhibitory effect of low concentrations of somatostatin was always evident. The inhibitory effects of somatostatin on the stimulation of aldosterone by increasing concentrations of angiotensin II are shown in Fig. 2. Without changing the basal aldosterone production, 10^{-10} and 10^{-9} M somatostatin reduced the maximal aldosterone responses to angiotensin II. Higher somatostatin concentrations (10⁻⁸ and 10⁻⁷ M) also decreased the maximal responses to angiotensin II, although basal aldosterone production was elevated due to the direct stimulatory action of the somatostatin preparation used in this experiment. Micromolar concentrations of somatostatin completely abolished aldosterone responses to angiotensin II concentrations up to 10^{-6} M.

To elucidate the mechanism by which somatostatin exerts its specific inhibitory effect on angiotensin II action, we analysed the interactions of synthetic somatostatin with adrenal receptors for angiotensin II and somatostatin. In 125 I-angiotensin II binding studies, several somatostatin preparations completely inhibited angiotensin II binding, but with varying half-maximum potencies ($\overline{\text{ID}}_{50}$) of $4.2 \pm 1.0 \times 10^{-8} \text{ M}$, $3.8 \pm 2.5 \times 10^{-7} \text{ M}$ and $5.1 \pm 1.2 \times 10^{-6}$ M for Beckman lots E0535 and B00926, and Peninsula peptides, respectively. These concentrations were similar to those required for stimulation of aldosterone production by the individual preparations, suggesting that interaction with the angiotensin receptor is responsible for their weak steroidogenic effects. This possibility was supported by the ability of the angiotensin II antagonist [Sar¹, Ala⁸]angiotensin II in three experiments (not shown) to inhibit the aldosterone responses to both 10^{-9} M angiotensin II and 10^{-7} M somatostatin, while the ACTH-stimulated aldosterone response was unchanged. The basis of the direct actions of certain somatostatin preparations on the angiotensin II receptor is not known, but such high-dose effects are not relevant to our main finding, that low concentrations of somatostatin consistently inhibit the aldosterone response to angiotensin II.

The specific inhibition of angiotensin-stimulated aldosterone responses by low somatostatin concentrations could not be explained by interaction with the angiotensin II receptor. Thus, nanomolar concentrations of somatostatin did not influence

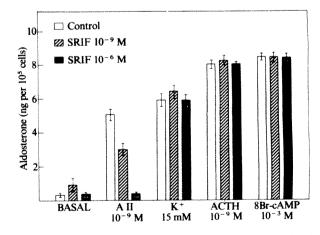


Fig. 1 Effect of somatostatin (Beckman, lot E0535) on basal and stimulated aldosterone production in collagenase-dispersed rat adrenal glomerulosa cells. Aliquots containing 1-2×10⁵ cells were incubated for 2 h and aldosterone production was determined by direct radioimmunoassay of the incubation media 14. Bars represent the mean and s.e. of three experiments. AII, angiotensin II; 8Br-cAMP, 8-bromo-cyclic AMP.

angiotensin II binding, yet significantly decreased the aldosterone responses to angiotensin II, suggesting that the peptide could act through specific receptors in the glomerulosa zone. In binding studies with ¹²⁵I-Tyr¹-somatostatin, specific highaffinity receptor sites for somatostatin were found in the adrenal capsule, which contains most of the zona glomerulosa layer (Fig. 3). In contrast, particulate fractions from the decapsulated adrenal displayed only 10% of the binding found in adrenal capsules (data not shown). In six experiments, the mean binding capacity for somatostatin was 329 ± 64 fmol per mg of adrenal capsular protein, and the association constant (K_a) was $0.9 \pm 0.3 \times 10^{10} \,\mathrm{M}^{-1}$. The binding of $^{125}\mathrm{I-Tyr}^1$ -somatostatin to adrenal capsular particles was saturable and specific, with no displacement by angiotensin II, [D-Ala⁶]gonadotropin releasing hormone, vasopressin, oxytocin and substance P, at concentrations up to 10^{-5} M. The specificity of the somatostatin receptor sites has been confirmed in studies which demonstrate that a series of somatostatin analogues displace 125I-Tyr1-somatostatin from adrenal capsular membranes with potencies that correlate with their ability to inhibit angiotensin II-stimulated aldosterone15

Somatostatin has been identified by immunofluorescence studies in specific cells of several tissues including thyroid⁹, pancreas^{11,13} and gastric mucosa^{12,13,16}, and local release of the peptide has been proposed to control the function of endocrine cells. Evidence for such a mechanism in the glomerulosa zone was sought by analysing the endogenous somatostatin content of the adrenal capsule. Tissues were homogenized in 2 M acetic acid, boiled for 5 min and centrifuged at 15,000g. The supernatant was lyophilized and the somatostatin content of the extract was determined by radioimmunoassay¹⁷. immunoreactive somatostatin in extracts from adrenal capsules was $1,817 \pm 200$ pg per mg, compared with $146 \pm 15, 7.6 \pm 1.3$ and 10.3 ± 2 pg per mg in extracts of decapsulated adrenals, urinary bladder and aorta, respectively. The values observed in adrenal capsule were comparable with those present in the pancreas and gastrointestinal tract¹², and were much higher than could be attributed to peptide binding to the specific receptor sites of the zona glomerulosa.

These data demonstrate the presence of somatostatin receptors and well defined actions of the tetradecapeptide in the

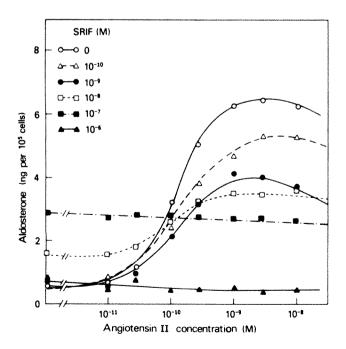


Fig. 2 Inhibition of aldosterone responses to angiotensin II (10⁻⁹ M) by somatostatin (SRIF) in collagenase-dispersed adrenal glomerulosa cells. Data points are the mean of duplicate incubations in one of three similar experiments.

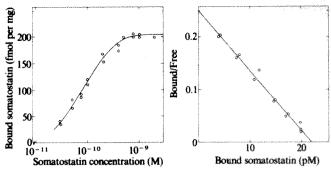


Fig. 3 Saturation curve and Scatchard plot of the binding of somatostatin (SRIF) to adrenal particles. Tyr¹-SRIF (Peninsula) was iodinated with ¹²⁵I by a modification of the technique of Hunter and Greenwood¹⁸, using prolonged incubation with low concentrations of chloramine T (1 µg). The reaction was terminated by addition of an excess of tyrosine, and the labelled peptide was purified by chromatography on a 0.7×14 cm Biogel P-2 column eluted with 0.01 M acetic acid. Specific activity of the tracer was 1,500-2,000 µCi per µg and maximum binding to excess receptors was 40-60%. Binding assays were performed by incubation of 50-100 µg of protein in 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl₂, 2 mM EGTA, 20 µg ml⁻¹ bacitracin, 50 µg ml⁻¹ Merthiolate and 1% bovine serum albumin in a total volume of 500 µl. Incubations were performed for 30 min at 20 °C, and bound ¹²⁵I-Tyr¹-SRIF was separated by filtration through Whatman GF/C glass fibre filters. Nonspecific binding was determined in the presence of 10⁻⁷ M unlabelled SRIF and this value (<1% of total radioactivity added) was subtracted from total binding to yield specific binding (8-15% of the total radioactivity added). Data points are the mean of duplicate incubations from a typical experiment.

adrenal glomerulosa zone. Somatostatin specifically inhibited the aldosterone responses to angiotensin II, and this effect was manifested at much lower concentrations of the peptide than those required to inhibit angiotensin II binding. Therefore, it is likely that somatostatin exerts its inhibitory effect on angiotensin II-induced aldosterone responses by interacting with specific high-affinity receptors and modulating the second messenger system that mediates the action of angiotensin II on aldosterone biosynthesis. The occurrence of high concentrations of immunoreactive somatostatin in the adrenal capsule suggests that, as in certain other tissues, somatostatin is locally secreted and could exert a regulatory action on target cell responses to endocrine stimulation. The highly specific inhibitory action of somatostatin on angiotensin II-stimulated aldosterone production could be relevant to the changing sensitivity of the adrenal glomerulosa zone to angiotensin II during the physiological control of aldosterone secretion.

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- 1. Hauger, R., Aguilera, G. & Catt, K. J. Nature 271, 176-178 (1978)
- Aguilera, G., Menard, R. & Catt, K. J. Endocrinology 107, 55-60 (1980). Aguilera, G. & Catt, K. J. Proc. natn. Acad. Sci. U.S.A. 75, 4057-4061 (1978).
- Aguilera, G., Schirar, A., Baukal, A. & Catt, K. J. Circulation Res. 46, Suppl. 1, 118-127
- Nichols, M. G. et al. Endocrinology 102, 485-493 (1978)
- Cowley, A. W. & McCaa, R. E. Circulation Res. 39, 788-797 (1976).
- Barzeau, P. et al. Science 179, 77-79 (1973).
 Vale, W., Rivier, J., Brazeau, P. & Guilleman, R. Endocrinology 95, 968-974 (1974).
- Kronheim, S., Berelowitz, M. & Pimstone, B. Clin. Endocr. 5, 619-623 (1976).
 Bolaffi, L. L., Reichlin, S., Goodman, D. B. P. & Forrest, J. N. Science 210, 644-646 (1980).
- Dubois, M. Proc. natn. Acad. Sci. U.S.A. 72, 1340-1343 (1975). Schlegel, W. et al. in Hormonal Receptors in Digestive Tract Physiology (eds Bonfils, S., Fromageot, P & Rosselin, G.) 361 (North-Holland, Amsterdam, 1977) Guillemin, R. & Gerich, J. E. A. Rev. Med. 27, 379-388 (1976).
- Douglas, J., Aguilera, G., Kondo, T. & Catt, K. J. Endocrinology 102, 685-696 (1978). Aguilera, G., Parker, D. & Catt, K. J. Endocrine Soc. 63rd a. Meet. abstr. 16 (1981).
- Larsson, L., Golterman, N., DeMagistris, L., Rehfeld, J. F. & Schwartz, T. W. Science 205,
- 17. Gerich, J., Greene, K., Hara, M., Rizze, R. & Petton, G. J. Lab. clin. Med. 93, 1009-1017
- 18. Hunter, W. M. & Greenwood, F. C. Nature 194, 495-496 (1962).

Gonadotropin releasing hormone stimulates calmodulin redistribution in rat pituitary

P. Michael Conn*, James G. Chafouleas†, Deloris Rogers* & Anthony R. Means†

- * Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710, USA
- † Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030 USA

Calcium (Ca²⁺) seems to have an informational role in many tissues. In particular, it fulfills the requirements of a second messenger for gonadotropin releasing hormone (GnRH)-stimulated luteinizing hormone (LH) release from the pituitary gonadotrope (see ref. 1 for review). Very little is known about the effect of this ion on intracellular targets or the mechanism by which Ca2+ mobilization stimulates LH release. One intracellular target for Ca2+ is calmodulin, a ubiquitous intracellular receptor that has been shown to modulate many cellular functions, including cyclic nucleotide and glycogen metabolism. protein phosphorylation, microtubule assembly and disassembly, Ca2+ flux, and the activities of NAD kinase, tryptophan 5' monooxidase and phospholipase A_2 (see refs 2-5 for reviews). We have now used a specific and sensitive radioimmunoassay to determine the quantity and distribution of calmodulin in the gonadotrope before and during GnRH-stimulated LH release. The data indicate that GnRH stimulates redistribution of calmodulin from the cytosol to the plasma membrane and suggest that the molecule may have a role in the mechanism of stimulus-secretion coupling.

Figure 1 shows the distribution of calmodulin in the subcellular fractions (expressed as a percentage of the homogenate) at the indicated dose and time after GnRH injection. There is an initial rise in the percentage of calmodulin associated with the plasma membrane which appears concomitantly with the depletion of cytosolic calmodulin. The increase occurs with a time course similar to that of secretion of LH into the blood. As the calmodulin begins to disappear from the plasma membrane fraction, its level increases first in the mitochondrial/granule and microsomal fractions and finally in the cytosol. There is also a dose-response relationship between plasma membrane accumulation of calmodulin and its cytosolic depletion (Fig. 2). Des¹GnRH²⁻¹⁰, which binds to the GnRH receptor with nearly 1,000-fold less affinity than GnRH itself⁶ and which had no efficacy in stimulating LH release in vivo, did not stimulate calmodulin redistribution (data not shown).

Calmodulin synthesis is constitutive in all systems examined (including the GnRH-stimulated pituitary)⁷. Accordingly, the redistribution reported here may indicate translocation between subcellular compartments. Accumulation of calmodulin at the plasma membrane at the expense of the cytosol might allow altered calmodulin control of regulatory functions at these loci. There is some evidence for such altered regulation by calmodulin in other systems. In the red blood cell, islet cell and adipocyte⁸⁻¹⁰ calmodulin-activated ATPases are located at the plasma membrane. In the case of the adipocyte, activation of Ca² ATPases seems to be hormonally regulated. Although calmodulin redistribution may be either a cause or a consequence of the secretory process, it does seem to have some role in secretion. Calmodulin has been found at the postsynaptic membrane, seems to mediate the Ca2+ effects on synaptic transmission 11,12 and may, accordingly, have a role in the release of neurotransmitters. In addition, trifluoperazine (Stellazine),

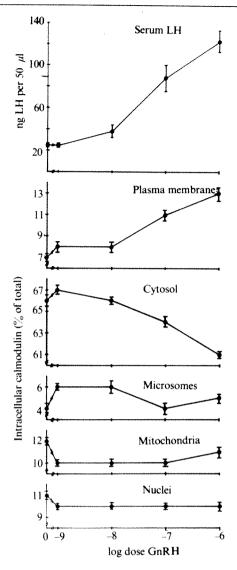


Fig. 1 GnRH concentration dependence of LH release and intracellular redistribution of calmodulin. GnRH (0.1-2 µg per rat, as indicated, obtained from the National Pituitary Agency) was administered by subcutaneous injection (0.1 ml in phosphate-buffered saline) to ovariectomized rats (Zivic-Miller, 5-7 weeks old, ovariectomized at 24 days). After 35 min the rats were killed by decapitation. Trunk blood was collected and serum LH determined by radioimmunoassay (materials from NIAMDD, standard RP1, antibody LHS-5, LH for iodination I-5, prepared by Dr A. Parlow). The assay is sensitive to < 1 ng per tube and cross-reacts < 1% with follicle stimulating hormone. Pituitaries were removed and homogenized (1 ml per pituitary) with a Dounce all-glass homogenizer (five strokes A pestle, three strokes B pestle) in ice-cold 0.5 M sucrose, 50 mM Tris-HCl pH 7.4, 25 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂ (0.5 M sucrose- TKMC). The suspension was filtered through organza cloth which was further washed with 1 ml per pituitary of 0.5 M sucrose-TKMC. This filtered suspension was the homogenate fraction. After saving an aliquot for assay, the remainder of the homogenate was centrifuged at $1,000g_{\rm ave}$ for 10 min in a Beckman TJ-6 centrifuge. The pellet (P₁) was suspended by gentle homogenization (two strokes of the B pestle) in 2.0 M sucrose-TKMC and further centrifuged at 20,000 gave for 45 min (Sorvall SS-34 rotor). The pellet (P2) and supernatant were designated nuclear and plasma membrane fractions, respectively. The supernatant of P1 (S1) was centrifuged at 12,000 gave for 20 min to collect the mitochondrial/granule fraction and the supernatant of this centrifugation further centrifuged at 100,000gave (Beckman type 40 rotor) to collect the microplasmal (pellet) and cytosolic (supernatant) fractions. Aliquots of the homogenate and subcellular fractions were assayed for DNA, protein and LH or for marker enzymes (aldolase, glucose-6-phosphatase, malic dehydrogenase, NADH cytochrome c reductase and 5' nucleotidase) using previously described methods (refs 21, 22). These analyses indicated < 10% cross-contamination between fractions. Other aliquots were stored at -70 °C, then heated and assayed for calmodulin as previously reported²³. The calmodulin concentration in the homogenate was 0.56 ± 0.04 ng per μ g protein. The values shown are means ± s.e.m.

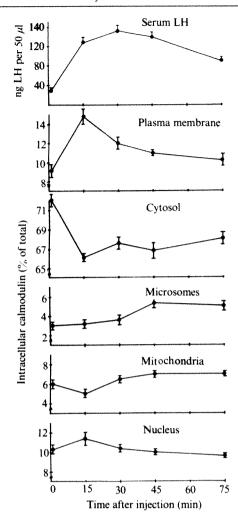


Fig. 2 Time course of LH release and subcellular calmodulin redistribution after a 2 µg injection of GnRH. Rats were killed at the indicated time. Subcellular fractions were prepared and assayed as described for Fig. 1.

which binds to calmodulin and inhibits some of its actions, also inhibits intestinal electrolyte secretion to the blood¹³

Although internalization of GnRH does not seem to be required for the release process¹⁴ the gonadotrope responses to this releasing hormone include receptor patching, capping and ligand internalization¹⁵, as observed for many cell-surface receptor-mediated systems. Recruitment of the clathrin-coated vesicles to the plasma membrane can be observed as coated pits sensitive to phenothiazine calmodulin inhibitors¹⁶ and a recent study has shown that calmodulin is a constituent of the coated vesicle¹⁷. Thus, its appearance at the plasma membrane may be associated with the recruitment of coated pits involved in the internalization process. Insulin, which is also internalized¹⁸, is associated with the translocation of glucose transport activity from the microsomal or Golgi fractions to the plasma membrane^{19,20}. Thus, the phenomena of redistribution of new activities to the plasma membrane may be a generalized occurrence for plasma membrane receptor-mediated events and suggests a mechanism by which calmodulin regulated events could be effected in the absence of additional synthesis.

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Note added in the proof: Since acceptance of this article, it has been shown²⁴ that neurotropic agents are non-competitive antagonists of GnRH-stimulated LH release. The drug concentration needed to inhibit 50% of stimulated release correlated well with the ability to inhibit enzyme activation by calmodulin

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- Conn, P. M. et al. Endocr. Rev. 2, 174–185 (1981). Cheung, W. Y. Science 207, 19–27 (1980). Klee, C. B., Crouch, T. H. & Richman, P. G. A. Rev. Biochem. 49, 487–516 (1980).
- Means, A. R. & Dedman, J. R. Nature 285, 73-77 (1980). Wang, J. H. & Waisman, D. M. Curr. Topics cell. Regulation 15, 47-105 (1979).
- Marian, J., Cooper, R. L. & Conn, P. M. Molec. Pharmac. 19, 399-405 (1981).
- Chafouleas, J. G., Conn, P. M., Dedman, J. R. & Means, A. R. Endocrinology A106, 289
- Larsen, F. L. & Vincenzi, F. F. Science 204, 306-308 (1979)
- Pershadsingh, H. A., McDaniel, M. L., Bry, C. G., Lacy, P. E. & McDonald, J. M. Nature 288, 492-495 (1980).
- Pershadsingh, H. A. & McDonald, J. M. Nature 281, 495-497 (1980).
 deLorenzo, R. J., Freedman, S. D., Yohe, W. B. & Maurer, S. C. Proc. natn. Acad. Sci. IIS A 76, 1838-1842 (1979)
- 12. Grab, D. J., Berzins, K., Cohen, R. S. & Siekevitz, P. J. J. biol. Chem. 254, 8690-8696
- 13. Ilundian, A. & Naftalin, R. J. Nature 279, 446-468 (1979)
- Conn, P. M., Smith, R. G. & Rogers, D. J. biol. Chem. 256, 1098-1100 (1981).
 Hazum, E., Cuatrecasas, P., Marian, J. & Conn, P. M. Proc. natn. Acad. Sci. U.S.A. 77, 6692-6695 (1980).
- 16. Salisbury, J. L., Condeelis, J. S. & Satir, P. J. Cell Biol. 87, 132-141 (1980).
- Linden, C. D., Dedman, J. R., Chafouleas, J. G., Means, A. R. & Roth, T. F. Proc. natn. Acad. Sci. U.S.A. 78, 308-312 (1981).
- Acad. Sci. U.S.A. 16, 508–512 (1981). Suzuki, K. & Kono, T. J. Biol. Chem. 254, 9786–9794 (1979). Suzuki, K. & Kono, T. Proc. natn. Acad. Sci. U.S.A. 77, 2542–2545 (1980)
- Cushman, S. W. & Wardzala, L. J. J. biol. Chem. 255, 4758-4762 (1980). Wray, W., Conn, P.M. & Wray, V. P. in Meth. Cell Biol. 14, 69-86 (1977)
- Schneider, W. in Selected Data for Molecular Biology and Biochemistry, K3-K14 (Chemical
- hafouleas, J. G., Dedman, J. R., Munjaal, R. P. & Means, A. R. J. biol. Chem. 254, 10262-10267 (1979).
- 24. Conn, P. M., Rogers, D. C. & Sheffield, T. Endocrinol. (in the press).

The expression-linked copy of surface antigen gene in Trypanosoma is probably the one transcribed

E. Pays, M. Lheureux & M. Steinert

Département de Biologie Moléculaire, Université libre de Bruxelles, 67, rue des Chevaux, 1640-Rhode St Genèse, Belgium

The antigenic specificity of the living trypanosome seems to be determined by the protein component of a unique glycoprotein species covering the whole surface of the parasite. During chronic infection, a single clone of trypanosomes may successively express a large repertoire of different variable antigen types (VATs). There are probably as many genes as variantspecific antigens (VSAs) (see refs 1-3 for reviews). The expression of the genes coding for the synthesis of these antigens is linked to genomic rearrangements involving duplication of the coding sequence and transposition of the additional copy The regulation of the expression of the VSA genes is operated at the transcriptional level⁷⁻¹⁰. It can thus be supposed that their transcription depends on the presence of the additional, transposed copy. We report here that this additional copy is in a chromatin configuration highly sensitive to pancreatic deoxyribonuclease, suggesting that it is the transcribed one.

The isolation of a cDNA clone coding for the AnTat 1.1 VSA of Trypanosoma brucei brucei (EATRO 1125, serodeme AnTar 1) has been described elsewhere. Detailed Pstl restriction analysis and hybridization with probes prepared from different parts of this cloned AnTat 1.1 cDNA enabled us to show that several copies of the AnTat 1.1 sequence are present in distinct bands of the genomic DNA (ref. 6 and Fig. 1). Of these, only a 6.4-kilobase (kb) and a 2-kb band seem to contain all the information present in the cDNA6 and to share a common SstI site 400 base pairs (bp) upstream of the coding sequence (Fig. 2 and manuscript in preparation); the other bands, which either contain incomplete sequences or show poor hybridization, are considered as imperfect copies of the the AnTat 1.1 gene⁶. The 3' site of all these PstI fragments is the only PstI site found in the cDNA, 340 bp from the 3' extremity (refs 6, 9 and Fig. 2). All these bands thus contain 5'-neighbouring genomic DNA as well as AnTat 1.1 information corresponding to the large (1,360-bp) 5' fragment of the cDNA. The remaining 3'-terminal Pstl sequence is not detected here, as our probe is the 550-bp HindIII-HindIII fragment (see Fig. 2). We have shown⁶ that the

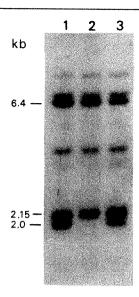


Fig. 1 Expression-linked copy of the AnTat 1.1 and AnTat 1.1b genes. The ³²P-labelled 550-bp *HindIII-HindIII* fragment of AnTat 1.1 cloned DNA was prepared as described previously^{6.9} and hybridized with Southern blots of *T. brucei brucei* nuclear DNA digested by *Psi*I. The DNA was extracted from bloodstream forms of the trypanosome expressing the VATs AnTat 1.1 (lane 1) or AnTat 1.1b (lane 3), or from procyclic forms derived from an AnTat 1.1 cloned population (lane 2) (see ref. 6 for details). The 2-kb band, which has clearly been lost in the procyclic trypanosomes, contains the additional, expression-linked copy of the VSA gene.

2-kb fragment is expression linked, being found only in the homologous AnTat 1.1 genomic DNA and not in DNA prepared from other variants of the same serodeme. The 6.4-kb Psfl fragment, present in the DNA of all the variants examined, is supposed to contain the basic copy of the AnTat 1.1 gene, that is, the one thought to be used as template to generate the expression-linked copy found in the 2-kb fragment⁶. It seems that the two copies are not contiguous, but rather that the additional copy has been transposed in a completely new genetic surrounding (Fig. 2). On the other hand, this additional copy is not extrachromosomal (data not shown). Finally, Hoeijmakers et al.⁴ have shown that the additional copy does not arise from DNA methylation of the target restriction site.

That the additional band is linked to transcription is already suggested by the fact that in all cases so far examined, a close correlation exists between the presence of the extra band and its corresponding mRNA (refs 4, 6, 9 and unpublished data). Moreover, we found that both the AnTat 1.1 mRNA and the 2-kb PstI additional band (Fig. 1, lanes 1, 3) are present in two clone populations of different lineage expressing the same AnTat 1.1 VAT (ref. 6 and manuscript in preparation). On the other hand, in procyclic forms of the trypanosome, in which the expression of the VSA is turned down¹¹, the 2-kb AnTat 1.1 additional band was not detected (ref. 6 and Fig. 1, lane 2),

although these procyclic trypanosomes were directly derived, by in vitro cultivation, from an AnTat 1.1 clone.

The only firmly established difference between active and inactive chromatin in eukaryotes is the differential sensitivity of their DNA to DNase I¹²⁻¹⁷. The transcribed sequences are indeed preferentially digested, probably because of the presence in active chromatin of either hyperacetylated histones¹⁸⁻²⁶ or some high-mobility group proteins²⁷⁻³¹. In protozoa in particular, DNase I selectively releases high-mobility group proteins from *Tetrahymena* nuclei³⁰.

We prepared nuclei from trypanosomes expressing the VAT AnTat 1.1 and submitted them to mild digestion by DNase I. As clearly shown in Fig. 3, among the AnTat 1.1-specific PstI bands, only the 2-kb additional sequence preferentially disappears

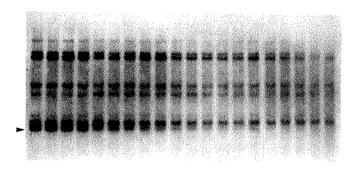
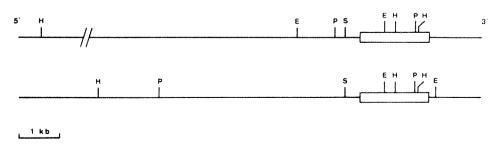


Fig. 3 Kinetics of the DNase I digestion of AnTat 1.1-specific sequences. Nuclei were purified from a clone population of trypanosomes expressing the VAT AnTat 1.1, by homogenization of the cells for 1 min at 23,000 r.p.m. in a Virtis 23 homogenizer, in 10 volumes of 0.32 M sucrose, 25 mM KCl, 3 mM MgCl₂, 20 mM Tris-HCl (pH 7.4) (medium A) at 4 °C. After centrifugation at 1,500g for 10 min the pellet was resuspended in the same volume of medium A, but with 0.2% Nonidet P40 (Shell), and homogenized by hand in a Potter apparatus. After centrifugation as above, the nuclei were washed twice in 10 mM NaCl, 3 mM MgCl₂, 10 mM Tris-HCl (pH 7.4) (medium B), then incubated at 20 °C for different periods as indicated, in 100-µl aliquots of medium B at 1 mg DNA ml⁻¹ with 80 ng ml⁻¹ DNase I (Worthington), previously freed from RNase by UMP agarose chromatography incubations were stopped by the addition of 5 volumes of 25 mM EDTA, 0.2% SDS and 250 µg ml⁻¹ proteinase K(Boehringer). After incubation for 15 h at 37 °C, the DNA in each sample was extracted by phenol/chloroform (1/1), chloroform and ultimately by ether, incubated with $200~\mu g~ml^{-1}$ RNase A (previously freed from DNase by heating for 10 min in a boiling water bath) then re-extracted as above and precipitated in ethanol at -20 °C. After centrifugation for 1 h at 16,000g, the DNA pellets were resuspended in 0.1 mM EDTA, 10 mM Tris-HCl (pH 7.5); 1 μg of each DNA sample was digested for 4 h at 37 °C with 10 units of PstI (Boehringer). The electrophoresis in 0.85% agarose gels, blotting on nitrocellulose filters, hybridization with a AnTat 1.1-specific cloned DNA probe and autoradiography were performed as described previously⁹. The different lanes contain, from left to right, a control DNA without DNase and samples incubated for 0, 5, 15, 30, 45, 60 s, 2, 3, 4, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 min with DNase. The arrow head indicates the additional band, containing the expression-linked copy of the VSA gene.

Fig. 2 Restriction maps of the basic (bottom) and additional (top) copies of the AnTat 1.1 gene and of their neighbourhood. Digestions with restriction endonucleases and hybridization of Southern blots of total genomic DNA digests with AnTat 1.1-specific probes were performed as described elsewhere E = EcoRI; H = HindIII; P = PstI; S = SstI. The 5' HindIII site to the left of the additional



copy is located 19 kb from the next *Hin*dIII site in the AnTat 1.1 coding sequence. The putative extent of the coding sequence (1,700 bp, see ref. 9) is indicated by an open box.

after DNase I treatment. Because of their larger target size, the largest bands are affected on long DNase exposure. However, despite its larger size, the 6.4-kb PstI fragment thought to contain the basic AnTat 1.1 sequence seems, among others, to be considerably less degraded than the expression-linked copy. If bands of similar size are compared, such as the 2.15- and the 2-kb fragments, the differential sensitivity to DNase I is particularly evident.

The chromatin of trypanosomes has not been extensively

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- Vickerman, K. Nature 273, 613-617 (1978).
- Cross, G. A. M. J. gen. Microbiol. 113, 1-11 (1979).
 Cross, G. A. M., Holder, A. A., Allen, G. & Boothroyd, J. C. Am. J. trop. Med. Hyg. 29, 1027-1032 (1980).
- 4. Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P. & Cross, G. A. M. Nature 284, 78-80 (1980).
- Borst, P. et al. Am. J. trop. Med. Hyg. 29, 1033-1036 (1980).
 Pays, E., Van Meirvenne, N., Le Ray, D. & Steinert, M. Proc. natn. Acad. Sci. U.S.A. 78, 2673-2677 (1981).
- 7. Lheureux, M., Lheureux, M., Vervoort, T., Van Meirvenne, N. & Steinert, M. Nucleic Acids
- Res. 7, 595-609 (1979).

 8. Hoeijmakers, J. H. J., Borst, P., Van den Burg, J., Weissmann, C. & Cross, G. A. M. Gene 8, 391-417 (1980)
- Pays, E. et al. Nucleic Acids Res. 8, 5965-5981 (1980).
- Agabian, N., Thomashow, L., Milhausen, M. & Stuart, K. Am. J. trop. Med. Hyg. 29, 1043-1049 (1980).
- Barry, J. D. & Vickerman, K. Expl Parasit. 48, 313-324 (1979). Weintraub, H. & Groudine, M. Science 193, 848-856 (1976).
- Garel, A., Zolan, M. & Axel, R. Proc. natn. Acad. Sci. U.S.A. 74, 4867-4871 (1977).
 Panet, A. & Cedar, H. Cell 11, 933-940 (1977).
- Bellard, M., Gannon, F. & Chambon, P. Cold Spring Harb. Symp. quant. Biol. 42, 779-791 (1978).

studied, but seems to be organized in typical nucleosomes 32,33 and to be made of the same histones as other eukaryotes³³. It can thus be reasonably assumed that in trypanosome nuclei also, the transcribed sequences are preferentially digested by DNase I. It is concluded that the additional, expression-linked copy of the VSA gene, which is in a chromatin configuration highly sensitive to DNase I, is most probably the one which is transcribed.

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- 16. Miller, D. M., Turner, P., Nienhuis, A. W., Axelrod, D. E. & Gopalakrishnan, T. V. Cell 14, 511-521 (1978).
- Zasioff, M. & Camerini-Otero, R. D. Proc. natn. Acad. Sci. W.S.A. 77, 1907-1911 (1980). Vidali, G., Boffa, L. C., Bradbury, E. M. & Allfrey, V. G. Proc. natn. Acad. Sci. U.S.A. 75,

- 2239-2243 (1978).

 9. Sealy, L. & Chalkley, R. Nucleic Acids Res. 5, 1863-1868 (1978).

 20. Simpson, R. T. Cell 13, 691-699 (1978).

 21. Davie, J. R. & Candido, E. P. M. Proc. natn. Acad. Sci. U.S.A. 75, 3574-3577 (1978).

 22. Oberhauser, H., Csordas, A., Puschendorf, B. & Grunicke, H. Blochem. biophys. Res. Commun. 84, 110-116 (1978).
- 23. Shewmaker, C. K., Cohen, B. N. & Wagner, T. E. Biochem. biophys. Res. Commun. 84, 342-349 (1978).
- 24. Mathis, D. J., Oudet, P., Wasylyk, B. & Chambon, P. Nucizic Acids Res. 5, 3523-3547
- (1776).
 Bonner, J., Wallace, R. B., Sargent, T. D., Murphy, R. F. & Dube, S. K. Cold Spring Harb.
 Symp. quant. Biol. 42, 851-857 (1978).
 Levy, B., Watson, D. C. & Dixon, G. H. Nucleic acids Res. 6, 259-273 (1979).
 Vidali, G., Boffa, L. C. & Allfrey, V. G. Cell 12, 409-415 (1977).

- Vidali, G., Boffa, L. C. & Alifrey, V. G. Cell 12, 409-415 (1977).
 Weisbrod, S. & Weintraub, H. Proc. natn. Acad. Sci. U.S.A. 76, 630-634 (1979).
 Gazit, B., Panet, A. & Cedar, H. Proc. natn. Acad. Sci. U.S.A. 77, 1787-1790 (1980).
 Hamana, K. & Zama, M. Nucleic Acids Res. 8, 5275-5288 (1980).
 Weisbrod, S. & Weintraub, H. Cell 23, 391-400 (1981).

- Borst, P. & Hoeijmakers, J. H. J. Plasmid 2, 20-40 (1979).
- Astolfi Filho, S., Martins de Sa, C. & Gander, E. S. Molec. biochem. Parasit. 1, 45-53 (1980).
- 34. Brison, O. & Chambon, P. Analyt. Biochem. 75, 402-409 (1976)

Structure of mouse metallothionein-I gene and its mRNA

Niall Glanville, Diane M. Durnam & Richard D. Palmiter

Howard Hughes Medical Institute Laboratory, Department of Biochemistry, University of Washington, Seattle, Washington 98195, USA

Metallothioneins are small cysteine-rich proteins that bind heavy metals such as zinc, cadmium, copper and mercury^{1,2}. Recent interest in these proteins has focused on the part they play in zinc metabolism and heavy metal detoxification1. Our interest in metallothionein genes stems largely from the observations that these proteins are inducible by both heavy metals and glucocorticoid hormones^{1,3}. To explore the regulation of these genes, we have isolated cDNA and genomic clones corresponding to mouse metallothionein-I (MT-I)4, and have used them to show that both inducers act at the transcriptional level in vivo and in a wide variety of cell lines⁵⁻⁸. We have also shown that the MT-I gene is amplified during selection for cadmium resistance⁵. To investigate the mechanisms of gene regulation, knowledge of the primary DNA sequence is necessary. Here we present the entire sequence of mouse MT-I gene along with ~300 bases of 5' flanking region that presumably includes promoter and regulatory sites. The 5' mRNA sequence, defined by S₁ nuclease mapping, was combined with sequences of the coding and 3' untranslated regions obtained previously to allow a computer prediction of the most stable secondary structure of MT-I mRNA.

The sequence of the mouse MT-I gene and its flanking regions is shown in Fig. 1a. Sequencing was done using the Maxam-Gilbert method9 with the strategy illustrated in Fig. 1b. Comparison of the genomic sequence with that of the cDNA clone⁴ shows that the coding region of the gene is interrupted by two introns as predicted by heteroduplex mapping4. The three exons of the gene are indicated by large capitals in Fig. 1a while the introns and flanking regions are shown by small capitals. The intron-exon junctions were deduced by comparing the genomic and cDNA sequences. Both introns obey the GT-AG rule originally proposed by Breathnach et al. 10—they start with the dinucleotide GT and end with AG. In addition, both have a 3

polypyrimidine tract similar to the polypyrimidine regions identified in many other introns¹¹. In many genes, introns lie between functional domains of the encoded protein 12-14. However, the 20 cysteine residues of metallothionein which coordinate seven metal atoms into two domains15 are distributed throughout the molecule (Fig. 1a), making it difficult to assign functional domains to the three exons.

The 5' end of exon 1 was deduced by S₁ nuclease mapping¹⁶. Total RNA isolated from cadmium-resistant Friend erythroleukemia cells was hybridized to a probe generated by cutting a plasmid carrying the genomic MT-I gene at its unique BgIII site (position +64, Fig. 1a) and labelling the 5' ends with 32 P. After S₁ nuclease digestion, the size of the resistant fragments was analysed by comparing it with a sequencing ladder of the Bg/II-AvaII fragment (+64 to -180, Fig. 1a). The results, shown in lane 1 of Fig. 2, indicate that the most abundant DNA fragment generated by S₁ nuclease comigrates with the G labelled +1 in Fig. 1a. Control experiments in which either MT-I mRNA or S₁ nuclease was omitted showed no DNA bands migrating in this region (lanes 2 and 3, Fig. 2). The concensus sequence, Y-C-A-Y (in which the A is position +1; Y represents unspecified pyrimidine nucleoside), derived from comparisons of 20 mRNA start sites¹⁷ is located three nucleotides downstream from the G labelled +1 in Figs 1a and 2. Perhaps steric hindrance from the mRNA cap structure retarded S₁ nuclease digestion of our DNA probe down to the last base-paired nucleotide. We obtained the same result when the S₁ nuclease digestion was done at either 37, 50 or 60 °C. This analysis indicates that the 5' end of exon 1 and the mature mRNA lies 74 nucleotides from the coding region of MT-I mRNA.

Analysis of the region 5' to exon 1 shows that, like many other eukaryotic genes that are transcribed by RNA polymerase B, there is a Goldberg-Hogness (TATAAA) sequence (boxed in Fig. 1a) located between nucleotides -23 and -28. The sequence GC_CCAATCT, which is also common to various eukaryotic genes 18, is not present in the 5' flanking region of the MT-I gene. The 5' region does, however, contain two large palindromic sequences centred at positions -55 and -103(underlined in Fig. 1a). We are now generating mutants in this region to determine which areas are involved in the regulation of MT-I gene expression.

Localization of the mRNA start site allowed us to write the complete sequence of MT-I mRNA (Fig. 3). Computer

TGAGTTCTCG TAAACTCCAG AGCAGCGATA GGCCGTAATA TCGGGGAAAG CACTATAGGG ACATGATGTT CCACACGTCA CATGGGTCGT CCTATCCGAG CCAGTCGT<u>GC CAAAGGGGGC</u>G GTCCCGCTGT GCACACTGGC GCTCCAGGGA GCTCTGCACT CCGCCCBAAA AGTGCGCTCG SCTCTGCCAG GACGCGGGC SCGTGACTAT GCGTGGGCTG GAGCAACCGC CTGCTGGGTG CAAACCCTIT GCGCCCGGAC TCGTCCAACG ACTATAAAGA GGGCAGGCTG TCCTCTAAGGC TCACCACGA CTICAACGIC CTGAGTACCI TCTCCTCACI TACTCCGTAG CTCCAGCTIC ACCAGATOTO GGANTOGACC CCAACTGCTC CTGCTCCACC GGTAAGACTC CCGATCCTTG GTCTTTAGAA TACCAAGTTG GGACCGCAGA GCGGAATCCC CGAGTTGTAS AGGCTTGGCG $\frac{180}{400}$ 7 GGAATAGGCA CCTTTAGTTG GCGATTCATT CCGGTTCTT<u>T CTAGA</u>ATCCG CTCTTGCAAA X ba 1 300 AGCCTTCATT AGTTACGAGT ATTGTCGAAC GGGTCCTTTG GCGGGGTTGG GGCTAGGATT TAGACGCGCA AATGTCCGGT ICCTGATCAC CCAGTTAGTG GGGACATCTG GGTTGAGTCC $$\it Ball$$ #20 CAGGCATTAC TAAACTTACT GTGAATTGCT TGAATTAAGA AAGAGGTGAA GGACCTITAT ### GTCTTGGGAC TCAAAGACAT AATCCCTGAC TTAACCTGTG AGGAGAAAAG TGGGGCTAGG T S S C A C K N C K C T S C K K 686 CACCAGCICC TGCGCCTGCA AGAACTGCAA GTGCACCTCC TGCAAGAAGA GTGAGTTGGG 220 ACACCTTGGG TGGCGGCTAA GGCTAGGGGC GGGGAACTCC TACAAAACTG GCTCTGAGAA 780 ATGTCCTTTG CTTCCCGGAG GCCATTGTAT TGTCTCGGGG ACAGAACTAT ACAGAGAACT A va IATTIAAAAAA ACCGAGGTCT TCTCTSTTGG GGACAGGAAG CAGAGGTCTT CAGCCAGGCT

SCOR S - SCOR P V G C S K C
GCCTCTTCCT CCTTCTTCTA GGCTGCTGCT CCTGCGGCTGC TCCAAATGTG CCCAGGGCTG TGTCTGCAAA GGCG<u>CCGCGG</u> ACAAGTGCAC GTGCTGTGCC TGAFGTGACG AACAGCGCTG CCACCACGTG TAAATAGTAT CGGACCAACC CAGCGTCTTC CTATACAGTT CCACCCIGIT TACTAAACCC CCGTTTTCTA CCGAGTACGT TAATAATAAA AGCCTGTTTG AGTETAACTE TEGTTTECTTE GTETEGTTTE SCAATAAGAA ACTEGEGTEA CTTGATAGTE 1808 TGGGGATCTG GTTTTGGACC CCCTCGTGCC TTTACCTCCG CCCTCTGGCC CTCACAGAGG GGTAATGTCT TIGGGTAAAG CCAAGCTATA TCCCATAAGC TICCTCATGG AAAACAGGTG
#tind III Puw II



Fig. 1 The DNA sequence of the MT-I gene. a, Sequencing was done by the method of Maxam and Gilbert⁹. Exons are shown in large print; introns and the regions flanking the gene are shown in small print. The sequences of commercially available restriction enzymes that recognize 6-base pair (bp) sequences are underlined and named. Possible regulatory sites at the 5' end of the gene as discussed in the text are also underlined. The TATAAA sequence, initiation and termination codons are boxed. The MT-I protein sequence is indicated above the exon sequences using the one letter code: A, Ala; C, Cys; D, Asp; G, Gly; K, Lys; M, Met; N, Asn; P, Pro; Q, Gln; S, Ser; T, Thr; V, Val. b, Sequencing was done from the restriction sites indicated. DNA fragments were either³² P-labelled at the 5' end using T4 polynucleotide kinase or at the 3' end using the large subunit of DNA polymerase I. Many of the sequence segments were determined on both DNA strands; all the sequence was analysed at least twice.

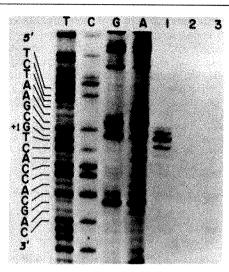


Fig. 2 Localization of the metallothionein-I mRNA start site by S₁ nuclease mapping. Lanes T, C, G and A: sequencing ladder from the Bg/II site (+64 in Fig. 1a). Labelling of the lanes from the sequencing gel has been transposed so that the mRNA sequence can be read directly. Lane 1, S1 nuclease-resistant products obtained by digesting a hybrid between a DNA probe labelled at the Bg/III site and MT-I mRNA. A genomic clone was digested at the unique BgIII site, treated with alkaline phosphatase, and labelled with $[\gamma^{32}P]ATP$ using T4 polynucleotide kinase; the specific activity of the probe was 4×10^6 c.p.m. per pmol of 5' ends. The probe was treated with proteinase K and phenol-chloroform extracted. Aliquots $(4 \times 10^5 \text{ c.p.m.})$ were ethanol-precipitated with 140 µg of total RNA from cadmium-resistant Friend erythroleukemia cells⁵ (35 ng of MT-I mRNA). The precipitate was dissolved in 25 µl of 80% formamide, 0.4 M NaCl, 40 mM PIPES buffer (pH 6.5), 1 mM EDTA, overlaid with paraffin oil, heated at 80 °C for 5 min and incubated at 50 °C for 12 h. The samples were then diluted 10-fold with 10 mM Tris-HCl, pH 7.5 and ethanolprecipitated. The nucleic acids were dissolved in 25 µl of 10 mM NaCl and then 2.5 µl of buffer containing 3 M NaCl, 300 mM NaOAc, 30 mM ZnOAc, pH 4.5 was added. S₁ nuclease (2 μ l, prepared by the method of Vogt²¹) was added and the sample incubated at 60 °C for 40 min. An aliquot (1 µl) was loaded onto the sequencing gel. Lane 2, conditions were the same as for lane 1, except that 140 µg of chicken calvaria RNA was hybridized with the probe. Lane 3, same as lane 1 except that no S₁ nuclease was added. A high-molecular weight band representing undigested ³²P-labelled DNA was visible on the gel but is not shown.

analysis 19 of this sequence showed that various similar secondary structures were possible for MT-I mRNA. The most stable secondary structure predicted is shown in Fig. 3. This structure has an overall free energy of -87 kcal, suggesting that it is fairly stable. The model shows that most of the MT-I coding region, including the initiation codon (AUG), is contained within the duplexed region of the molecule, whereas the stop codon (UGA) is exposed in a loop. Note that the positions where RNA splicing occur (arrows, Fig. 3) are located very close to each other at the base of loop structures. It has been suggested that genes may evolve by addition or subtraction of exons²⁰. In order for this mechanism to yield a functional protein, the reading frame of the exons as well as a stable secondary and tertiary structure of both the protein and its mRNA must be preserved. Inspection of the secondary structure model of MT-I mRNA (Fig. 3) shows that the absence of exon 2 would have little effect on mRNA secondary structure. In addition, the reading frames of exons 1 and 3 are preserved without exon 2 (Fig. 1a). Thus, a plausible model for the evolution of MT-I gene might involve the primordial existence of an uninterrupted gene consisting of the nucleotides now represented by exons 1 and 3. This gene might then have been interrupted by an intron into which exon 2 was later inserted. Cell transformation techniques should allow us to reverse this process as a way of testing the hypothesis of exon addition.

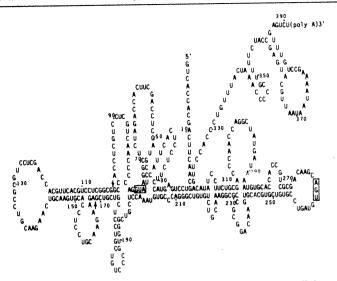


Fig. 3 Computer model of the secondary structure of MT-I mRNA. Analysis was done using the computer program developed by Pipas and McMahon¹⁹. The initiation codon (AUG) and the termination codon (UGA) are boxed. Splice points are indicated by arrows.

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- 1. Kägi, H. R. & Nordberg, M. (eds) Metallothionein (Birkhauser, Basel, 1979).

- Kagi, H. R. & Nordberg, M. (eds) Metailoninonein (Birkinauser, Basel, 1979).
 Cherian, M. G. & Goyer, R. A. Life Sci. 23, 1-10 (1978).
 Karin, M. & Herschmann, H. R. Science 204, 176-177 (1979).
 Durnam, D. M. et al. Proc. natn. Acad. Sci. U.S.A. 77, 6511-6515 (1980).
 Beach, L. R. & Palmiter, R. D. Proc. natn. Acad. Sci. U.S.A. 78, 2110-2114 (1981).
 Mayo, K. E. & Palmiter, R. D. J. biol. Chem. 256, 2621-2624 (1981).
 Durnam, D. M. & Palmiter, R. D. J. biol. Chem. 256, 5712-5716 (1981).

- Hagar, L. J. & Palmiter, R. D. Nature 291, 340-342 (1981)
 Maxam, A. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560-564 (1977).
- Breathnach, R. et al. Proc. natn. Acad. Sci. U.S.A 75, 4853-4857 (1978) Lewin, B. Cell 22, 324-326 (1980).
- Sakano, H. et al. Nature 277, 627-633 (1979). Lomedico, P. et al. Cell 18, 545-558 (1979).
- 13. Stein, J. P. et al. Cell 21, 681-687 (1980).
- Otvos, J. D. & Armitage, I. M. Proc. natn. Acad. Sci. U.S.A. 77, 7094-7098 (1980).
- Weaver, R. & Weissman, C. Nucleic Acids Res. 5, 1175-1193 (1979). Corden, J. et al. Science 209, 1406-1414 (1980).
- Benoist, C. et al. Nucleic Acids Res. 8, 127-142 (1980)
- Pipas, J. M. & McMahon, J. E. Proc. natn. Acad. Sci. U.S.A. 72, 2017-2021 (1975).
- Gilbert, W. Nature 271, 501 (1978). Vogt, V. M. Eur. J. Biochem. 33, 192-200 (1973).

Recombination between short **DNA** homologies causes tandem duplication

Thomas Edlund & Staffan Normark

Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

The ampC gene of Escherichia coli K-12 codes for a B lactamase which can hydrolyse the β -lactam ring of ampicillin^{1,2}. Ampicillin resistance is strictly related to ampC gene copy number thus we have been able to isolate ampicillin-resistant mutants carrying multiple ampC repeats3.4. We have isolated on a plasmid a segment of chromosomal DNA carrying multiple ampC repeats, and compared the nucleotide sequence of the region joining repeat units to the sequence of the DNA segments that fused to create the joint. The fusion had occurred within a 12-base pair (bp) sequence of perfect homology. We suggest that recombination between randomly occurring short homologies (12-13-bp long), could be a general mechanism to generate tandem duplications in the size range of 10 kilobases (kb).

The properties of E. coli K-12 mutants carrying multiple copies of the ampC gene have been described elsewhere^{3,4}. We isolated amplified mutants that were derivatives of an ampC up-promotor mutant resistant to ~20 μg ml⁻¹ ampicillin⁵. By reciprocal recombination between a ColEl-ampC hybrid plasmid, pNU1 (ref. 4), and the chromosome of two ampCamplified mutants, plasmid derivatives were isolated which carried multiple copies of the respective ampC duplication. One of these, derivative pNU8, carried multiple ampC genes of repeat size 9.8 kb. The repeat was found to have both its end points within the chromosomal DNA carried by plasmid pNU1 (ref. 4) thus plasmid pNU8 could be used as a source of DNA for the segments involved in the formation of the novel joint in the 9.8-kb repeat. The only difference between restriction fragment patterns obtained for pNU1 and pNU8 was the presence of an additional joint fragment in the latter. The restriction enzyme PvuII was used to generate a length of DNA small enough for sequence analysis but which also carried the joint fragment. Figure 1 shows the PvuII sites on plasmid pNU1. We concluded that the 9.8-kb repeat of pNU8 had one end point in the PstI4-PvuII5 fragment (fragment A) and the other in the PvuII₉-Pst₁ fragment (fragment B) of pNU1. Thus, the PvuII joint fragment (fragment C) of pNU8 consists of parts of fragments A and B.

The strategy for sequencing relevant stretches in fragments A, B and C is described in Fig. 2 legend. By comparing the sequences of these stretches (Fig. 3), it was found that the two DNA segments that had recombined contained a 12-bp long sequence of perfect homology, which was also present within the novel joint of fragment C. Thus, the 9.8-kb duplication had occurred by a recombination event at some point within these 12-bp sequences of fragments A and B.

E. coli strain Gllal is usually resistant to 20 μg ml⁻¹ of ampicillin. Mutants of Gllal resistant to 40 or 60 µg ml⁻¹ of

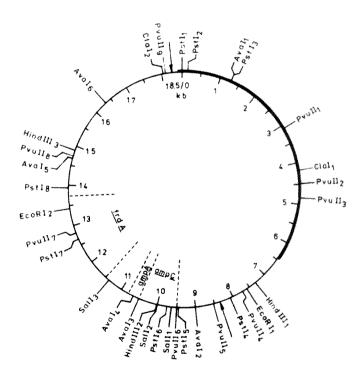


Fig. 1 Physical restriction fragment map of plasmid pNU1. The location of the ClaI and PvuII sites within pNU1 were determined by double digestions with these enzymes and the enzymes previously used to map pNU1 (ref. 4). The restriction endonuclease cleavage sites are numbered, starting from the PstI1 site at the top. The thick-lined segment represents DNA from ColE1. Arrows indicate the end points for the 9.8-kb repeat unit. Locations are given for the frdA and ampC genes as well as the ampA control region.

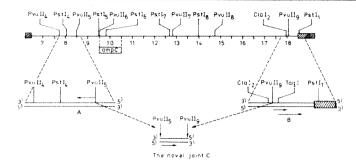


Fig. 2 Strategy for sequencing the novel joint of the 9.8-kb repeat, and the DNA segments involved in its formation. The numbered solid line at the top represents chromosomal DNA carried by plasmid pNU1. Hatched areas represent ColE1 DNA. The box indicates the position of the β -lactamase structural gene, ampC. The TaqI site within the PvuII₉-PstI₁ fragment was located by the mapping technique of Smith and Birnstiel⁸ using purified $ClaI_2$ -AvaI₁ fragment of pNU1. For this purpose the fragment was end-labelled at the ClaI 5' terminus. The PvuII₅-PvuII₉ fragment carrying the novel joint was purified from plasmid pNU8 whereas the other fragments sequenced were from pNU1. Arrows indicate the direction and extent of sequencing runs, using the Maxam-Gilbert method⁹.

A	3,	T CATAATTGACGGAC CAACACCACGCG T G G C G T T G G T G A A G T A T T A A C T G C C T G G T T G T G G T G C R C A C C A C C A C	A S A 3'
C	5°	A G G G G C C G G T A T C G T C A A C A C C A C G C G T G G C G C C A T A G C A G T T G T G G T G C G C A C C G C G A C C A C T	A G A 3'
В	5°	AGGGGCCGGTATCGT CAACACCACGCG CGTAACCGAGTC	A G A ³ '

Fig. 3 Comparison of nucleotide sequences within fragments $Pst_4-PvuII_5$ (A) and $PvuII_9-PstI_1$ (B) of pNU1, and the PvuII 'joint' fragment (C) of pNU8. Boxed nucleotides represent a 12-bp perfect homology present in all three fragments. In fragment A this sequence was located ~90 bp from the $PvuII_5$ end, and in fragment B 230 bp from the $PvuII_9$ end. A recombination event within these 12 bp produced the novel joint.

ampicillin were isolated; this level of resistance corresponds to that shown by defined mutants carrying an amp duplication or triplication. By further selection for ampicillin resistance, amplifiable derivatives resistant to between 600 and 1,000 µg ml⁻¹ of ampicillin were obtained from individual Amp⁻⁴⁰ or Amp⁻⁶⁰ clones. Ten amp-amplified mutants were isolated in this way. The sizes and end points of their respective repeat units were determined by cleavage of chromosomal DNA with restriction endonucleases and the fragmentation patterns compared with that of pNU1. This ColE1 hybrid plasmid carries 12 kb of chromosomal DNA surrounding the amp gene. The

Table 1 Effect of size of homology on the length of DNA required to contain expected numbers of homologies

Homology		No.	of homolo	gies	
size (bp)	1	4	9	16	25
10	2*	3	4	5	6
11	3	5	7	9	11
12	5	9	13	17	21
13	9	17	25	33	41
14	17	33	49	65	81

Calculations were based on the fact that in a random sequence the number of homologies are proportional to the square of the length of the DNA segments expected to contain homologies.

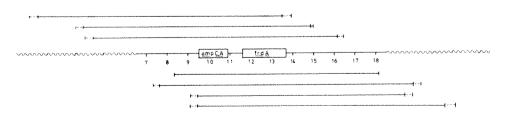
* The sizes of DNA fragments are given in kilobase pairs.

repeat copy number of the mutants was from 30 to 50 as judged from their ampicillin resistance, allowing restriction fragments of repeated chromosomal DNA larger than 400 bp to be detected. The sizes and end points of the respective repeats are given in Fig. 4. The end points could be accurately determined for seven amplified mutants; none of these had the same two end points. The sizes of the repeats varied between 9 and 13 kb. The remaining three mutants carried larger repeats (~18 kb). However, as their end points were situated outside the chromosomal DNA carried by pNU1, it was not possible to determine their exact position. However, we concluded that the end points of the ten different repeats were all located within 20 kb of chromosomal amp DNA.

A similar ampicillin selection procedure was performed on a recA1 derivative of Gllal. Chromosomal DNA from 15 independently isolated Amp^r-300 derivatives was digested with several restriction endonucleases. No amplified fragments were observed in any of the mutants. These results suggest that the frequency of generation of amp duplications is decreased in a recA1 background to such an extent that other types of ampicillin resistant mutants will predominate after the selection. On average, two homologous 12-bp sequences of any composition will be found within a random DNA sequence of 4 kb. This was deduced from the equation $x^2 = 4^n$, where n is the number of homologous base pairs and x is the number of base pairs which should contain a duplicate of any composition of n base pairs in length. This equation is an approximation which is valid if $x \gg n$.

In the *amp* system duplications may not occur within the *ampC* gene, which constitutes about 1 kb. Thus, on average, a duplicate of any 12-bp sequence will be found once in 5 kb. The general formula for this system will therefore be $(x-1,000)^2 = 4^n$. The size of *amp* DNA required for different numbers of homologies with sizes ranging from 10 to 14 bp is given in Table 1; twenty-five 12-bp homologies and nine 13-bp homologies would be expected within a 21-kb and a 25-kb random sequence, respectively. In addition, our sequence and distribution data suggest that unequal recombination between any homologies close to 12 bp may be a general mechanism for

Fig. 4 Distribution of amp repeats in seven independent Amp^r mutants of E. coli K-12. The size and end points were determined by cleavage of chromosomal DNA with a series of restriction endonucleases. The amplified fragments were compared with the fragmentation patterns of plasmids pNU1 and pNU8. The



numbered solid line indicates chromosomal DNA carried by pNU1; wavy lines represent surrounding chromosomal DNA. Horizontal bars above and below the genetic map indicate the extent of different amp duplications. Dashed lines indicate the extent to which the respective end point was mapped. The approximate locations for the ampC and the frdA genes are indicated by boxes. A represents the control sequence region for ampC.

generating tandem duplications. Our results also suggest that recombinations between such small homologies are promoted by the recA protein.

The novel joint described here was formed by recombination between DNA segments carrying a 12-bp perfect homology but with no other obvious homologies. This implies that the opportunity to form stable base pairing is an important feature of the mechanism that gives rise to tandem duplications. Promoter structures and possibly other controlling sequences which exist in many copies on the bacterial chromosome, could provide homologies for duplication, thus allowing catastrophic deletions. However, available sequence data on promoters indicate that they have been built up to contain recognition regions only a few base pairs long, interrupted by non-homologous stretches of nucleotides⁶. This strategy should prevent recombination and thus avoid deleterious deletion. In fact, it has been shown that intragenic deletions within the lacI gene can occur at repeated sequences as short as 5 bp?

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- Burman, L. G., Park, J. T., Lindström, E. B. & Boman, H. G. J. Bact. 116, 123-130 (1973).
 Normark, S. & Burman, L. G. J. Bact. 132, 1-7 (1977).
 Normark, S., Edlund, T., Grundström, T., Bergström, S. & Wolf-Watz, H. J. Bact. 132,

- 912-922 (1977).

 4. Edlund, T., Grundström, T. & Normark, S. Molec. gen. Genet. 173, 115-125 (1979).

 5. Jaurin, B., Grundström, T., Edlund, T. & Normark, S. Nature 290, 221-225 (1981).

 6. Siebenlist, U., Simpson, R. B. & Gilbert, W. Cell 20, 269-281 (1980).

 7. Farabaugh, P. J., Schmeissner, U., Hofer, M. & Miller, J. H. J. molec. Biol. 126, 847-857
- Smith, H. O. & Birnstiel, M. L. Nucleic Acids Res. 3, 2387–2398 (1976).
 Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560–564 (1977).

Temperature-dependent ΔC_p^0 generated by a shift in equilibrium between macrostates of an enzyme

Harvey F. Fisher, Alan H. Colen & Richard T. Medary

Laboratory of Molecular Biochemistry, Department of Biochemistry, University of Kansas School of Medicine and the Veterans Administration Medical Center, 4801 Linwood Blvd, Kansas City, Missouri 64128, USA

Substantial negative heat capacity changes $(\Delta C_n^{\theta'})$ have frequently been observed to accompany the formation of protein-ligand complexes^{1,2}. Glutamate dehydrogenase³ and horse liver alcohol dehydrogenase4, however, have been reported to form binary complexes with coenzyme with negligible $\Delta H^{0\prime}$ and only small ΔC_{ν}^{0} s. Although many intriguing mechanisms have been proposed to account for the observed phenomena, there is little direct experimental evidence available which might provide a basis for evaluating the contributions of ΔC_p^{0} s of complex formation from the various mechanistic sources or even for distinguishing between them. However, if, as Eftink and Biltonen⁵ have suggested, a shift in equilibrium between macrostates contributes significantly to an observed $\Delta C_p^{0\prime}$ for a given reaction, it should be possible to characterize such a system by measuring the temperature dependence of the ΔC_p^{0} . Despite this, few studies have determined ΔH^{0} values at more than two temperatures. We have now measured the temperature dependence of the ΔH^{0} (and, thereby, that of the ΔC_{p}^{0}) of the formation of an enzyme-reduced coenzyme complex in an attempt to provide such a basis and have found that the entire $\Delta C_p^{0\prime}$ of complex formation is accounted for by a temperatureinduced shift of an equilibrium between the different forms of the free enzyme.

The temperature dependence of the calorimetrically measured ΔH^{0} for the reaction written as

$$E+NADPH \rightleftharpoons E-NADPH$$
 (1)

is shown in Fig. 1. Although the free enzyme is stable up to a temperature of ~50 °C (ref. 6), the E-NADPH complex is markedly more heat-labile,7 and reliable measurements of the formation of this complex cannot be made at temperatures above 35°C using attainable flow rates. Precautions and controls described in Fig. 1 legend, however, assure us of the validity of measurements up to 35 °C.

If the formation of the E-NADPH complex proceeded according to reaction (1) unaccompanied by a measurable ΔC_i^c the data of Fig. 1 would lie on a horizontal straight line, because $(\partial \Delta H^{0i}/\partial T)_p = \Delta C_p^{0i}$. If the reaction proceeded with finite temperature-independent ΔC_p^0 , the data would lie on a straight line with a slope equal to ΔC_p^0 . It is apparent from Fig. 1 that the data correspond to neither simple case. The plot shows a marked curvature, the $\Delta C_p^{0'}$ approaching 0 at low temperatures and increasing to a value of -450 cal K⁻¹ mol⁻¹ at higher temperatures. This behaviour can be explained on the basis of a temperature-sensitive equilibrium between 'implicit' macrostates of at least one reaction component⁸.

Consider a binding reaction which is thought to be expressed by reaction (1) but which, in fact, involves the following equilibria:

$$\begin{array}{c|c}
E' \\
\kappa_2 \parallel \\
NADPH + E & \rightleftharpoons E-NADPH
\end{array}$$
(2)

where

$$K_2 = \frac{[E]}{[E']}$$
 and $K_1 = \frac{[E-NADPH]}{[E][NADPH]}$

We assume that neither step involves a measurable ΔC_p . If step 2 is itself accompanied by a substantial ΔH^{0i} , and has a ΔG^{0i} such that $K_2 = 1$ within or near the experimental temperature range, the observed ' $\Delta H^{0\prime\prime}$ ' for reaction (1) will vary from $\Delta H_1^{0\prime}$, the value at temperatures at which the protein is entirely in the E form, to a value $(\Delta H_1^{0\prime} + \Delta H_2^{0\prime})$, at temperatures at which the protein is entirely in the E' form. By definition, the resulting temperature dependence of ΔH^{0i} will be interpreted as a ΔC_p^{0i} effect; and, if measurements are performed over a sufficient range of temperatures, the ΔC_p^{0r} itself will be found to be temperature dependent. This will be so even if, as we have assumed, both $\Delta H_1^{0\prime}$ and $\Delta H_2^{0\prime}$ are themselves temperature independent. We have portrayed the ligand as binding to only one form of the enzyme for ease in following the argument. In practice, no such distinction can be made from equilibrium measurements.

The dependence of ΔH^{0} on temperature for reaction (2) is

$$\Delta H^{0i} = \Delta H_1^{0i} + \frac{\Delta H_2^{0i}}{1 + K_2}$$
 (3)

where $K_2 = \exp[\Delta H_2^{0'}(T - T_2)/RTT_2]$ and T_2 is that temperature at which $\Delta G_2^{0'} = -RT \ln K_2 = 0$.

The solid line in Fig. 1 shows the best fit of equation (3) to the data with the parameters $\Delta H_1^{0\prime} = 4.73 \pm 0.28 \text{ kcal mol}^{-1}$, $\Delta H_2^{0\prime} = -20.1 \pm 2 \text{ kcal mol}^{-1}$ and $T_2 = 43.6 \pm 1 \,^{\circ}C$. The magnitude of the apparent ΔC_p^0 generated by the effect of

varying temperature on this implicit equilibrium is8

$$\left|\Delta C_p^{0r}\right| = \left|\left(\frac{\partial \Delta H^{0r}}{\partial T}\right)_p\right| = \frac{K_2(\Delta H_2^{0r})^2}{(1 + K_2)^2 R T^2} \tag{4}$$

For a reactant isomerization (such as reaction (1)), the sign of ΔC_p^{0r} is negative. For a reaction sequence involving a product isomerization step, however, the sign of ΔC_p^{0} is positive.

In Fig. 2 we have plotted ΔC_p^{0} as a function of temperature according to equation (4) using the best-fit parameters and algebraic sense of Fig. 1.

Because the sign of the ΔC_p^{0} is negative, if there is a single (implicit) isomerization in the overall reaction, it must involve one of the reactants rather than one of the products. An attempt to fit the data to an equation similar to that for reaction (2) expanded to include an isomerization of the E-NADPH complex as well as one of E, indicated no contribution from that added feature. Thus, although there may be an isomerization of the enzyme-ligand complex, evidently such an equilibrium either is not poised in or near the experimental temperature range or must not involve a substantial ΔH^{0} . As reaction (2) is symmetrical in E and NADPH, an isomerization of the ligand could account for the data as well as an isomerization of E. While NADPH is known to exist as an equilibrium mixture of folded and unfolded forms, the T_2 for this process is below 20 °C and its ΔH^{0} is too small to make an appreciable contribution to the observed process9. The attempted inclusion of finite intrinsic ΔC_p^{0} terms for steps 1 and 2 of reaction (2) also failed to improve the fit of the equation to the data.

We conclude, therefore, that the observed ΔC_n^{0} of the formation of the glutamate dehydrogenase-NADPH complex is fully accounted for by a poised isomerization of the free enzyme involving a very large enthalpy change that is largely compensated by an opposing large entropy change, the step in itself

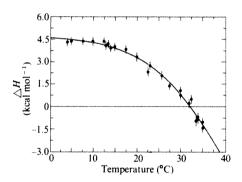


Fig. 1 The temperature dependence of ΔH^{0} of the glutamate dehydrogenase-NADPH complex in 0.1 M phosphate buffer, pH 7.6. The reaction heats, q, were determined in a flow microcalorimeter using apparatus, reagents and procedures as previously described³. The enzyme concentration was usually ~90μM (active sites). At each temperature, calorimetric measurements were made at saturating concentrations of NADPH starting at 20 × KD and extending to $200 \times K_D$. A plot of q versus [NADPH] was linear with a slope of $0 \pm 6 \times 10^{-4}$ kcal per mol NADPH in each case. As noted in the text, while the free enzyme is stable at 50 °C, its reduced coenzyme complex denatures rapidly above 37 °C. The following controls and precautions assure us that the data presented are unaffected by errors due to denaturation. (1) The solution emerging from the calorimeter was assayed for enzymatic activity. The activities so measured agreed with those of the initial solution to within 5% and showed no trend indicating denaturation. As the solution remains at an elevated temperature for some time after leaving the sensing chamber of the instrument, any time-dependent denaturation would be magnified several-fold by the time the solution actually emerged from the instrument. (2) The value of q was unchanged on raising the flow rate from 10 μ ls 13 μ ls $^{-1}$. Had there been any significant time-dependent do . Had there been any significant time-dependent denaturation, the value of q would have differed because the two flow rates correspond to different residence times. The solid line is calculated from a nonlinear least-squares fit to reaction (3). The ΔH^{0} , value at 15 °C differs from that reported prevously using a batch calorimeter. The batch value (and the very small ΔC calculated from it) must now be presumed to be erroneous.

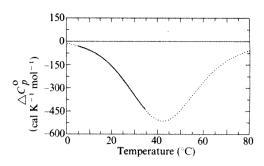


Fig. 2 The temperature dependence of ΔC_p^{0} of the glutamate dehydrogenase-NADPH complex. The line is calculated from equation (4) using parameters from Fig. 1. The solid line portion of the curve indicates the region covered by experimental data.

involving no detectable $\Delta C_p^{0\prime}$ and H⁺ transfer to or from the buffer not contributing significantly to the measured enthalpy change.

A similar mechanism, involving a temperature-induced shift between multiple forms of the free enzyme, is consistent with the recently reported large ΔC_p^{\dagger} in the glutamate dehydrogenase-catalysed reaction⁸. We cannot say how general the phenomenon reported here may be, but we note that ΔC_p^{0} s reported for complexes of other dehydrogenases at 20 °C are of a similar magnitude to that of glutamate dehydrogenase at its maximum near 40 °C. Thus, the differences between the ΔC_p^{0} s of the various enzymes may simply reflect differences in the T_{2} s of otherwise similar mechanisms.

Received 13 January; accepted 29 April 1981.

- 1. Sturtevant, J. M. Proc. natn. Acad. Sci. U.S.A. 74, 2236-2248 (1977).

- Sturtevant, J. M. *Proc. nam. Acad. Sci. U.S.A.* 74, 2236–2248 (1977).

 Biltonen, R. L. & Langerman, M. *Meth. Enzym.* 61, 287 (1979).

 Subramanian, S. Stickel, D. C. & Fisher, H. F. *J. biol. Chem.* 250, 5885–5889 (1975).

 Subramanian, S. *Biophys. Chem.* 7, 375–378 (1978).

 Effink, M. & Biltonen, R. L. in *Biological Microcalorimetry* (ed. Beezer, A. E.) 396 (Academic, London, 1980).
- Olsen, J. R. & Anfinsen, C. B. J. biol. Chem. 202, 841-856 (1952).
- Grisolia, S., Quijada, C. L. & Fernandez, M. Biochim. biophys. Acta 81, 61 (1954). Colen, A. H., Medary, R. T. & Fisher, H. F. Biopolymers 20, 879-889 (1981).
- Catteral, W. A., Hollis, D. P. & Walters, C. F. Biochemistry 8, 4032-4036 (1969).

Corrigendum

In the letter 'Competition relatedness and efficiency' by J. R. W. Harris, Nature 292, 54-55, the parameter x in equation (2) should be replaced by z.

Errata

In the article 'Seismotectonics of the El Asnam earthquake' by M. Ouyed et al., Nature 292, 26-31, the legend to Fig. 6 was reproduced incorrectly in some copies of *Nature*. It should read: a, The 1.30-m left lateral slip measured on a ploughed field at Zebadja near the centre of the fault. b, Southern segment of the fault. Typical pressure ridges showing the uplift of the northwestern block (to the left), and collapse due to gravity. Extension cracks follow the compression axis.

In the news item 'Yugoslavian nuclear power: Locals late reacting', Nature 291, 446, the name of the head of the Croatian Commission for the Human Environment was given incorrectly. It should read Marko Branica.

MATTERS ARISING

Grand unification magnetic monopoles inside the Earth

CARRIGAN1 has suggested that the equilibrium between the magnetic and gravitational forces would lead to these monopoles being trapped in two positions on the magnetic axis, one each side of the centre, depending on their polarity. However, his discussion is incorrect in several respects. First, in the Earth's core, the gravitational field is roughly proportional to radius r. If the magnetic field were due to a central dipole it would be proportional to r^{-3} , and there would be (radial) equilibrium on the axis at $\sim r =$ $0.18 R_{\rm e}$, within the fluid core, as he states. But the magnetic field is far from being dipolar, and within the core the dipole and non-dipole fields are comparable in magnitude; even at the core surface there are already ~14 positions2 where the field is radial, not just the two he assumed.

Second, in fact, the magnetic field is produced by electric currents in the core; reasonable current distributions give a dipole radial component of field which increases only very slowly with depth. This would give an 'equilibrium' radius of $\sim 0.007 R_{\odot}$ only 50 km from the centre, and well within the solid inner core. Third, 'equilibrium' calculations apparently considered only radial forces, and for his dipole 'equilibrium' the monopole is unstable to infinitesimal tangential displacements. Stable equilibrium would require complicated fields with a most unlikely coincidence of magnitude and topology.

Finally, as Carrigan points out, in the core there are probably toroidal fields much larger than the poloidal dipole fields. However, these toroidal fields have no radial component, and will be largely latitudinal, so will not "result in large axial fields". What they will do is make it even less likely that there will be any stable positions of purely radial field. The inner core also will have large toroidal fields³.

It does seem most unlikely that there will be any significant trapping of monopoles by the mechanism Carrigan suggests.

F. J. Lowes

School of Physics, The University, Newcastle upon Tyne, NE1 7RU, UK

- 1. Carrigan, R. A. Jr Nature 288, 348-350 (1980).
- Barraclough, D. R. & Malin, S. R. C. Geophys. J.R. astr. Soc. 65, 467-473 (1981).
- 3. Gubbins, D. J. geophys. Res. (in the press).

CARRIGAN REPLIES—Because of the limitations of space I could not emphasize the model nature of the calculation. However, I did point out that the dipole

model was not a proper picture of the interior of the Earth. I did not say a monopole of one polarity would occupy a single region of equilibrium but rather that GUMMs of one polarity would interchange with those of the other polarity. I did not touch on one aspect of the situation, the fact that GUMMs would probably not move rapidly through solid material in the interior of the Earth. If this is so it represents a third effective force. Static or dynamic equilibrium would be a result of all three effective forces.

In view of the limits of the picture—little is known about GUMM energy loss processes, there is incomplete knowledge of the interior of the Earth and little understanding of the accretion mechanism—it did not seem appropriate to develop a more comprehensive picture. The main point I wished to make was that even a relatively few GUMMs of opposite polarities could contribute a spectacular amount of energy.

RICHARD A. CARRIGAN JR

Fermi National Accelerator Laboratory, PO Box 500, Batavia, Illinois 60510, USA

Estimated speed of a giant bipedal dinosaur

FROM observations of locomotion in diverse living animals, including ostrich, bird, man, horse and elephant, Alexander obtained a relationship between speed, stride length (λ) and body size (expressed by h, height at the hip). This relationship seems to hold true, at least in general terms, for large and small animals, both bipeds and quadrupeds, at gaits from walk $(\lambda/h < 2.0)$ to run $(\lambda/h > 2.0)$. To estimate the speeds of certain dinosaurs Alexander applied this relationship to their tracks, where λ could be measured directly and h could be estimated from the size of the footprints. He found that the estimated speeds were rather low—between 1.0 and 3.6 m s⁻¹ (3.6 and 13.1 $km h^{-1}$).

Alexander's method has since been applied to various other dinosaur tracks²⁻⁵, but few of these have yielded speed estimates greater than about 4.2 m s⁻¹ (15.0 km h⁻¹). A notable exception was provided by Russell and Béland², who calculated that a short section of trackway in the Mesaverde Formation (Cretaceous) of Colorado⁶ had been made by a dinosaur running at a speed of 7.54 m s⁻¹ (27.1 km h⁻¹). The Colorado dinosaur was apparently a giant bipedal ornithischian (probably an ornithopod of the family Hadrosauridae), with h being

~3.44 m and a live body weight of ~11 tonnes2. Russell and Béland based their calculations on data published by Brown⁶, whose account of the Colorado trackway is rather ambiguous; in one place Brown referred6 to a "fifteen foot stride", while in another he mentioned that the dinosaur "stepped 15 feet 2 inches". A stride is commonly understood to comprise two consecutive steps, or paces7, though the terms 'stride' and 'step' have sometimes been used synonymously. Evidently Brown was referring to a step (or pace), for the distance of 15 feet was measured between two footprints which identified as those of left and right feet. Russell and Béland apparently followed Brown's interpretation: they presumably took the figure of 15 feet to represent a step (left footprint to right footprint) and doubled it to obtain the figure of 9.25 m which they cite for stride length²

I have examined photographs of the Colorado footprints (supplied by Dr E. S. Gaffney), and I suspect that Brown's interpretation of them may be wrong (see Fig. 1). The two footprints identified by Brown as left and right are very similar,

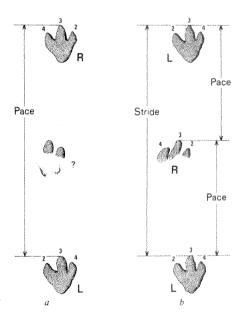


Fig. 1 Diagram illustrating two interpretations of ornithopod dinosaur trackway from the Cretaceous of Colorado (American Museum of Natural History, no. 3650). Each footprint has the digits numbered and is identified as left (L) or right (R). The footprints are represented by natural casts on the under-surface of a sandstone bed, so that left and right appear transposed. a, Brown's interpretation⁶, indicating a pace of ~15 feet (4.57 m); the surface feature marked? is not regarded as part of the trackway. b, Revised interpretation, indicating a pace of ~7.5 feet (2.29 m).

and they may well be prints of the same (left) foot. Both prints seem to show the same pattern of asymmetry-weak outward curvature of digit 3, and an angle of divarication between digits 2 and 3 which is slightly greater than that between digits 3 and 4. Mid-way between these two footprints is a poorly defined surface feature which was regarded by Brown⁶ as an amalgam of two footprints from other dinosaur tracks. This feature is probably an incomplete print of the ornithopod's right foot; it is about the same width as the other two footprints and shows traces of three digits with an appropriate pattern of divarication. The three footprints have a pattern of digit spacing (digit 2 slightly more divergent than digit 4) which is characteristic of many ornithopod footprints5 and which is consistent with their identification as left, right and left. This interpretation also reveals distinct positive (medial) rotation of the footprints, an arrangement which is common in the trackways of other bipedal dinosaurs (see, for example, refs 8 and 9); no such footprint rotation is evident in Brown's interpretation.

According to the revised interpretation (Fig. 1b) Brown's measurement of 15 feet would actually represent a stride (between successive prints of the same foot). Using Alexander's method¹, it may then be calculated that the Colorado ornithopod was walking (with λ/h of 1.34) at a speed of 2.4 m s⁻¹ (8.5 km h⁻¹). The highest speeds so far estimated from dinosaur tracks are in the region of $3.6-4.3 \text{ m s}^{-1}$ (13.0-15.5 km h⁻¹), and these are attributed to small bipedal runners with h < 1 m (refs 1,5). There are no reports of tracks indicating that larger bipedal dinosaurs were capable of achieving a running gait (with $\lambda/h >$ 2.0).

I thank Dr E. S. Gaffney (American Museum of Natural History) for providing a description and illustrations of the Colorado footprints.

RICHARD A. THULBORN

Department of Zoology, University of Queensland, St Lucia, Queensland 4067, Australia

- 1. Alexander, R. McN. Nature 261, 129-130 (1976).
- Alexander, R. McN. Nature 261, 129-130 (1976).
 Russell, D. A. & Béland, P. Nature 264, 486 (1976).
 Tucker, M. E. & Burchette, T. P. Palaeogeogr. Palaeoclimatol. Palaeoecol. 22, 195-208 (1977).
 Coombs, W. P. Q. Rev. Biol. 53, 393-418 (1978).
 Thulborn, R. A. & Wade, M. Lethaia 12, 275-279 (1979).
 Brown, B. Nat. Hist. 41, 190-202, 235 (1938).

- Sarjeant, W. A. S. in The Study of Trace Fossils (ed. Frey, R. W.) 283-324 (Springer, New York, 1975).
 Currie, P. J. & Sarjeant, W. A. S. Palaeogeogr. Palaeoclimatol. Palaeoecol. 28, 103-115 (1979).
- 9. Newman, B. H. Biol. J. Linn. Soc. 2, 119-123 (1970).

RUSSELL REPLIES—Brown clearly uses 'stride' in the sense of step in his description1: "The tracks... (show) the right and left foot in normal stride where the giant

had stepped 15 feet". He also describes a third footprint, made when the animal had stepped on a firmer substrate. The steps defined by the three footprints would presumably have been of comparable length or Brown would not have referred them to the same trackway. In view of the symmetry of hadrosaur footprints² and Brown's presence at the site where the trackway was excavated, prudence suggests that his interpretation remains as plausible as Thulborn's proposed alternative.

DALE A. RUSSELL

Paleobiology Division, National Museum of Natural Sciences, Ottawa, Canada K1A OM8

1. Brown, B. Nat. Hist. 41, 190-202, 235 (1938). 2. Langston, W. Nat. Hist. Pap. natn. Mus. Can. 4, 1-9 (1960).

Inhibition of haemoglobin S gelation and water structural effects

BENESCH AND BENESCH¹ recently proposed an interesting hypothesis concerning the mechanism of action of various hydrophobic molecules which inhibit the gelation of sickle cell haemoglobin (HbS). They suggested that this hydrophobic class of sickling inhibitors may act on water structure rather than interact directly with HbS. The physical explanation provided by Benesch and Benesch¹ for the 'melting' of the HbS gel at low temperatures seems very plausible. They suggest that the creation of more polyhedral void spaces in water at lower temperatures and the consequent coalescence of 'filled' (with nonpolar moieties) and 'empty' polyhedral cages is responsible for weakening the hydrophobic forces. However, they extend this reasoning to suggest that the inhibition of polymerization of HbS by a variety of compounds containing hydrophobic residues is also "brought about by competition between the water polyhedra filled with small hydrophobic molecules and those attached to the protein, rather than a direct interaction between the hydrophobic residues and the protein which should be stereospecific". The two situations are not physically analogous.

The clumping of 'empty' and 'filled' polyhedral cages leads to the relief of hydrophobic interactions while clumping of filled cages with other filled cages produces hydrophobic interactions2. This will apply equally whether the cages are filled with nonpolar segments from the protein or the small hydrophobic molecules. When the latter are added to a solution containing the HbS polymer, some existing water polyhedral void spaces will be further occupied and the resulting encounter of cages filled with small hydrophobic molecules with those filled with nonpolar side chains of the protein should provide the stimulus for a direct (hydrophobic) interaction of the small molecule with the haemoglobin nonpolar side chains, in competition with the side chain-side chain interaction. Gel melting can occur, in such cases, by a substitution of protein-protein hydrophobic interaction with protein-small molecule hydrophobic interaction. This interaction may be weak or strong, depending on the nature of the molecule and the side chain. If, in addition to this hydrophobic interaction, ionic or hydrogen bonding is possible, the direct interaction will be further strengthened. Such a possibility has been postulated for a molecule like benzyl alcohol³, although there has been no direct proof of this.

The attempts to increase the effectiveness of hydrophobic antisickling agents by structural modifications have failed so far. not because the agents are incapable of direct interaction with the protein but because of stronger macromoleculemacromolecule interactions in the polymer.

S. SUBRAMANIAN

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases. National Institutes of Health, Bethesda, Maryland 20205, USA

- 1. Benesch, R. & Benesch, R. E. Nature 289, 637 (1981).
- Stillinger, F. H. Science 209, 451 (1980).
 Ross, P. D. & Subramanian, S. Biochem. biophys. Res.

Commun. 77, 1217 (1977).

Matters Arising

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Liposomes, Drugs and Immunocompetent Cell Functions edited by Claude Nicolau and Alain Paraf

August/September 1981, xiv + 194pp., £14.00 (UK only)/\$34.00, 0.12.518660.6

The chapters of this volume reflect the multidisciplinary nature of the meetings by discussing liposome research from a number of different angles. The main topics considered are the physico-chemical properties of liposomes and their design, the use of liposomes to transport molecules into a variety of cells, the improvement of their stability, the use of virus envelopes to introduce material into cells, immunological targeting of liposome and transmembrane signalling via membrane bound enymes. In discussing these topics, the contributors not only reappraise existing knowledge but also present new findings. Areas of new research covered include the transfer of genetic material, the use of liposomes in vivo and in vitro and targeting with monoclonal antibodies.

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Rhône — Poulenc Round Table Conferences

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The purpose of the Rhône-Poulenc Round Table Conferences is to bring together a number of widely known scientists from universities and the pharmaceutical industry who are engaged in common research. The second conference was held in Paris in February 1980, and was an attempt to obtain a fully up-to-date assessment of the most important aspects of the use and development of antibacterial agents. Its proceedings are contained in the present book, which comprises 31 chapters by 44 leading experts.

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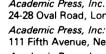
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This is the first book dealing exclusively with x-ray microanalysis of biological specimens. It not only presents the basic theory and operation of the instruments used for producing, measuring and interpreting x-ray signals, but also describes in detail the preparation of bulk specimens, sectioned specimens and fluid specimens.

The book includes both qualitative and quantitative analysis of biological materials and it discusses application of x-ray microanalysis to pathology, physiology and cytochemistry.

Instrumentation is presented in a concise manner and methodology in detail. Almost all instruments are covered, including TEM, SEM and STEM. Instructions are given on how to produce, measure and interpret x-ray signals and emphasis is given to the aspects of instrumentation that are of practical use. In its comprehensive compilation of methods for preparing biological specimens the advantages and disadvantages of alternate methods are discussed.

READERSHIP: Electron Microscopy, Biology, Biochemistry.

LEVEL: Undergraduate and graduate students, researchers and research technicians.

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BOOK REVIEWS

Food aid, self-sufficiency and the new technology

Barbara Huddleston

In Seeds of Plenty, Seeds of Want, Andrew Pearse sets the stage for consideration of a number of new directions for food and agriculture policy in the 1980s. What to do about food aid is one of them. The subject is currently embroiled in controversy, much of it created by the uneven income distribution effects of Green Revolution technology, which food aid has more often than not reinforced.

How did food aid come to be what it is, and what changes can we expect in its use as an instrument of foreign policy and consequent impact on developing Third World economies? For answers to these questions, the interested reader should surely not miss Mitchel Wallerstein's new book, Food for War — Food for Peace. His is, however, a historian's book, and the book of a political scientist more interested in the forces which influence the level and allocation of food aid flows than in those which affect its impact on development.

Wallerstein's book is divided into four parts and 12 chapters. Part I presents a fairly straightforward, factual summary of the origin and subsequent evolution of US food aid policy and Part II does the same for other bilateral donors and the UN system. An important insight growing out of the historical material on US policy is the perception that in periods of crop surplus domestic agricultural policy concerns have dominated the political process, whereas in periods of shortage foreign policy concerns have prevailed. This means that if agricultural experts are correctly reading future trends, and world grain markets do not return to a situation of chronic surplus, foreign policy considerations will be even more important than before in fixing the level and direction of US food aid flows.

Economic development in friendly Third World countries has been and will continue to be an important objective of American foreign policy, and food aid will probably continue to be an important instrument for attempting to achieve this aim among others. In Part III, Wallerstein presents case material for each major recipient country, showing how the food aid programme had served one or more of the following foreign policy purposes: to give economic support to Cold War allies; to secure military bases; to exercise diplomatic leverage in tense international hotspots; to maintain friendly relations with UN voting allies. He also discusses the

Seeds of Plenty, Seeds of Want: Social and Economic Implications of the Green Revolution. By Andrew Pearse. Pp.262. ISBN 0-19-877150-9. (Clarendon/Oxford University Press: 1980.) £7.50, \$22.50. Food for War — Food for Peace: United States Food Aid in a Global Context. By Mitchel B. Wallerstein. Pp.312. ISBN 0-262-23106-9. (MIT Press: 1980.) \$30, £18.60.

interaction between the use of food aid to foster US trade interests and the withholding of it as a factor in US economic and commercial relations with Third World countries. An important aspect of Wallerstein's treatment is his focus on the individual concerns and policy interests of each of the four Presidents who have exercised control over US food aid policy since the shift toward its greater use for foreign policy purposes in the early 1960s. The effect of personality and temperament was clearly important, and this suggests that while the categories Wallerstein uses may be appropriate for the specific circumstances of the past, they will not necessarily fit the future so neatly.

The final part of the book treats issues relating to the role of multilateral versus bilateral food aid in the context of international politics. There are some cogent reasons for both donors and recipients to prefer that some portion of the overall flow of food aid be channelled through multilateral assistance agencies. However, both international politics and the sluggishness of the US bureaucracy constrain the amount which can be effectively administered in this way. An alternative for the future which Wallerstein suggests, and which is already a glimmering in the eyes of some of the more forwardlooking participants in the food aid debate, would be for the International Wheat Council to play an increased role in the coordination of food aid flows, and for donors to come together more frequently through aid consortia for agricultural development. This mechanism would encourage the working out of the respective roles of food aid and other foreign assistance in complementary and developmentally effective fashion, without requiring donors to give up the foreign policy benefits they stand to gain from maintaining bilateral food

programmes.

Like many good historians, Wallerstein uses his material to weave a story which is full of complexity and detail, bringing into focus the pattern of past events without prejudging the future. In a sense, Pearse's book is also historical, although he tries so hard to be true to the results of each piece of research which he reports that the story he has to tell suffers somewhat. While it is not an easy book to read, Seeds of Plenty, Seeds of Want does have some important points to make. It is a book about smallness versus largeness, about cultivation for a livelihood versus cultivation for a profit, about the old way of doing things versus the new. It raises issues and suggests problems arising out of Green Revolution strategies of agricultural development which concern aid donors and recipients

Pearse's volume grew out of a joint undertaking of the UN Research Institute for Social Development/UN Development Programme (UNRISD/UNDP) which he directed, the brief of which was to examine the socio-economic effects of the Green Revolution. The work began in 1970 and was conducted by a number of researchers, each working on an individual case study or particular aspect of the subject. Much of the research was completed by 1974 and many of the case-study results have subsequently been published in their own right. Nevertheless, the project directors rightly felt that an analytical and interpretative summary of the main conclusions and their policy implications would be in order, and Pearse's book is the result.

Since the volume is a summary of previously published reports of individual research efforts, some of them dating back several years, its analysis and conclusions do not always reflect the latest results available; nor is reference made to similar kinds of studies done by workers who were not part of the UNRISD study. This is unfortunate, since some later work modifies and amplifies the findings of the early UNRISD pieces, and the book would have had more general value if it had set the UNRISD material in the context of the larger body of work which addresses questions relating to the impact of Green Revolution technology and means of improving its effectiveness as a tool for agricultural development. Even among those who originally contributed, there is growing recognition that, as the full effects of new technologies continue to work themselves out, dynamic changes are beginning to occur which will provide new economic opportunities and more widely distributed income growth than previously thought.

This difficulty does not detract from the main thrust of the argument, however. Pearse states frankly that his objective, and that of his collaborators in the UNRISD studies, is "to strengthen those forces likely to press for non-elitist development" (p.219), and the concluding part is particularly successful in creating the basis for doing this. The first two parts attempt to draw generalizations from the specific case studies that were part of the UNRISD project. Here Pearse meets with less success — the material is organized somewhat arbitrarily around a number of themes commonly associated with Green Revolution impact analysis. However, some of the studies are quite situation specific and of doubtful general relevance.

In Part III the critical issues are summarized in Pearse's own words, and here both the nature of specific difficulties and the lines of possible policy change are spelled out with great clarity. It is not simply that the rich are getting richer and the poor poorer. More importantly, the economics of agricultural development are such that the most effective techniques for increasing agricultural production result in a reduction in welfare for small farmers where there is land scarcity and labour surplus. Pearse does not oversimplify and make the mistake of asserting that a return to subsistence agriculture can solve the food problem; he recognizes that production growth cannot occur without commercialization of farming. But he points out that under the early Green Revolution strategy, the number of sublivelihood households increased, with no comparable increase in alternative earning opportunities for the growing number of landless labourers.

Thus he recommends the different strategy of concentrating on the poor but aspiring farmer as initial adopter of new agricultural technologies, and focuses on questions of appropriateness of technologies, institutions, and price and planning policies for implementing such a strategy. Out of the questioning of the old approach and the recognition of previous failures has arisen a new "food systems" approach to planning which some countries are already beginning to pursue and which is being supported by further research at UNRISD. With its focus on the distributional as well as the yield-increasing results of agricultural development strategy, the new approach promises to open up different avenues for seeing and doing which give hope that one day a book such as this will no longer be necessary.

Barbara Huddleston is a Research Fellow at the International Food Policy Research Institute, Washington DC.

Astronomical, in content and in effort

Michael Salt and Joseph Needham

Chung-Kuo Ta Pai K'o Ch'üan Shu (The Greater Encyclopaedia of China). Vol.1 T'ien Wên Hsüeh (Astronomy). Pp.650. (Greater Encyclopaedia of China Publishing House, 1-A Wai Guan Dong Jie, Beijing, 27 (Peking), China: 1981.) \$30.

"Your writing's grown weedier and weedier:
Produce more, or we've no further need o' ya",
His publishers warned.
Their advice wasn't scorned:
He's at work on an encyclopaedia.

At the present time we seem to be flooded with encyclopaedic compilations. Last year, there was the new *Britannica*, and the 16-volume *Dictionary of Scientific Biography*. This year has seen the new and revised edition of *Grove*. In popular astronomy alone, half-a-dozen different compendia are available.

But China is different, for there has been no grand encyclopaedia produced in that great culture in modern times at all. The present book is the first of 80 volumes that will cover archaeology, law and jurisprudence, history, literature, philosophy, religion, economics and many other subjects. It deals not only with contemporary astronomy but also with its history. Astronomy is one of the few sciences for which it is possible to write a single, continuous, historical account reaching from antiquity to the present day, as was done by R. Wolf in his Handbuch der Astronomie; ihrer Geschichte and Litteratur of 1890. Now Chang Yü-Chê and his 20 colleagues have accomplished the same.

Physically, this first volume is of excellent quality. The binding (of our hardback copy, at least) has survived the destructive testing to which so many Chinese books succumb in transit; the paper is thin but substantial; and the print is clean and easy to read. The numerous intext photographs and diagrams, too, are of admirable quality. So are the beautiful coloured plates, clustered together at intervals throughout the book. Several hours of serious reading have revealed only two misprints, though in a volume of one and a half million (Chinese) characters, there must presumably be others. But our first and enduring impression is that proof-readers and publishers have done their utmost to ensure an accurate text, handsomely presented.

On the whole, the book is easy to use. An English index to entries rescues those whose technical Chinese is insufficient to give them accurate bearings in the vastness of the field. A glossary of proper names, in Roman and Cyrillic scripts, promises help with the transliterated scientists who feature in the text. This promise is not per-

haps fully carried out, however, for several names (e.g. de Broglie, Lorentz, Narlikar and Planck) are not given in the glossary. This list is really a conversion table from the Western forms to the Chinese forms, and one has to be forewarned that in the main index the alphabetical order follows the Chinese form of the name, not the original Western one. It is a pleasure to see that due credit is given to the great scientific men such as Chang Hêng, Tsu Ch'ung-Chih, I-Hsing and Kuo Shou-Ching, who figure in the history of astronomy; though we cannot say we like the practice of giving entirely imaginary portraits of them (as in Pl.5), recalling the Renaissance busts of Aristotle and Galen with which the older historians of biology and medicine used to ornament their books. There are also plenty of plates and text-figures showing ancient Chinese instruments, and pages of old Chinese books, all well explained, but they do not in any way impede the exposition of the most modern theories and the most recent knowledge. Finally, starmaps identify the traditional Chinese constellations in terms of those used scientifically today.

There are minor inconveniences. One of these arises from the purely alphabetical arrangement of the entries, which follows the sounds of the Pin-yin romanization. Another concerns the diagrams. In a work arranged by subject, a few well-placed and well-chosen diagrams can be made to serve an entire section, but here diagrams are sometimes lacking in places where they would be helpful. For instance, in the article on relativistic cosmology (p.444), there is no diagram to illustrate the different values of k in the three models of the Universe; but a suitable graph is given in the entry on the age of the Universe (p.519). Unfortunately, though, there is no crossreference.

Cross-references to entries within the volume are achieved by a simple convention; a different Chinese type-face in the text. In practice, though, this is not rigorously applied. For instance, the entry on the "problem of the advance of Mercury's perihelion" (p.333) mentions Le Verrier and Newcomb, but fails to indicate that each of these men is himself the subject of an entry (pp.204, 244).

The matter of entries leads to two more important questions. First: in an encyclopaedia of astronomy, how much physics, say, or mathematics, should be included? Second: how much background knowledge, and what level of understanding, should the compiler expect of his readers? These questions face every author. But for the encyclopaedist, they are of paramount importance.

As regards coverage, we are not competent to judge whether or not all the growing points of this prolific subject are

adequately covered. We were, however, slightly disappointed to find no separate articles on quantum theory, on the special or general theories of relativity, or on tensor analysis. One wonders why such topics as these are not separately expounded when the scintillation counter (p.288), photoneutrino processes (p.96) and metric (p.64) each feature individually? Future volumes will no doubt supply the missing information, but for the present our thirst for understanding is not slaked.

The second question is also difficult to answer on the basis of the present volume. In general, the articles are well written. Some, indeed, are admirable, both in exposition and content. Others are less good, either failing to link the subject in hand with cognate subjects, and with the general principles involved; or attempting to cover basic theory, the history of the subject and its interrelations, all in one and the same exposé. Moreover, not all articles start from the same intellectual baseline. In one, for instance, π and G are glossed, but in another, a partial differential equation is

given, without preliminaries or explanation. This case suggests that this volume is intended primarily for scholars and research workers. But if so, why do most of the articles lack bibliographies? And why are such references as are given always to books rather than to research papers?

But these are early days. We must not focus disproportionately on comparatively minor shortcomings. Subsequent volumes will surely make further improvements even on the high standard that this first volume has set. This work is of a very high standard, quite dazzling in production. Contributors and editors alike should rightly feel proud of all that they have achieved within three years. We look forward eagerly to the remainder of what promises to be a magnificent compilation.

Michael Salt and Joseph Needham are at the East Asian History of Science Library, Cambridge, UK. The most recent of Dr Needham's volumes in the series Science and Civilisation in China was published late last year.

Prehistoric Sahara of green and plenty

Martin Williams

Prehistory of the Eastern Sahara. By F. Wendorf and R. Schild. Pp.414. ISBN 0-12-743960-9. (Academic: 1980.) \$65, £24.20.

DEFUNCT lakes and rivers, plant and animal fossils, Neolithic villages and rock paintings testify to when the Sahara was, in certain parts at least, a green and pleasant land. Nowhere is the contrast between present wasteland and prehistoric plenty more stark than in the Western Desert of Egypt. For six seasons, from 1972 to 1977, a multidisciplinary team from Egypt, Poland and the United States laboured to reconstruct the life-style, economy and habitat of the Palaeolithic and Neolithic peoples who once inhabited the now-arid wastes between Libya and the Nile.

The book opens with a short, clear account of field and analytical methods. Four profusely illustrated chapters detail the archaeological finds and stratigraphy in each of the localities studied, after which the geographically and chronologically discrete strands are woven into two rich tapestries depicting the former environments and human exploitation of the Western Desert. A lucid, thoughtprovoking discussion of the origin of food production in northern Africa concludes the main section of the book. Ten specialist appendices follow, including evaluations of Quaternary deposits (marred by the unreadable Figure A1.1), radiocarbon dating problems, artefact and pottery analyses, the fossil record, and a scanning electron microscope study of cereal grains.

What conclusions emerge from these new data? The most interesting, best documented evidence relates to the Terminal Palaeolithic and Neolithic sites near Gebel Nabta, 100 km west of Abu Simbel. At Nabta playa three Holocene lake phases date to ≥ 9000 , ≥ 8600 and 7000-5800 BP. Terminal Palaeolithic sites persist until the end of the first moist phase; Neolithic sites appear at the close of the ensuing dry phase. Early cattle, sheep and goat remains date respectively to 9300, 8100 and 7000 BP. Attempts by the early desert dwellers to adapt to an increasingly harsh Holocene climate culminated in domestication of animals and plants over a thousand years before food production became widespread in the adjacent Nile valley.

The final exodus of Neolithic herders from the dying grasslands and shrinking lakes of the Saharan interior must have had repercussions beyond the confines of the desert. In this context, the recent migrations of starving herds and emaciated nomads during the latest sahel drought reflect the tragic but inexorable late Holocene desiccation of our greatest desert.

A fine testimonial to the dedication of the archaeologists and to the organizational skills of the two authors, this clearly written, well-produced volume deserves to be widely read by prehistorians and students of the Quaternary.

Martin Williams is Associate Professor in Earth Sciences and Director of the Quaternary Research Unit at Macquarie University, Australia.

Blossoming Balkans

P.J. Grubb

Flowers of Greece and the Balkans: A Field Guide. By Oleg Polunin. Pp.592 + plates. ISBN 0-19-217626-9. (Oxford University Press: 1980.) £40, \$125.

THE latest in Oleg Polunin's series of illustrated guides to European plants is a most attractive introduction to the Balkan flora, which is the richest in Europe, amounting to more than 5000 species. Over 500 of these are illustrated in colour photographs and some 350 others in blackand-white drawings. The plan of the book is essentially the same as that adopted in the earlier Flowers of South-west Europe (OUP, 1973), produced jointly with B.E. Smythies. There is a general account of the climate, geology, flora and vegetation, an introduction to each of the "plant hunting regions", and then brief diagnoses of about 2000 species (320 pp.), followed by indexes of names and places, and an exhaustive bibliography. A new departure is the index of popular names used in Yugoslavia, Bulgaria and Greece. There are 22 absolutely superb colour plates of representative landscapes, but my copy lacks numbers 1 to 4 — an unreasonable fault in a book costing £40!

The author conveys his "feel" for the country and his enthusiasm for the plants very well. His accounts of particular regions have been vetted by local botanists, and those of the several areas I have visited myself ring true. The drawings are excellent, and the photographs constitute the usual mixture, some being of little value for critical identification but others are perfect. Whether the accounts of genera that are nowhere illustrated will be of any use to those who have not seen the plants concerned elsewhere, I doubt.

The weakest part of the book is the ecological account, particularly the section on forest and scrub communities, and it would have been better to have followed more closely the scheme of Horvat, Glavac and Ellenberg, whose map of vegetationtypes has been used as the basis for the simplified map lining the covers. More precise thumb-nail sketches of the ecology of each species described would also have been possible. The worst fault is the emphasis on 1920s' ideas of "migration routes" of plants, and the failure even to mention the appreciation built up in the 1960s and 1970s of the dramatic changes that occurred in Mediterranean communities during the Ice Ages, when steppe seems to have replaced much of the forest.

Despite its minor blemishes, this book will be an invaluable guide in the Balkans for those who already have a good knowledge of northern and central European plants.

P.J. Grubb is a University Lecturer in Botany at the University of Cambridge.

Life of an archaeologist extraordinary

Peter Warren

The Find of a Lifetime: Sir Arthur Evans and the Discovery of Knossos. By Sylvia L. Horwitz. Pp.278. ISBN 0-670-13575-5. (Viking: 1981.) \$14.95. To be published in the UK in October by Weidenfeld & Nicolson, price £9.95.

FEW subjects could be more compelling for a biographer than Sir Arthur Evans. The reasons for this are not simply external: his passionate devotion, based on deep historical study and extensive topographical research, to the freedom of the Slav peoples; his re-foundation, almost creation, of the Ashmolean Museum; his virtually personal discovery of the Minoan, Bronze Age civilization of Crete, with its complex yet graceful society; and his wealth, so generously given over to recreating the Minoans for all the world. All these stand as beacons for any biographer. But what gives the subject a yet stronger appeal is the astonishing range and quality of Evans's mind, that of a true Renaissance man, as learned and appreciative in numismatics, botany, Balkan topography and history, folklore, Belgic and Roman settlement in Britain, as in Minoan archaeology.

Yet one turns to a new biography with some trepidation, since this work was done in a most perceptive and scholarly way by Joan Evans in Time and Chance (Longman, Green, 1943), published within two years of Evans's death at the age of ninety. As Sir Arthur's half-sister, Joan Evans had access to - indeed was an intimate part of - family history, which made her book a masterpiece. Since that time, too, the archaeologist W.A. McDonald has included a lengthy chapter on our subject in his excellent survey, Progress into the Past. How, then, does Sylvia Horwitz fare? The answer is that hers is very good and sympathetic account. While its lighter tone and air of a rapid read mean it in no sense replaces Time and Chance, its qualities are clear. It is accurate, a few smallish errors apart; it is lively and enjoyable, clear though brief on archaeological and Balkan political issues; it does not disguise Evans's autocratic and imperious attitudes, nor his generosity. The most perceptive sections are those examining the effects upon Evans of the death of his mother when he was six, of the sister closest to him and of his wife Margaret, daughter of the Liberal and historian Edward Freeman, after only 15 years of marriage. The author's suggestions are convincing, that these losses made Evans deeply withdrawn and much more reticent about personal feelings than was usual even for a high Victorian, while at the same time, in pursuing his scholarly and political interests, and with children, he was extrovert, generous and always vigorously engaged.

The book makes good use of the 40 years

since Evans's death and the publication of Time and Chance by describing how his theories and interpretations have fared after two more generations of archaeologists and Cretan discoveries. With the exception of his views on Mycenae, the huge edifice of thought he erected, on religion, art, architecture and Cretan prehistory, remains the firm basis of contemporary Minoan understanding. While reaction to his Minoan colonization of the Greek mainland was natural, given the rich vein of Mycenaean discoveries explored by A.J.B. Wace and a distinguished line of Greek archaeologists, the extent of Cretan artistic and economic influence throughout the south Aegean and southern Peloponnese now appears to be much greater than critics of Evans's views could then allow. His dating of the destruction of Knossos as a palace, at around 1400 BC, and all that that means for Aegean prehistory, came under detailed attack from Professor L.R. Palmer some years ago; but a large majority of archaeologists has confirmed the essential correctness of Evans's and his assistant Mackenzie's views. Thermoluminescence dating may one day provide a close date for the burning of the Linear B tablets, at the heart of that controversy, in the fire which consumed the palace.

Unlike recent studies on Schliemann, Sylvia Horwitz's account is thoroughly



Sir Arthur Evans — a true Renaissance man.

sympathetic. She admires the vast nervous energy, the astonishing yet repeated flair for understanding an object or its function from exiguous evidence, often confirmed years later by new discoveries. She found no need for a severe critique of this eminent Victorian. Her account can be warmly recommended, for itself and as a preliminary to *Time and Chance*. We should recall only that as much is to be found of political reporting from remote Bosnian fastnesses or richly flowered Ragusan gardens as of the splendours of the House of Minos.

Peter Warren is Professor of Ancient History and Classical Archaeology at the University of Bristol, and currently Director of Excavations at Knossos.

Ordered amino acids brought to book

Linda Fothergill

Handbook of Protein Sequence Analysis: A Compilation of Amino Acid Sequences of Proteins with an Introduction to the Methodology. 2nd Edn. By L.R. Croft. Pp.628. ISBN 0-471-27703-7. (Wiley: 1981.) £38, \$104.50.

This second edition of Croft's compilation of amino acid sequences of proteins is strikingly different from its predecessor, both in format and content. Disappointingly, it is no longer loose-leaf, thereby losing the useful flexibility of the first edition. It is obviously no longer possible to slip in annual supplements — a most attractive feature in a field such as protein (and nucleic acid) sequence determination that expands at such a dauntingly rapid rate. Sadly, also the useful transparent overlay to show residue numbers is no supplied, although extraordinary practice of designating N-terminal acetyl groups as residue one has been perpetuated in the second edition.

A new feature of this second edition is that approximately one-third is devoted to protein sequence determination methodology. Much of this section is admirably full of practical detail — something unfortunately often lacking in primary publications. There are some gaps however (HPLC separation of peptides is not mentioned for example), and a more extensive, separate publication would seem more satisfactory. Moreover, I suspect that a great many of those consulting a handbook of sequences are not particularly interested in the methodology.

A comparison of this handbook with Dayhoff's Atlas of Protein Sequence and Structure (Supplement 3 to Vol.5, 1978) shows that Croft's book is more up-to-date, but that it includes fewer homologous sequences from related species, no fragments and less extensive notes on the proteins.

Croft's book will be indispensable in providing the most up-to-date handbook of protein sequences currently available; but please, for the next edition, return to the loose-leaf format with annual supplements!

Linda Fothergill is a Research Officer in the Department of Biochemistry, University of Aberdeen.

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PROFESSOR OF PHYSICS

and wish to fill this position as soon as possible. A candidate for this position will be an outstanding scientist with an established international reputation.

Applications, accompanied by a current CV and the names of three referees, should be forwarded to: Dr J C Irwin, Chairman, Department of Physics, Simon Fraser University, Burnaby, BC, Canada V5A 1S6. (604) 291-3160/3154. (NW749)A

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts AL5 2JQ

STATISTICIAN

To work on the design and analysis of groups of experiments done by the Agricultural Development & Advisory Service of the Ministry of Agriculture, Fisheries and Food. Scope for original work; interest in computing and techniques of data handling essential.

Oualifications: Degree with statistical component. At least five years relevant post-qualifying experience is required for appointment to Higher Scientific Officer.

Appointment in the grade of Scientific Officer (£4,809 — £6,480) or Higher Scientific Officer (£6,075 £7,999) according to qualifications and experience.

Apply in writing to the Secretary naming two referees and quoting Ref 458 by 6th August 1981. Further (9087)A details on request.

City Hospital

SENIOR CYTOGENETICIST Full-time. BASIC GRADE CYTOGENETICIST

The above appointments are in the South-Trent Sub-Regional Cytogenetics Service currently housed in the Maternity Unit at the City Hospital, Nottingham, but will shortly be moving to more extensive accommodation on the same site

The successful applicants will be expected to share in all standard laboratory procedures including culturing, harvesting, slide making, staining and appropriate analysis of preparations from blood, amnistic fluid, bone marrow and other tissues. The diagnostic workload is heavy but it is hoped that, with these two new appointments all members of staff will have some time for personal work. One post is available immediately and one from 1st October, 1981

For the Senior Grade post, preference will be given to those candidates with a wide range of experience in diagnostic chromosome analysis.

For the Basic Grade post, a graduate with a reasonable degree in one of the Biological Sciences is required. Preference will be given to candidates whose degree course or postgraduate work includes a large Genetics component and/or tissue culture experience.

Further information about these posts can be obtained by ringing Dr Pat Cooke on Nottingham (0602) 608111 Extension 2785 or by visiting the Department.

Application forms are available from Personnel Services, Valebrook House, Sherwood Hospital, Hucknall Road, Nottingham. Tel: (0602) 625459 anytime.

Closing date: 30th July, 1981.

NORTH NOTTINGHAM HEALTH DISTRICT(T)

Neuropharmacologist

Greenford, Middlesex

A vacancy has arisen in our Pain Project Group in the Pharmacology Department for a neuropharmacologist to extend our investigations into the role of Substance P in pain.

The vacancy would be suitable for a pharmacology/physiology graduate who will be completing PhD studies this year, or has one or two years post-doctoral experience, in a relevant subject. Some knowledge of electro-physiological techniques would be desirable but is not essential.

The Company offers excellent conditions of employment, including a subsidised canteen, non-contributory pension scheme, bonus schemes and an active sports and social club. Generous assistance will be given with relocation where appropriate.

Please write or telephone for an application form to: Miss E. M. Butler, Personnel Department, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE. Tel: 01-422 3434, ext. 2707, quoting ref. U.378.

Glaxo Group Research Ltd

(9110)A

BIOCHEMICAL PHARMACOLOGIST

Graduate — Biochemistry and/or pharmacology Experience/interest in study of neurotransmitters

We have a place for such a person to work with our pharmacologists on ligand binding studies.

Syntex Research Centre is the research division of Syntex Pharmaceuticals Limited, part of the multi-national Syntex Corporation. Our conditions of employment include a 37 hour week, bonus opportunities, Bupa and non-contributory pension, we are situated in pleasant rural conditions on a University campus on the outskirts of Edinburgh.

If you want to join our research effort please write for an application form to:

Mr A.B. Cran, Director of Personnel.



Research Park, Heriot-Watt University,

(9104)A

The University Wollongong **DEPARTMENT OF BIOLOGY** Lecturer in Ecology

(Limited term appointment of 2 years)

The appointee will be responsible for a third year course in Ecology and will be expected to contribute to other courses in the biology syllabus. Applicants should possess a higher degree and preference may be given to a candidate with post-graduate experience in Ecology. It is expected that the appointee should take up the appointment on 1st February, 1982. A tenurable lectureship in this field may be obtained from Professor A. D. Brown, Chairman, Department of Biology.

Commencing salary, according to qualifications and experience will be in the range \$A19,821 \$A26,037 per annum. Fares to Wollongong for the appointee and dependents will be paid and a relocation allowance is payable. The University will consider contributing to the cost of repatriation on conclusion of the appointment.

Applications should contain full details of qualifications, employment history, research interests, publications list, and the names and addresses of three referees and should be forwarded to the University Secretary, the University of Wollongong, Box 1144, PO Wollongong, NSW 2500, Australia. Please mark envelope 'Confidential Appointment' Applications close 1st September 1981. (9077)A

FISH PHYSIOLOGY POST DOCTORAL RESEARCH

Position available August 1, 1981 (flexible) to study corticosteroid dynamics and physiology in adult Atlantic salmon

Salary \$14,000. Second year possible. PhD with experience in radioimmuno assays preferred.

During the Canadian postal strike send curriculum vitae and two letters of reference to

NATURE Box NW753 15 East 26th Street Suite 1503 New York, NY 10010

Otherwise applications should be sent to: Dr Melvin Wiesbart, Dept of Biology, St Francis, Xavier University, Antigonish, Nova Scotia, Canada B2G 1C0 (NW753)A

KING'S COLLEGE **HOSPITAL** RESEARCH ASSISTANT/

New graduate with Honours degree in Biochemistry or Chemistry required to work on the development of immunoassays for use in reproductive medicine.

BIOCHEMIST

Starting salary: £5,873 pa (under review).

For job description and application forms contact District Personne Department, King's College Hospital Department, King S College Prospital Denmark Hill, London SE5 9RS Telephone: 01-737 0348 (24 hours Ansaphone) quoting reference no DP/202. Informal enquiries may be made by contacting Professor Colins on 01-274 6222 Ext. 2568.

Post tenable to 31st March 1982 in the first instance, and subject to annual renewal. (9097)A

RESEARCH AND DEVELOPMENT PERSONNEI **NUCLEAR WASTE MANAGEMENT** Systems Waste Geotechnical Engineering Chemistry Development **Assessment Immobilization** Research

Challenging opportunities exist at the Whiteshell Nuclear Research Establishment for individuals to participate in an expanded research and development program for the immobilization and disposal of nuclear wastes.

The program offers a unique opportunity to participate in team-oriented R&D with wide scope for initiative, responsibility and collaboration with other scientific groups in Canada and abroad.

We require interested and/or trained personnel for the areas of:

SYSTEMS ASSESSMENT:

numerical analysis, hydrogeological modelling and risk

assessment

WASTE IMMOBILIZATION: GEOTECHNICAL RESEARCH: CHEMISTRY:

materials science, metal corrosion, glass and ceramics geology, hydrogeology and geomechanics

geochemistry. hydrogeochemistry, chemistry actinides, chemical processing and applied chemistry

ENGINEERING DEVELOPMENT: chemical, mechanical, geotechnical, instrumentation

and civil

The Whiteshell Nuclear Research Establishment is located in a wooded area on the banks of the Winnipeg River 105 km northeast of Winnipeg and employs approximately 900 people. It has modern laboratories and equipment, is organized as a multi-discipline research centre and undertakes a wide range of scientific and engineering activities.

Starting salaries are commensurate with qualifications and experience. We have a comprehensive benefits package and relocation assistance is provided.

Men and women who may be interested in these positions and possess the necessary qualifications should apply in writing giving full particulars to: Personnel Supervisor, Atomic Energy of Canada Limited — L'Energie Atomique du Canada, Limitee WHITE-SHELL RESEARCH ESTABLISHMENT, PINAWA, Manitoba, R0E 1L0; or you may call (204) 753-2311. Please indicate your area of interest on your letter of application.



Atomic Energy Research Company

L'Énergie Atomique of Canada Limited du Canada, Limitée Société de Recherche

Senior Behavioural Pharmacologist

Eli Lilly & Company is one of the world's largest producers of ethical pharmaceutical, animal health and agrochemical products. At our U.K. Research Centre, situated near Camberley in Surrey, we are actively engaged in basic research and development on a variety of projects.

A vacancy has arisen for a SENIOR BEHAVIOURAL PHARMACOLOGIST to lead a small section of three people within a multi-disciplinary C.N.S. group.

This group has a commitment to basic research and our laboratories are well equipped with on-line computer control and testing equipment.

This challenging appointment would ideally be suited to a Ph.D. candidate with 2-3 years' experience in research.

The compensation package includes a competitive salary, noncontributory pension and life assurance schemes, B.U.P.A. membership, 21 days' holiday and flexible working hours. Assistance with relocation costs will also be considered, where appropriate.

Please write to Sue Smith, Personnel Manager.

(9113)A



LILLY RESEARCH CENTRE LTD.

ERLWOOD MANOR, WINDLESHAM SURREY, TEL: (0276) 73631.

Midland Centre for Neurosurgery and Neurology

Basic/Senior Biochemist

Applications are invited from graduates in Biochemistry and related sciences for the above full time vacancy in the Clinical Chemistry Section of the Pathology Department at the above hospital.

A wide range of routine and special investigations is carried out, and participation in research will be encouraged.

User experience with one or more of the following analytical methods would be an advantage, although not essential: G.L.C., H.P.L.C., I.R., Spectroscopy, Radio isotope assay, isotachophoretic analysis.

Whitely Council conditions of service and salary applicable to this post. The Midland Centre for Neurosurgery and Neurology (81 beds) is a regional centre for the treatment of neurological and neurosurgicl diseases, situated approximately 4½ miles north-west of Birmingham. An excellent motorway network provides quick and easy access to many surrounding rural and residential areas.

Interested applicants may visit the department by appointment — telephone Mr R.A. Westhead, Principal Biochemist, on 021-558 3232

Applications in writing, stating age, qualifications, previous experience, together with the names and addresses of two referees to the Hospital Secretary, Midland Centre for Neurosurgery and Neurology, Holly Lane, Smethwick, Warley, West Midlands.

Closing date for receipt of applications: 31st July 1981.(9064)A

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF GENETICS

Applications are invited from men and women for the post of Research Assistant in the above department, funded by the Science Research Council, to work on a project on mitotic errors in Aspergillus nidulans. Experience in fungal genetics desirable but not essential. Tenable for three years from a date to be arranged. Initial salary up to £6,880 a year.

Details from Professor J. A. Roper, the Department of Genetics, the University, Sheffield S10 2TN to whom applications, with the names of two referees, should be sent by 14 August 1981. Quote ref. R609/G. (9081)A

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT IN BIOCHEMISTRY

Applications are invited for this postdoctoral post to work on the reconstruction of the insulin receptor and glucose transporter in bilayer systems. Salary on Range IA starting at £6,880 pa.

Applications with curriculum vitae and names of two referees to Dr M N Jones, Department of Biochemistry, The University, Manchester M13 9PL. (9058)A

OTAGO Dunedin, New Zealand IPORARY LECTURER I

TEMPORARY LECTURER IN CLINICAL/MEDICAL MICROBIOLOGY

UNIVERSITY OF

Applications are invited from science graduates for a temporary post as Lecturer in Clinical/Medical Microbiology. This is a one-year appointment which could be extended to 18 months.

The appointee will be expected to: (1) Act as a consultant in medical microbiology to the Otago Hospital Board. It is anticipated that this consultation should occupy about 50% of the time; (2) Contribute to the teaching of the University Department of Microbiology and to join in on-going research project or undertake independent research.

Salary: NZ\$19,140 — \$23,520 per annum, depending upon qualifications and experience. A particularly well suited and qualified applicant could be appoined at the level of Senior Lecturer.

A portion of the salary may be paid as a direct grant for fares and travel expenses of the appointee and his or her family. The residue will be subject to new Zealand income tax.

Further particulars are available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University, PO Box 56, Dunedin, New Zealand.

Applications close on 31 August (9085)A

POST-DOCTORAL RESEARCH ASSOCIATES

Positions are available immediately for recent graduates in cell biology and biochemistry to work in the areas of intracellular binding and internalization of gonadotropins.

The candidates should have experience in processing tissues and isolated subcellular organelles for EM examination, their morphological assessment, autoradiography — electron microscopy or biochemical techniques related to the above project.

Qualified candidates contact: Dr Ch V Rao, Dept of OB/GYN, 436B MDR Bldg, University of Louisville, Louisville, Ky 40292. (NW752)A

POSTDOCTORAL ASSOCIATE. Position available September 1981 or after. Postdoctoral Associate to work on rapid kinetics or hemoglobin and the study of protein conformation. Salary commensurate with experience. Send résumé to: Dr Quentin H Gibson, Section of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, New York 14853. An Equal Opportunity/Affirmative Action Employer. (NW754)A

RESEARCH ASSOCIATE

Position available immediately for PhD to work on biochemical and physiological mechanisms in the crystalline lens in relation to cataract development. Experience in membrane or protein biochemistry would be most advantageous. Send curriculum vitae to University of Colorado Health Sciences Center, 4200 E 9th Ave, Denver, Colorado 80262, USA. The University of Colorado is an equal opportunity employer. (NW750)A

UNIVERSITY OF DUNDEE Department of Biochemistry POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a Post-Doctoral Research Assistant to work on an SRC supported project investigating the structure of hydrogenase from E coli. The project involves protein chemical characterisation of the isolated and membrane bound enzyme and the cloning of its structural gene. Experience in membrane protein characterisation and/or recombinant DNA technology would be advantageous. The appointment is available from 1 October 1981 or as soon as possible thereafter and will be for a period of up to three years. Applications, accompanied by a curriculum vitae and the names of two referees should be submitted by August 6th 1981 to K. M. Cocker, Assistant Personnel Officer, The University, Dundee DD1 4HN, from whom further particulars are available. Please quote ref. EST/44/81J. (9099)A

Assistant Professor

Applicants are invited for a tenure track position at the rank of Assistant Professor. Candidates possessing an M.D. or Ph.D. several years of postdoctoral experience, and a strong commit-ment to developing an independent research program are sought. Applicants with research interests in neuropharmacology, toxicology, or molecular biology are preferred, but applications from outstanding candidates in any area of pharmacology are welcomed. Responsibilities include participation in departmental responsibilities for medical and graduate student training.

Inquiries, which should include a curriculum vitae, synopsis of current research interests, list of publications, reprints of selected publications, and the names of three references should be addressed to: Professor Cheng-Wen Wu, Chairman, Faculty Search Committee, Department of Pharmacological Sciences, School of Medicine, Health Sciences Center, SUNY Stony Brook, Stony Brook, NY 11794.

SUNY Stony Brook is an equal opportunity/affirmative action employer. AK#92A. (NW746)A

Instrumentation Specialist

University of Petroleum & Minerals Dhahran, Saudi Arabia

The University of Petroleum & Minerals, Dhahran, Saudi Arabia, is seeking for its chemistry department a full-time specialist to service the department's major instrumentation-Varian XL 200 ft NMR superconducting spectrometer, ENRAF Nonius CAD-4 automatic diffractometer, RIBERMAG GC-MS and emission spectrograph. New acquisitions will include EPR and ENDOR spectrometers.

Applicants should have a least a B.S. in electronics, or a B.S. degree in chemistry, physics or chemical engineering with training in electronics, and be knowledgeable in the operation and maintenance of computer-interfaced instrumentation. Experience with magnetic resonance is preferred. Responsibilities will include the maintenance, troubleshooting and repair of these instruments.

Minimum regular contract for two years, renewable. Competitive salaries and

allowances. Air-conditioned and furnished housing provided. Free air transportation to and from Dhahran each year. Attractive educational assistance grants for schoolage dependant children. All earned income without Saudi taxes. 10½ months duty each year with 45 days vacation with salary plus other fringe benefits as per policy.

Apply with complete résumé on academic, professional and personal data, together with photograph, list of references, copies of certificates, diplomas and degrees and home and office addresses and telephone numbers to:

Dean of Faculty and Personnel Affairs, University of Petroleum & Minerals, P.O. Box 144, Dhahran International Airport.

Dhahran International Airport Dhahran, Saudi Arabia.

(9109)A

Hoechst &



is planning to extend considerably its gene technology group in Frankfurt (Main), Germany involved in basic research with special regard to health care.

We are inviting applicants for

Molecular Biology

with several years experience in recombinant DNA research and nucleic acid biochemistry.

Salary will be competitive and in accordance with experience. Applicants should have basic knowledge in German language. Applications, including curriculum vitae (with a description of previous experience) should be sent to:

Hoechst Aktiengesellschaft, Personalabteilung T, Referat Naturwissenshaftler, Postfach 80 03 20. 6230 Frankfurt (Main) 80

(W380)A

SYNCHROTRON RADIATION RESEARCH MRC/SERC JOINT APPOINTMENT

The Medical Research Council and the Science and Engineering Research Council have agreed jointly to appoint a Post Doctoral Research Associate to work at the SERC Synchrotron Radiation Source (SRS) at the Daresbury Laboratory. This appointment provides a unique opportunity for the right man or woman to make an original contribution to an expanding field of research. The successful applicant would be expected to take responsibility for supporting the exploitation of the SRS in the field of biological and medical applications of small angle x-ray scattering and to participate in the research programme. Projects already approved include work on proteins, muscle and viruses, and the appointee would be expected to act as a focal point for these activities and to advise and assist other SR users in this area. An experimental station is being commissioned and will be available for some of this work but the appointee would be expected to take part in the design, construction and operation of further equipment. For this reason, the appointee should have a good understanding not only of the application of SR to biological problems but also of electronic methods of x-ray detection and data collection. The ability to work as a member of a team and to have an enthusiasm for the development of new techniques are essential qualities.

An appointment will be made at a salary between £5862 and £9015 per annum fixed according to age, qualifications and experience. The post will be available for a fixed term of three years and will be superannuable.

Closing date: 31st August 1981.

For further information please write to or telephone Warrington (0925) 65000 Dr. P. J. Duke (Ext. 460). Applications should be sent together with curriculum vitae and the names and addresses of two referees, quoting reference number DL/765/

The Personnel Officer

DARESBURY LABORATORY

Science & Engineering Research Council Daresbury, Warrington WA4 4AD.

(9108)A

DURHAM DEPARTMENT OF PHYSICS

UNIVERSITY OF

SENIOR RESEARCH ASSISTANT IN PHYSICS

Applications are invited from candidates of post-doctoral status with experience in the fields of solid state physics, applied physics or materials science for an appointment as Senior Research Assistant tenable for three years from 1 October 1981.

Initial salary on Range 1A £6,070 -£8,515 plus superannuation.

The appointment is a full-time research appointment and the successful candidate will be engaged in a programme of investigation on the magnetic properties of structural steels sponsored by British Gas with a substantial financial committment from them.

Applications (three copies) naming three referees should be sent by 31 July 1981 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE from whom further particulars may (9095)A be obtained.

OUEEN ELIZABETH COLLEGE Kensington (University of London)

LECTURER IN NUTRITION

Applications are invited for a Lectureship in Nutrition to take part in the teaching of undergraduates, MSc students and research students and to undertake research in an appropriate field. The Department of Nutrition has excellent facilities and has long been a centre of distinction in this field since it was established as the first teaching Department of Nutrition in Europe

In the present circumstances the appointment will be made initially for a three-year period with the possibility of permanency at a later date. Salary in the range £6,070 to £12,860 plus London allowance of £967 pa.

Application forms and further particulars from the College Secretary, (N) Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH. Tel: 01-937 5411 ext 209. Closing date: 7 August 1981. (9105)A

UNIVERSITY OF OSLO

ZOOLOGICAL INSTITUTE

POST DOCTORAL **POSITION**

Available immediately for a theoretically oriented population ecologist in a Scandinavian project on the population ecology of bark beetles. (Ips typographus). Experience in developing and analyzing simple differential — and difference equation models are essential. Good training in entomology is required.

The appointment will initially be for one year but with the possibility of extension to a total of three years. The appointee will work in close cooperation with empirists and other theoreticians working on Ips typographus (as well as other animal groups).

Applications, including a curriculum vitae and names of two referees should be sent as soon as possible to Dr Nils Chr. Stenseth, Zoological Institute, University of Oslo, PO Box 1050, Blindern, Oslo 3, Norway.

(W377)A

ST JUDE CHILDREN'S RESEARCH HOSPITAL

DIVISION OF **IMMUNOLOGY MOLECULAR**

> AND CELL **BIOLOGY**

Postdoctoral research traineeships are offered in a multidisciplinary research institute. (1) Biochemistry structure and functions of biologica membranes; biological regulatior mechanisms with emphasis or hormonal action; receptor functions and cellular messengers; and contro of normal and neoplastic growth (W Y Cheung, M Morrison and (W Y Cheung, M Morrison and G Schobaum); (2) Immunology: regulation of antibody formation macrophage functional heterogeneity and characterization of macrophage receptor functions and structures (F L Adler, M Fishmar and W S Walker); (3) Pharmacology: human and animal cancer chemotherapy; drug-induced changes ir DNA replication and repair synthesis; genetic, biochemical and physiological approaches to analysis of mechanisms of drug resistance in human cells (T P Brent, A Fridland and A Welch), and (4) Virology molecular biology and epidemiology of viruses; eukaryotic gene expression and regulation (A Granoff, D Kingsbury and R C

Applicants must be citizens of permanent residents of the US Please send curriculum vitae, name of three references and a brief state ment of career objectives to Dr N Fishman, Division of Immunology St Jude Children's Research Hospital, 332 N Lauderdale, PO Box 318, Memphis TN 38101.

(NW732)A

UNIVERSITY OF DURHAM

DEPARTMENT OF PHYSICS

Applications are invited for a POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIF IN PHYSICS

for two years, starting as soon a possible after 1st September 1981 to work on a collaborative project with the Max Planck Institute for Radio astronomy, Bonn on the interpretation of the continuum radie emission of the Galaxy. Candidate should preferably have experience is cosmic rays, radioastronomy of plasma astrophysics and an interes in computing.

Initial salary in the range £6,070 to £7,290 per annum on Range 1A plu superannuation.

Applications (3 copies) namin three referees should be sent by 3 August 1981 to the Registrar an Secretary, Science Laboratories South Road, Durham DH1 3LE. (9107)A

N I H E National Institute for Higher Education, Dublin

This Institute is the newest in Ireland's higher education system and is funded by the Irish Government through the Higher Education Authority as are the Universities. It places equal emphasis on its teaching research activities. Applications are invited for the following ossition in the School of Biological Sciences:

LECTURER IN MICROBIOLOGY

The School of Biological Sciences s at present making a major input o the Institute's degree course in Analytical Sciences. A number of additional biology based teaching programmes are currently being proposed. The School is engaged extensively in industry supported esearch activities and the successful applicant will be incouraged to contribute to these projects or to initiate independent esearch. The expansion of the nstitute will ensure that new staff nave the opportunity to contribute o the design and inception of new eaching and research pro-grammes. Candidates should be vell qualified academically and lave research interests in aspects if microbial biochemistry, physioligy, cell culture.

Salary Scale: Lecturer: IR£10,974 - IR£15,318; Assistant Lecturer: R£8,561 - IR£10,514.

application forms and further letails are available, on written aquest, from the Personnel Office, lational Institute for Higher ducation, Glasnevin, Dublin 9, reland. (9082)A

SUNDERLAND POLYTECHNIC

Faculty of Pharmaceutical Sciences DEPARTMENT OF

PHARMACOLOGY LECTURER II IN PHARMACOLOGY

(Temporary for one year ommencing 1st September, 1981) lary scale: Lecturer II £6,462 — 624 Bar £10,431 per annum.

The post will involve responsibility teaching students, both in lectures 1 practical classes, courses in ysiology and pharmacology to BSc armacy, BSc Pharmacology, and c Combined Studies in Sciences.

There are several active research ups within the department and the cessful candidate will be encourd to either participate in one of se groups or develop his/her own parch interest.

An application form and further ticulars may be obtained from the sonnel Officer, Sunderland Polynnic, Langham Tower, Ryhope ad, Sunderland SR2 7EE. Closing e31st July, 1981. (9072)A

Magnetic Materials Technologist

New Materials for Data Recording

IBM United Kingdom Laboratories Limited, based at Hursley near Winchester, are looking for a scientist, either male or female, with at least three years experience in the study of magnetic materials. You will work with a team developing materials and processes relevant to the fabrication of magnetic recording heads and disks.

The team, which is part of the Advanced Technology function, has significant interactions with other IBM Research and Development groups in Europe and the USA and the successful applicant would be expected to develop these contacts.

Applicants should hold a PhD or be able to demonstrate research experience, in one of the Physical Sciences and will have a sound theoretical and practical knowledge of magnetics technology. Familiarity with vacuum and plasma processing techniques would be advantageous.

As a Company making a substantial contribution to the country's economyover £600m in the last 10 years–1BM employs 15,000 men and women at over 40 locations throughout the UK. Some 4,000 work at our two manufacturing plants and another 1,500 here at the Hursley laboratory.

In addition to an attractive salary, a substantial employee benefits package is offered which includes a non-contributory pension scheme, free life assurance and BUPA membership. Relocation assistance will be provided where appropriate.

If you are interested in the above vacancy, please write for an application form to Lionel Hobbs, Personnel Officer, IBM United Kingdom Laboratories Limited, Hursley Park, Winchester, Hampshire SO21 2JN. Please quote ref: (N/92286)



(9114)A

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

Chemistry & Biochemistry Division

SCIENTIFIC OFFICER

A Chemist is required in the Chemistry & Biochemistry Division to join a team to study the naturally occuring potentially toxic compounds in vegetables. The aim of the research project is to identify and determine such toxic compounds in varieties of vegetables currently grown or being developed in the UK.

Qualifications: A first or upper second class Honours degree in Chemistry.

Salary: On a scale £4,809 — £6,480 (under review). Non-contributory superannuation scheme.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting reference 81/18.

Closing date: 7th August 1981. (9075)A

INSTITUTE OF CHILD HEALTH 30 Guilford Street,

London WC1N 1EH

DEPARTMENT OF HAEMATOLOGY AND ONCOLOGY

RESEARCH ASSISTANT

required immediately in Oncology Laboratory for continuation of 5 year research project on isolation of 5 year research project on isolation of recombinant genomic and cDNA clones for human α feto-protein (α FP) and albumen. The project is co-ordinated from the Institute with recombinant DNA work under the supervision of Dr Kay Davies and Professor Bob Williamson of the Department of Biochemistry, St. Mary's Hospital Medical School, W1

Pre-doctoral and post-doctoral applicants with suitable experience considered. Initial salary: pre-doctoral £6,252 pa; post-doctoral £7,037 pa (both inclusive of London Weighting). Appointment available until March 1983 with possibility of renewal.

For further information telephone Dr Jon Pritchard on 01-405 9200 Ext. 248 (9.30am — 5.30pm) or 01-771 6683 (8.00 — 10.00pm). (9094)A

PROFESSORSHIP in the Clinical Smell and Taste Research Center at the University of Pennsylvania. Established scientist working in olfactory neurophysiology in Physiology and/or Otorhinolaryngology Department. Based in VA Hospital. Preferably with an MD degree. Write to Dr James B Snow, Jr, Department of Otorhinolaryngology and Human Communication, 5th Floor Silverstein, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104 and provide three letters of reference. The University of Pennsylvania is an Equal Opportunity/Affirmative Aetion Employer.

UNIVERSITY OF CAMBRIDGE

DEPARTMENT OF METALLURGY AND MATERIALS SCIENCE

POSTDOCTORAL ASSISTANT

To work with Dr D J Fray in an investigation into novel cell designs and electrolytes for electro-winning from chloride solutions. The project is intended to make a significant contribution to energy saving. The appointment will be for three years at a commencing salary of £6,070 pa.

Applications, which should include the names and addresses of two referees, should be sent to The Secretary, Department of Metallurgy and Materials Science, Pembroke Street, Cambridge CB2 3QZ, not later than 1 August 1981. (9069)A

OF EDINBURGH

DEPARTMENT OF
GENETICS

POST DOCTORAL

RESEARCH FELLOW

A post is available for a post-doctoral research fellow (salary in the 1A range) from October 1st 1981 to April

15th 1984. The project involves

assessing the effect of teratogens on cells in primary cultures and corre-

lating the data with effects obtained

in vivo. The techniques used in this

work include developmental biology,

Synthetic Organic Chemistry Pharmaceutical Research

We would like to hear from Synthetic Organic Chemists who will complete their Ph.D. this year or who have up to three years academic or post doctoral industrial experience.

The successful candidates will be expected to play an important role in the design and synthesis of novel compounds for biological evaluation, and will work as members of a multi-disciplinary project team in close collaboration with biologists.

The company, which has a very large investment in research and development, has its modern research laboratories in Milton Keynes where housing for rent or purchase is available. The site is within easy reach of London and other major cities.

Salaries and conditions are of the high order to be expected from a world leader. Benefits include a contributory pension scheme with free life insurance, free membership of P.P.P., subsidised staff restaurant and generous allowance for relocation where necessary.

Please write or telephone for an application form to:

Adrian Forrest, Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

(9060)A

Hoechst



histology, cell culture, protein electrophoresis, use of radioactive tracers, etc. Training will be given as necessary but experience with high resolution electrophoresis or cell culture would be important.

The applicant would be associated with a team working on cellular and molecular problems of cell differentiation.

Applications with CV and the names of two referees to the Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL. Please quote Reference 5034, (9092)A

MEDICAL RESEARCH COUNCIL

ENVIRONMENTAL EPIDEMIOLOGY UNIT

RESEARCH ASSISTANT

The Environmental Epidemiology Unit of the Medical Research Council based at Southampton General Hospital has a vacancy for a Research Assistant. This is a new Unit whose main interest is determining environmental causes of disease in

The successful applicant will assist the Director with surveys mainly in the field of cancer. The work may include interviewing patients and travelling within England. A numerate graduate with at least one year's experience in survey work or an appropriate MSc will be preferred.

The appointment will be in the Research Assistant Grade 1B, for a period of three years and salary will be in the range £5,285 — £7,700, depending on age, qualifications and experience. The post will be superannuable under the Universities Superannuation Scheme.

Further particulars and application forms from the Secretary to Professor E D Acheson, MRC Unit in Environmental Epidemiology, South Lab/Path Block, Level F, Southampton General Hospital, Southampton. (Tel: Southampton 777222 Ext. 3984.) Closing date for applications August 14th. (9098)A

LOTHIAN HEALTH BOARD

METABOLIC UNIT

Western General Hospital, Edinburgh

Applications are invited for the appointment of a:

SENIOR GRADE BIOCHEMIST

in the above Unit which is concerned with the management of, and research into endocrine, metabolic and nutritional disorders. Previous relevant experience required. Suitable candidates may register for a higher degree and may take part in the MCB training programme run by the Departments of Clinical Chemistry. Salary on the scale £7,674—£9,921. Whitely Council conditions of service apply.

Further particulars may be obtained by contacting the Secretary, Metabolic Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Telephone 031-332 2525, extension 365.

Applications, which should be typewritten, giving particulars of age, qualifications and previous experience, together with the names, addresses and telephone numbers of two referees should be lodged with the Secretary, 11 Drumsheugh Gardens, Edinburgh as soon as possible, but not later than two weeks from the date of this advertisement. (9063)A

GUY'S HOSPITAL Basic Grade Physicist

Required to join the Department of Clinical Physics and Bioengineering. The person appointed will work primarily in non-ionising radiation aspects of Medical Physics inlcuding clinical instrumentation and physiological measurement. Applicants should have a good degree in physics or electronic engineering. Salary in the range of £5,873 to £7,637 (inclusive), depending on qualifications and experience.

Gratorex, Dept. of Clinical Physics and Bioengineering. Application forms from the Personnel Officer, Guy's Hospital, St Thomas Street, London SE1. Tel: 01-407 7600 Ext. 3471 quoting reference P/30. (9070)A

GENETIC resources conservation. A vacancy exists at the International Institute of Tropical Agriculture, Ibadan, Nigeria, for a scientist to co-ordinate the activities of the Institute's Genetic Resources Unit. The successful applicant must have a doctorate in the field of crop science, botany, or genetics and previous experience of crop breeding and/or genetic resources conservation. As well as the collection, maintenance, evaluation, documentation and preservation of large amounts of food legume and rice germplasm, the Unit Co-ordinator and his staff work closely with the Institute's and other crop improvement programs to promote the use of germplasm. The Unit runs an annual training course for African Technicians in many aspects of crop genetic resources conservation. Applications direct to: Director for Administration, International Institute of Agriculture, Oyo Road, PMB 5320, Ibadan, Nigeria. (9068)A

Commissioning Editor

Academic Press seeks a new Commissioning Editor with publishing experience and preferably with a background in or experience of physics, earth sciences, materials sciences or engineering.

Please write with full career information to:

Anthony Watkinson, Academic Press Inc. (London) Ltd., 24-28 Oval Road, London NW1 7DX (9112)A

KING'S COLLEGE London DEPARTMENT OF BIOPHYSICS POSTDOCTORAL

POSTDOCTORAL RESEARCH ASSISTANTS

- 1) Biochemist with experience in nucleic acid and protein chemistry or enzymology to study the mechanism of chromatin transcription in vitro. Post tenable for 2 years from 1 September 1981. Salary in the range £6,070 to £8,515 plus £967 London allowance. USS.
- 2) Cell Biologist or Biochemist with experience in tissue culture and recombinant DNA techniques to study the structure and expression of cloned genes in eukaryotic cells. Post tenable for 2 years from 1 September 1981. Salary in the range £6,070 to £9,335 plus £967 London allowance, USS.

Please reply as soon as possible, with a curriculum vitae and the names and addresses of two referees, to Dr H J Gould, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL. (9051)A

THE ROYAL VETERINARY COLLEGE University of London

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY POSTDOCTORAL VIROLOGIST/ IMMUNOLOGIST

A postdoctoral worker is required to assist in investigations of the pathogenesis of a herpesvirus-induced paresis in horses. The two-year, grant-aided project offers a salary in the range of £6,299 — £8,095, plus £967 London Allowance per annum. Entry point would be according to experience and qualifications. Further information about the work can be obtained from Dr N Edington.

Application form and further letails obtainable from Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU (Tel: 01-187 2898 Ext. 264). (9074)A

WASHINGTON STATE UNIVERSITY

FACULTY FOR INSTITUTE OF BIOLOGICAL CHEMISTRY

An outstanding scientist is being sought to fill a permanent faculty position in the Institute. This person will be expected to conduct a vigorous research program in biological chemistry relevant to agriculture and participate in graduate teaching. Salary and rank are open to negotiation. Review begins September 1, 1981.

Send nominations and applications, including a curriculum vitae, a short description of research goals and a list of three or more references to P E Kolattukudy, Director, Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164.

Washington State University is an equal opportunity/affirmative action employer. (NW735)A

OREGON STATE UNIVERSITY

SCHOOL OF OCEANOGRAPHY IGNEOUS/METAMORPHIC PETROLOGIST

Faculty position, 12-month, tenuretrack. Candidate expected to develop strong research program emphasizing the application of latest theoretical, experimental and geochemical methods to petrological problems, particularly those related to the formation of oceanic crust, islands, and/or volcanic arcs.

Rank is Assistant/Associate Professor. Salary: \$23,000-\$38,000 commensurate with experience.

Send résumé and names of three references by 1 September 1981 to: G Ross Heath, Dean, School of Oceanography, Oregon State University, Corvallis, Oregon 97331.

OSU is an Affirmative Action/ Equal Opportunity Employer. (NW755)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street/Gower Street, London WC1E 7HT

POSTDOCTORAL RESEARCH FELLOWS

Applications are invited for two posts in the Department of Medical Protooology for biochemical parasitologists to investigate: 1) Metabolism in nalaria parasites; 2) The mode of action of antiprotozoal drugs. Candidates hould have a strong background in biochemistry and experience in cultivation echniques, preferably with parasitic protozoa.

The posts are initially for one year, commencing October 1981 or earlier, 71th a possibility of renewal. Initial salary not less than £7,037 gross depending 10 qualifications and experience.

Applications, with *curriculum vitae* and the names and addresses of two eferees, should be sent as soon as possible to The Senior Assistant Secretary at 1e School. (9062)A



National Research Council Canada Conseil national de recherches Canada

RESEARCH OFFICER

The Canada Centre for Space Science of the National Research Council of Canada requires a Research Officer. As a Space Scientist in the Scientific Planning and Evaluation Group, the duties will include:

- Assisting in the evaluation and processing of proposals received by the CCSS for the rocket and balloon program, the satellite program and supporting ground-based experiments.
- Participating in the planning of scientific campaigns forming part of the above programs and in scientific working groups associated with the experiments.
- Perform studies pertaining to monitoring and evaluating the functioning of the above programs as and when required.
- Participating in CCSS national and international committees and working groups as required.
- Participating as an investigator (experimental or theoretical) in CCSS associated scientific programs.
- Performing other associated duties as required.

Preference will be given to candidates having a PhD in Physics with several years' experience in space science or equivalent research experience in space science.

Salary — Up to \$30,430 per annum depending on qualifications.

Apply in writing to the Employment Officer, National Research Council of Canada, Ottawa, Ontario, K1A 0R6. In reply, please quote CCSS-81-2-N.

(NW747)A

Canadä

UNIVERSITY OF ZÜRICH (Switzerland) FACULTY OF VETERINARY MEDICINE

invites applications for the position of

PROFESSOR in VETERINARY PHYSIOLOGY

Applicants should have a higher degree, a PhD or an equivalent qualification in veterinary physiology and teaching experience at university level. They are expected to perform and to direct experimental research, to teach physiology (in German) to undergraduate veterinary students and to be head of the institute of veterinary physiology.

Salary range: SFr. 99 300 to 128 200 (1SF_E = 0.5 US\$).

Applications including a curriculum vitae and a list of the applicant's published papers should be addressed to the Dekanat der Veterinärmedizinischen Fakultät der Universität Zürich, Winterthurerstr. 260, CH 8057 Zürich (Switzerland) to arrive no later than October 1, 1981.

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES DEPARTMENT OF DEVELOPMENTAL BIOLOGY RESEARCH FELLOW

The successful applicant will be expected to undertake research on the developmental biology of plants. The Department has special interests in plant hormones, tissue culture, and cell biology. Applications in all these areas will be considered but preference will be given for work on aspects of development utilising ultrastructural and/or immunological approaches. Applications should include a summary of research interests and proposals, together with the names and addresses of three referees.

Closing date: 11 September 1981.

DEPARTMENT OF POPULATION BIOLOGY RESEARCH FELLOW

The successful applicant will work in the fields of developmental molecular biology or genome structure. Current research is concerned with developmental aspects of the genome of *Drosophila* using cloning, sequencing and genetic techniques. The applicant would be expected to contribute to studies analysing developmental mutants or developmental facets of genome structure in *Drosophila* or mammals. The position will not be available until June 1982.

Closing date: 11 September 1981.

FACULTY OF SCIENCE DEPARTMENT OF PHYSICS LECTURER

The successful applicant will hold a PhD and should be prepared to teach undergraduate courses at all levels and will be required to participate in experimental research in the department using pulsed and c.w. dye lasers to study the interactions of their radiation with atomic systems. A person with a good theoretical background in quantum mechanics of such interactions would be preferred.

The appointee should be able to take up duty without delay.

Closing date: 30 September 1981.

Salary will be in accordance with qualifications and experience within the ranges: Lecturer \$A19,821 — \$A26,037 p.a.; Research Fellow \$A19,821 — \$A25,871 p.a. Current exchange rages are \$A = \$US1.14 = £Stg.0.57p.

Unless otherwise stated, appointment as Research Fellow will be for up to three years with possibility of extension to five years. Lecturer: four years in the first instance, with possibility, after review, of reappointment to retiring

Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation benefits are available for applicants eligible to contribute. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Registrar, P.O. Box 4, Canberra ACT 2600, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, before submitting applications

(9100)A

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)

CENTRE FOR HUMAN GENETICS

CYTOGENETICIST

There is a vacancy for a Cytogeneticist (Basic Grade) at the above Unit. The work is part of a Regional diagnostic service and will include tissue culture and the identification of chromosome abnormalities.

Applicants should have a relevant Science degree.

Salary Scale £5,346 per annum rising to a maximum of £7,110 per

Applicants wishing to visit the Laboratory or requiring further information should telephone Mr A.M. Potter at Sheffield (0742) 667333.

Applications, together with curriculum vitae and the names and addresses of two referees, should be sent to the District Personnel Officer, B Floor, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.

Closing date for applications: 24th July 1981.

(9067) A

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOW IN

BIOLOGICAL SCIENCES

This post in the Chloroplast Research Group is part of a SERC grant for the study of the role of light in controlling chloroplast development in higher plants, with specific reference to the involvement of phytochrome in the expression of nuclear genes encoding chloroplast proteins. Experience with phytochrome, radioimmunoassay or nucleic acid hybridization would be an advantage.

The post is tenable from 1st October 1981 on the Research Range IA scale: £6,070 to £10,575 pa.

Applications (no forms) should include a curriculum vitae, and the names of two referees to the Academic Registrar, University of Warwick, Coventry CV4 7AL and quote Ref No 48/A/81/0. Informal enquiries to Dr J Bennett, Department of Biological Sciences by 7th August, 1981. (9102)A

THE UNIVERSITY OF MANCHESTER FOETAL EPITHELIAL **PHYSIOLOGY**

Applications are invited for the post of

TEMPORARY LECTURER

held jointly between the Departments of Physiology and Child Health. Research will be on ion transfer across the placenta mainly in sheep or pigs. Teaching duties will be in the Department of Physiology. This is a new venture and the initial appointment will be for a term of three years. The possibility exists for the appointment to be made substantive if the joint appointment is a success. Initial salary range pa: £6,070 — £7,290.

Particulars and application forms (returnable by September 1st) from the Registrar, The University, Manchester M13 9PL. Quote ref. (9004)A 127/81/N.

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) DEPARTMENT OF MEDICAL BIOCHEMISTRY

POST-DOCTORAL RESEARCH OFFICER

Applications are invited for the above 2-year appointment to work with Dr A K Campbell and Dr G R A Hunt on the application of nuclear magnetic resonance spectroscopy to the study of cell injury. Starting salary up to £6,070 on the scales for University Research and Analogous staff.

Candidates should have completed the work for a PhD in chemistry, biochemistry or a related discipline. Previous experience of NMR is an advantage, but not essential.

Further particulars (quoting reference No C38/4/37) from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (tel no 0222/755944 ext 2296). Closing date for application 25th July, 1981 (9089)A

UNIVERSITY OF LIVERPOOL DEPARTMENTS OF

GENETICS AND BOTANY **TECHNICIAN** (GRADE 3)

to work on the genetic analysis of the bacterium Streptomyces. Candidates should have experience in microbiology, genetics or biochemistry, and an interest in microbial genetics.

Minimum qualifications ONC plus three years experience, but suitable for recent graduate.

Salary within range £4,672 -£5,473 pa.

Application forms can be obtained from the Registrar, The University PO Box 147, Liverpool L69 3BX Quote Ref. RV/850/N. (9080)A

STUDENTSHIPS

The Hatfield Polytechnic School of **Natural Sciences RESEARCH IN** GEOLOGY, CHEMISTRY, **CROP PROTECTION**

Applications are invited from appropriately qualified graduates for the following posts:

SRC CASE Studentship

Investigations of road-making materials in South-West England.

SRC CASE Studentship

Chemistry of cement forming compounds.

Postdoctoral Fellowship Research Assistantship

Both into novel methods of meta recovery from complex ores.

Research Studentship

Crop Protection.

Application forms and further details from the Staffing Office. The Hatfield Polytechnic, PO Box 109, College Lane, Hatfield, Herts (Hatfield 68100, ext. 309).

Please quote reference 414.

Closing date: 27th July, 1981 (9071)F

UNIVERSITY OF ABERDEEN DEPARTMENT OF CHEMISTRY

SRC CASE STUDENTSHIP Molecular Sieve Precursors

A three year grant, in association with Unilever Research, is availab for research into the mechanism formation of zeolite molecul-sieves. The holder will form part an active group working in the are and will gain experience of a varie of techniques.

The grant, which is subject to the usual SRC requirements (Briti nationality, first or upper secon class honours degree), carries a sm supplement above the usual SF

For further details, contact Dr I Dent Glasser, Department Chemistry, University of Aberdee Meston Walk, Old Aberdeen A 2UE, as soon as possible. (9090)F

THE OPEN UNIVERSITY

SRC Research **Studentships**

A SRC studentship is available from 1st October, 1981 for a student to undertake biological/biophysical research on regular tissues (muscle, cornea, possibly lens) using biochemical and electro-physiological methods, and light and X-ray diffraction, including high intensity synchrotron X-ray diffraction and neutron diffraction.

The work would be with the Biophysics Research Group (Chairman, Professor G. F. Elliott) of the Oxford Research Unit. Our laboratory is well equipped and based in a pleasant country mansion with wooded grounds on the outskirts of Oxford.

We are looking for a graduate with a first (or upper second) class degree in biological sciences, chemistry/biochemistry or in physics with some biological interests.

Please contact Professor Elliott, Dr Zehra Sayers or Dr Else Bartels by letter or telephone. (OXFRU, Foxcombe Hall, Boars Hill, Oxford; 0865 730731). Please quote Please quote reference (SO/3). (9084)F

THE UNIVERSITY **OF SHEFFIELD DEPARTMENT OF HUMAN METABOLISM AND CLINICAL BIOCHEMISTRY**

Applications are invited for a Postgraduate Research Studentship tenable from 1 October 1981. The successful applicant would work in one of the following areas: 1) Intracellular control mechanisms of pituitary hormone secretion; Neuroendocrine control in the hypothalmic-pituitary hormone secretion; 3) Mechanisms of inflammation and control of proteinase and prostaglandin production by human chondrocytes in relation to arthritis; 4). Vitamin D metabolism in man in health and disease; 5) Biochemical control of human blood platelet function. The Department has excellent facilities for laboratory and clinical work, with emphasis on endocrinology, connective tissue biology, cancer research and throm-bosis. Other topics are available.

Applications, including curriculum vitae and the names of two referees, should be sent to Professor R. G. G. Russell, Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX (tel: 0742 26484, Ext. 3037) as soon as possible. Quote Ref. R610/G. (9111)F

UNIVERSITY OF WARWICK

SERC/CASE STUDENTSHIP IN

BIOLOGICAL SCIENCES

Applications are invited from microbiologists or biochemists for a SERC/CASE Studentship in the Department of Biological Sciences. The project concerns the application of molecular biological techniques to the taxonomy of the C1 compound utilizing bacteria, particularly those of industrial importance.

The University supervisor will be Dr C S Dow and the co-operating body (Torry Research Station — National Collection of Industrial Bacteria) supervisor will be Dr D M Gibson.

Applicants should write direct to the Departmental Secretary, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL enclosing a curriculum vitae and the names of two referees by 7th August, 1981. (9101)F

UNIVERSITY OF **SOUTHAMPTON**

Clinical Pharmacology -Faculty of Medicine

TWO RESEARCH **STUDENTSHIPS**

leading to the degree of PhD are available to study: 1. The biochemical pharmacology of human lung mast cells, and; 2. Metabolism of drugs by the intestinal microflora. Applicants who have or expect to obtain a first or upper second honours degree in biochemistry or biochemical pharmacology should send a current c.v. and the names of two referees to Dr M K Church, Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO94XY as soon as possible, from whom further details may be obtained. (9096)F details may be obtained.

UNIVERSITY OF **BATH**

SCHOOL OF BIOLOGICAL SCIENCES SRC CASE **STUDENTSHIP**

Applications are invited from graduates with an upper second or first class degree in Plant Sciences or a related subject for a CASE Studentship to study the effects of virus infection on clonal variation in woody ornamentals.

The work will be done in collaboration with Long Ashton Research Station and the joint Horticultural Trades Association/National Farmers' Union Nursery Stock Committee.

Applicants should send curriculum vitae and the names and addresses of two referees to: Dr R G T Hicks, School of Biological Sciences, University of Bath, Claverton Down, Bath BA2 7AY, from whom further details may be obtained. (9066)F

CONFERENCES and COURSES

LOTHIAN REGIONAL COUNCIL NAPIER COLLEGE

DEGREE OF MASTER OF SCIENCE THE BIOLOGY OF **WATER MANAGEMENT** (CNAA)

A 12 months full-time course: 60% of course time taught; 40% of course time research.

Subjects: Natural Quality of Water Resources: Pollution Causes; Pollution Effects; **Pollution Control**

Entrance requirements: An honours degree in a biological subject or an equivalent qualification

Further information may be obtained from: Dr K J Anderson, Head of Department of Biological Sciences, Napier College, Colinton Road, Edinburgh EH 10 5DT (Tel: 031-447 7070)

(9065)C

CONFERENCES continued on page xxx

UNIVERSITY OF ABERDEEN

DEPARTMENT OF **AGRICULTURE**

Cytokinin production in nodulated legume cuttings

SRC CASE STUDENTSHIP

Applications are invited for a threeyear SRC Case Studentship to be held in the Department of Agricultural Biochemistry, University of Aberdeen in conjunction with Dr T L Wang of the John Innes Institute, Norwich. The project will involve studies on the legume-bacteria symbiosis and its influence on production cytokinin and metabolism. Approximately equal time will be spent in Aberdeen and in Norwich.

Applicants should have or expect to obtain a first or upper second class honours degree in biochemistry. chemistry or the biological sciences and an interest in plants.

Applications with the names of two referees should be sent as soon as possible to Dr T Stuchbury, Department of Agricultural Biochemistry, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD from whom further details (9091)F can be obtained.

FELLOWSHIPS

UNIVERSITY OF **ANTWERPEN** DEPARTMENT OF CHEMISTRY **POSTDOCTORAL**

FELLOWSHIP

available 1 October 1981 or as soon as possible thereafter in a laboratory specializing in analytical chemistry and applications of microanalysis (ion microscopy (SIMS), laser microprobe mass analysis and electron microprobe).

Candidates with experience in EMPA or SEM will be preferred. The position involves research with a new fully equipped Superprobe 733 on particle analysis for environmental applications. Appointment is for 2

Salary: 60,740-68,220 BF/month (before income tax).

Additional information: telephone Profs F Adams or R Gijbels, Belgium 31/28.25.28 ext. 213 or 214 or administration ext. 166.

Send resumé, statement of research interests and two references to University of Antwerpen, UIA, Director of the Personnel, Universiteitsplein 1, 2610 Wilrijk, Belgium. (W378)E

continued on page xxx

FELLOWSHIPS



THE HARTLEY FELLOWSHIPS of the UNIVERSITY OF SOUTHAMPTON

Applications are invited for three Fellowships normally tenable from 1 October 1982, for up to two years research, in any field for which facilities are available within the Faculties of Arts, Science, Engin-eering & Applied Science, Social Sciences, Law, Educational Studies, Medicine and Mathematical Studies

Fellows will normally be appointed from persons of high promise whose records show evidence of capacity for original research, and who have up to five years postdoctoral experience or equivalent research record.

Salary will be related to age and experience and be within nationally agreed ranges for research staff in universities. (No provision for travel or removal expenses).

The closing date for applications for the 1982 competition is 1 October

Details and application forms from Mr. E. D. Gordon, Secretary, Committee for Advanced Studies, Academic Registrar's Department The University, Southampton SO9 5NH. Please quote Ref. N. (9073)E

UNIVERSITY OF WARWICK

Compton Scattering Studies of Electron Density

A SRC supported two year

POSTDOCTORAL FELLOWSHIP is available in the Department of Physics, for gamma-ray Compton scattering studies of charge density in transition metals and their alloys. Most of the experiments will be carried out using the recently commissioned ²⁴¹Am Compton spectrometer at Warwick, but some of the experiments are scheduled for the high energy Compton spectrometer at the Rutherford SRC Laboratory. The successful applicant will play a major role in the planning, execution and computational analysis of the experiments, working with a graduate research assistant under the direction of Dr M J Cooper.

The appointment, to commence on or before 1st October 1981, will be made at the second or third point of the Research Range IA scale: (currently £6,475 — £6,880 pa respectively).

Informal enquiries to Dr Cooper, Department of Physics; application Department of Frigores, approached forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No. 47/A/81/0. Closing date for receipt of applications 7th August, 1981.

(9061)E

UNIVERSITY OF WARWICK

RESEARCH FELOWSHIP

IN

VIROLOGY/BIOCHEMISTRY/ **IMMUNOLOGY**

Our recent work suggests that antigenic modulation is responsible for the neutralization of influenza virus by antibody, since monoclonal anti-haemagglutinin antibody inhibits the activity of the internal virion transcriptase.

We are seeking applicants with a background in biochemistry/virology/ immunology to study the molecular aspects underlying this phenomenon, assisted by a research technician and as part of a large group working on related topics under the direction of Dr N J Dimmock.

The post is funded by the Medical Research Council for three years, on the Research Range IA scale: £6,070 to £10,575 pa. Informal enquiries to Dr N J Dimmock, Department of Biological Sciences.

Applications (no forms) giving details of age, qualifications and experience together with the names of two referees to the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref No: 48/2A/81/0 by 7th August, 1981. (9103)E

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY Babraham, Cambridge CB2 4AT

POST DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a post doctoral appointment financed for a period of up to 3 years by the National Research Development Corporation in one of the following fields:
i) Chemical carcinogemesis and cell transformation in vitro;

ii) Hybridoma production. Both areas will involve study of animal and/or human cells secreting hormones or antibodies.

Candidates should have a 1st or upper 2nd class honours degree and at least 2 years post-graduate experience in molecular biology and/or immunology and aquaintance with cell culture techniques.

Salary in Higher Scientific Officer scale (under review) £6,075 to £7,999 Starting pay according to experience non contributory pension scheme.

This post can be discussed in more detail with Dr W Mason on Cambridge (0223) 832312. Application forms from the Secretary of the Institute quoting reference NRDC1. Closing date: (9106)E 31.7.81.

EMBO

European Molecular Biology Organisation LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1981 AWARDS

Next deadline: August 15, 1981

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are awarded initially for one year, but subject to review of progress by the selection committee, they are usually renewed for a second year. In cases of exceptional scientific merit renewal for a third year is possible. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is August 15, 1981. Successful candidates will be notifed of their awards immediately after the meeting of the selection committee which is on October 23, 1981.

Application forms and further details may be obtained from Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.

(W370)E

UNIVERSITY OF **ABERDEEN**

DEPARTMENT OF MICROBIOLOGY **POSTDOCTORAL** RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral Research Fellowship to join a group led by Professor W A Hamilton working on the fouling and corrosion of steel structures in the North Sea. This is a joint project with the School of Mechanical and Offshore Engineering at Robert Gordon's Institute of Technology and is financed by the SERC Marine

Appointment will be from 1st October 1981 and will be for one year in the first instance.

Technology Directorate.

Salary within Range 1A £6,070 — £10,575 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by Friday 7 August (9083)E

UNIVERSITY OF SOUTHAMPTON CHEMISTRY DEPARTMENT **POSTDOCTORAL** RESEARCH FELLOWSHIP IN ORGANIC ELECTROCHEMISTRY

Fellowships are available in Synthetic Organic Electrochemistry. One position will require extensive industrial collaboration. Salary on 1A scale, minimum starting point £6,880 pa, plus superannuation.

Applicants, having experience in electrochemistry or synthetic organic chemistry should send CV and the names of two reverees to Dr J M Mellor, Department of Chemistry, University of Southampton, High-field. Southampton SO9 5NH. field, Southampton SO9 Please quote Ref. N. (90 (9059)E

MEDICAL COLLEGE OF ST. BARTHOLOMEW'S **HOSPITAL**

University of London **BIOCHEMISTRY DEPARTMENT** POSTDOCTORAL

RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral research fellowship sup-ported by the British Diabetic Association to work with Dr J B Clark on the role of insulin in the regulation of energy and neurotransmitter metabolism at the nerve ending (synaptosome). The post is available for 3

Applications, with a curriculum vitae and names of two referees, should be sent as soon as possible to Dr J B Clark, Department of Biochemistry, Medical College of St. Bartholomew's Hospital, Charter-house Square, London EC1M 6BQ. (Tel: 01-253 0661, Ext. 22). (9079)E

years starting immediately at a salary

of £6,475 pa + £967 pa LW.

CONFERENCES and **COURSES**

continued from page xxix

INTERNATIONAL DIOXIN CONFERENCE

Washington, D.C. 25-29 October 1981 Information: Box 209, Rockville, MD 20850. Tel. 301: 468-2500 × 409. (NW756)C

Please mention

nature

when replying to these advertisements

Definitive, Complete, Authoritative

NATURE announces the publication in 1981 of the Annual Directory of Biologicals and Disposable Chemicals. The directory will be available for distribution to all NATURE subscribers in the Autumn of 1981. It will also be marketed separately, offering an even larger distribution.

A New Buyers guide

The basic thrust of the directory will be to provide NATURE subscribers with a definitive reference catalogue from which they may purchase the chemicals and disposables listed.

From research conducted around the world the NATURE marketing staff has determined the need for a directory of biologicals, disposable chemicals and gases. Probably more than any other scientist, the NATURE reader represents the largest potential market for the sale of these chemicals.

Every attempt will be

made to produce a catalogue that will reference the worldwide market of chemical manufacturers, distributors, suppliers, and exporters.

No instrumentation or apparatus listings will be included in this directory; its sole purpose is to provide detailed information for our readers as to where they may secure the biologicals, chemicals and gases for use in their

laboratories.
The directory will be cross-referenced and include chemical classifications, product classifications, geographical location of suppliers and other referenced information as needed.

NATURE Directory of Biologicals



What's in It?

The directory will include (but not be limited to) these biological reseach chemicals

- immunochemicals
 - cell lines
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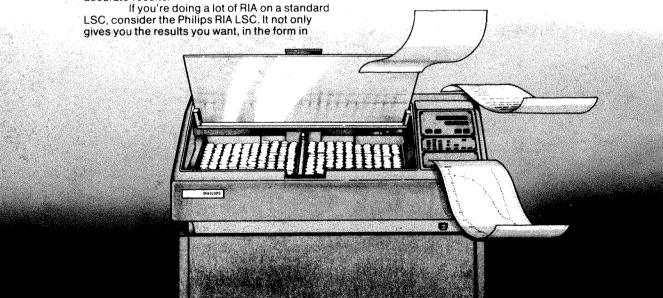
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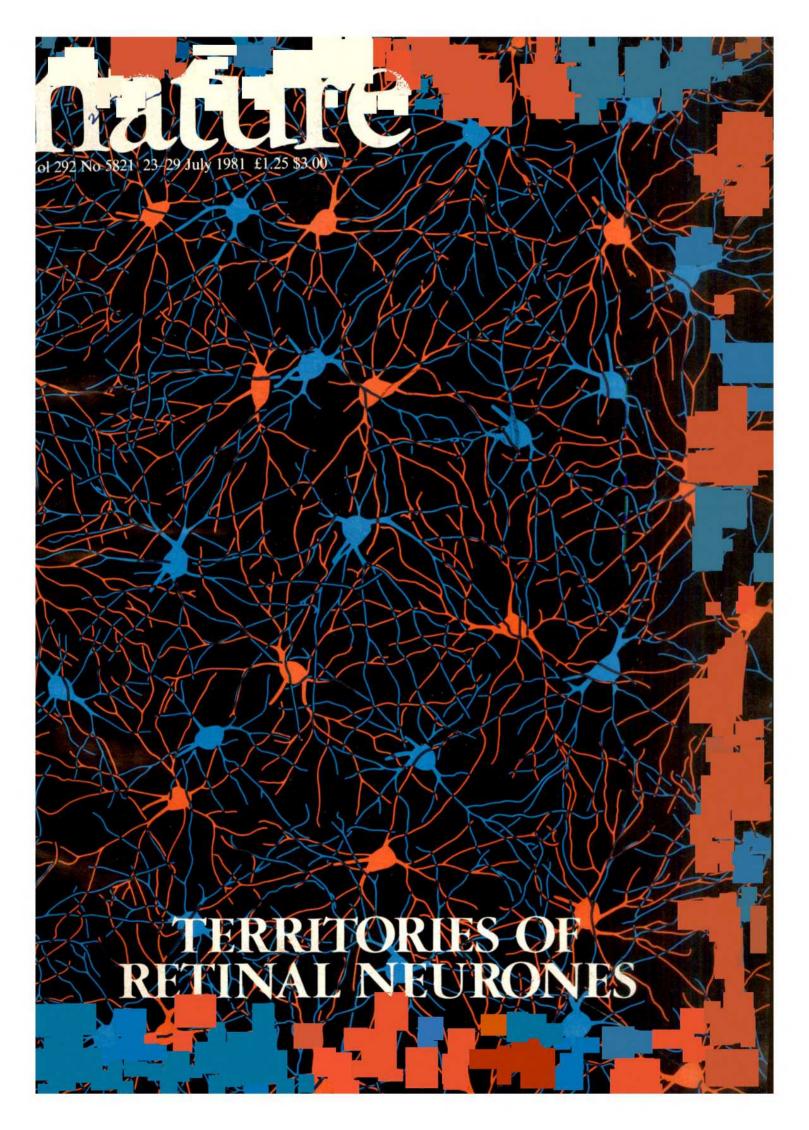
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Figure 2.5 '(3²P)-tRNAPhe (0.5 μg; 2×10⁵ DPM/μg) in reaction buffer (5μ]; 25 mM sodium citrate pH 5.0, 6.5 M urea, 0.05% XC, and BPB) was digested with BRL* RNAse TI (2 units—track A; 0 units—track B) for 15 minutes at 55°C. The reaction mixture was then heated to 90°C for one minute, chilled on ice, and analyzed in a 10% and analyzed in a 10% acrylamide gel as des-cribed in Figure 1. Noted above are cleavage posi-tions from the 5' end of **tRNAphe**

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electrophoresis (at 50-60°C) the gel was exposed to X-ray

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Figure 1. Purified 5' [32P] -IRNA (0.1 μ g; 1 \times 106 DPM/ μ g) was incubated a second

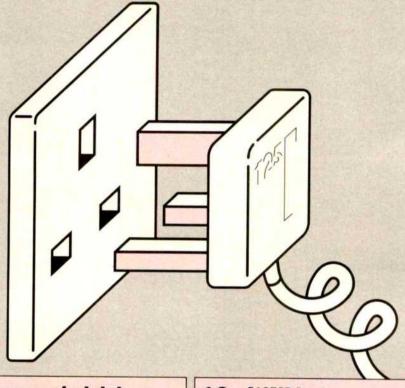
time in the presence (track B)

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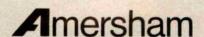
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(1) HOCHBERG, R.B. Science, **205**, pp.1138-1140, 1979

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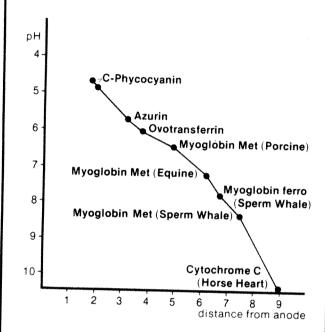
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Only self-help for British universities

The Committee of Vice-Chancellors and Principals is right to have lobbied the British government last week with its argument that a slower contraction of the university system would help not merely the universities but the government itself (see page 281). But nobody should be surprised if the government chooses not to respond. For ever since it was announced nearly a year ago that the allocation of public funds to the University Grants Committee would be reduced over three years by 8.5 per cent, it has been clear that the agenda of the dispute between the government and the universities is partly a hidden agenda. The government is behaving as if large parts of the university system are effete, and that the time has come to teach them a lesson. In public, however, the government merely says that higher education must share in the sacrifices expected, at times of economic decline, by other publicly supported institutions. It would be better for all concerned if the hidden agenda were now made public. The plight of the universities is worsened because the British government's secret opinion of their usefulness is broadly based. The universities have long since lost the wholehearted friendship of the major political parties. Student rebellions in the 1960s, although modest by international standards, are well remembered. The rash of new undergraduate courses that came in at the same time helped to undermine the reputation of the university system as a whole. That many academics and institutions stood out against these excesses is forgotten, unjustly but understandably. And even though some of the surviving industries in Britain which owe their chances of future prosperity to graduates from the universities are quick to acknowledge their dependence, the public impression of the utility of the system is more accurately shaped by the numbers of graduates in sociology and related fields who leave their graduation ceremonies to join

The issue of academic tenure, the substance of the vicechancellors' complaint last week, is more contentious than the universities appear to understand. When the number of people unemployed in Britain is certain to increase above three million, it is hard to see emerging a wave of public sympathy for academics who lose their jobs. Indeed, the system of academic tenure is widely resented, even though it may be an essential assurance of freedom in scholarship - and, technically, required by the statutes under which many universities are established. Why should academics have a guarantee of lifelong employment when other people are being thrown out of work in droves? Against this public view, it would require a Hercules among ministers to persuade his colleagues in the British government to provide anything like a fund of £250 million to help academics see in the sunset years. Mr Mark Carlisle, Secretary of State for Education, is unlikely to fight for the vice-chancellors' case as they would wish. Such a fight would in any case expose him to the charge of maladministration — the muddle now emerging is, after all, one that he and his officials should have anticipated. Thus the most likely outcome of last week's meeting is that, for the time being, the government will let the universities stew in their own juice. If, in due course, the cost of academic redundancy threatens to loom large but the general level of unemployment has not declined, the government could think of overriding the provisions of tenure in academic contracts by means of legislation. As things are, the universities are virtually powerless. They have no choice but to seek out ways in which they can help themselves.

The first need is of self-help in the impending crisis. If the vice-chancellors' estimate of the number of academics likely to be

thrown out of work is anything like correct, the chances are that many people with a potentially important contribution still to make to scholarship and teaching will find themselves on a labour market in which their skills are not appreciated. Is it beyond the wit of the universities collectively at least to set up machinery for making sure that such displaced academics are not needed elsewhere in the system? And since all three parties to such an arrangement — the displaced academic and the two institutions between which he might be shuttled - stand to benefit, would not a fund to which all universities contribute help to smoothe the transfer of people between institutions? It is disappointing that the vice-chancellors seem so quickly to have abandoned hope that such schemes can function. It is also disappointing that they have not yet formally explored with the unions more radical departures from present arrangements — the replacement of annual by tenmonth contracts, for example, or the feasibility of a negotiated reduction of salaries, at least in the institutions most directly threatened. The sheer rigidity of the present arrangements, under which academic (and other) university salaries are nationally uniform and tightly linked with age is in itself one of the causes of present troubles. In present circumstances, there is every chance that a majority of university teachers would prefer flexibility and even hardship to the collapse of the institutions to which they belong. That chance should be explored, and quickly

Another immediate need is that universities should work out ways of sharing resources with each other. The University Grants Committee has pointed to some of the more obvious possibilities the pooling of equipment or the merging of departments between universities that happen to be close to one another. Other benefits could flow from joint appointments, the designation of some departments in some universities as places whose research equipment is regarded as a freely accessible pool. More important, in the contracting system there should be ways of pooling students, graduate students in particular. Some of the universities that survive the years ahead will be more lopsided than they should be. Some areas of study will have disappeared. Some departments, even whole universities, may find it necessary to give up in-house research. So the British university system, traditionally too indifferent to the need that students should have some freedom to explore different fields of study, needs to find ways of helping students as well as teachers move. And because it would be intolerable that students' chances of becoming graduate students should be determined by the chance that the university to which they happen to belong can keep its graduate programmes in being, there is an urgent need of national arrangements for placing bright people. This is a problem for the research councils.

It is not, however, too soon to ask what plans should be laid against the period after 1984-85, when last month's cuts have taken their full effect. For the causes of the present crisis will still then persist. Lacking coherence as a system and the capacity internally to be decisive even on academic policy, the university system of the late 1980s will be just as vulnerable as it has proved to be in the past few weeks. The most urgent need is that the system of public and private support for university education should be arranged in such a way that individual universities have a more explicit incentive to provide a valued service for their students and the communities in which they are embedded. Universities that are popular with students for good academic reasons should be helped financially — and not penalized (as the University Grants Committee is now threatening) if they attract more than their quota of home students. Good research, even as

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crudely measured by the volume of successful research grant applications, should qualify for a bonus.

Breaking with tradition, the University Grants Committee should also make explicit the basis on which its allocations of funds are made, so that universities know how most successfully to compete for what funds there are. And something should be done to clarify, simplify and ideally cheapen the present cost to public funds of supporting students in universities. One of the causes of the government's discontent, and the immediate cause of the restriction on student numbers now announced, is the cost of paying student fees and maintenance grants, using local authorities as paying agents. These arrangements, dating back to the days when students were considered to be the salt of the earth, are certainly not the most economical ways of helping to encourage the spread of university education. The anomalies which they include are often inequitable as between one student and another. One of their consequences is that British universities are chronically short of student accommodation. The arrangements should be re-examined, whatever noise the National Union of Students makes.

But why should the universities take the initiative in helping the government to save money, when the government has been so beastly? That is a hollow argument. The objective is not economy but independence. For all its faults, the British university system is a remarkable and valuable institution. The most immediate danger is that it will be so demoralized by the three painful years ahead that its capacity for self-renewal will be permanently impaired, as will be its capacity to persuade those who must support it that even its present role, even in present circumstances, is indispensable. The most cruel irony is that the British university system has been so mindlessly attacked when it is more than ever apparent that Britain lacks many of the skills that the universities could cultivate.

Clinch River runs again

During President Carter's tenure at the White House, the fast reactor site at Clinch River in Tennessee seemed destined to become a special kind of white elephant — a collection of unused equipment surrounding a half-begun concrete structure that would for all time be a monument to the fancied folly of the 1960s that it would be possible economically to win power from a nuclear reactor making more fuel than it consumed. Now, the prospects are changing. Against expectations and the recent voting record of the key committees in the House of Representatives, Congress appears to be ready to see work at the reactor site begin again (see Nature, 16 July). President Reagan, having spoken fondly of Clinch River during his election, dutifully included in his budget in March a request to Congress for funds to start building again at Clinch River. But the issue has never seemed important enough to the Administration to justify the risks of a serious fight. In the end, local interests in Congress have given the Administration what it was asking for without blood being spilled. The embarrassment, for President Reagan, is that he must now ask the Department of Energy to brush up its policies in other parts of the domestic nuclear energy programme.

Although the completion of Clinch River has for some time seemed to be an acid test of the willingness of the United States to commit itself to fast reactor technology, appearances are false. The Clinch River design is more than a decade old; much has since been learned, especially in France, about the design and operation of fast reactors. The escalation of the cost of the project, by a factor of three or thereabouts, is a measure not so much of the optimism of its original designers as of the need for changes of design after construction had begun. The enforced four-year delay will not have helped. Clinch River may yet be a white elephant of a different kind, a representation of an earlier technology. Even so, it will provide some practical experience of the operation of nuclear reactors cooled by liquid metal (sodium). And it will be another spur to the continuing wrangle in the United States about the rights and wrongs of nuclear power.

Technically, Clinch River is a demonstration plant, intended to demonstrate to utilities and their customers that electricity can be produced safely from fast reactors and that, in due course, fast reactors could be built economically. The demonstration would have been more helpful if the design had been less out of date. But in the assessment the utilities will eventually have to make, one crucial piece of information will be missing. The economic case for breeder reactors of any kind (which need not be fast reactors) is that they can use plutonium as fuel. When President Carter took fright at the prospect of the "plutonium economy", the extraction of plutonium from spent fuel rods taken from existing reactors was suspended. The Administration's new and sensible policy on the proliferation of nuclear weapons (see page 281) will help to liberate the Department of Energy from the traditions of the recent past, but there is a lot to be done before the economics of the plutonium fuel cycle can be tested. Completing Clinch River will, however, be a nonsense if that is not quickly done.

The wider implications of Clinch River in the nuclear debate are not at this stage easy to foresee. The most serious argument in recent years against breeder reactors has been economic; the price of uranium has risen so much less quickly than that of fossil fuels, and the cost of electricity is so much less dependent on the cost of fuel in nuclear reactors than in fossil power plants, that the potential advantages of breeder reactors (fuel at no cost at all) are not worth striving for. So why not put the huge cost of development into more imaginative exploits? The argument is respectable, and deserves attention. Its flaw is its assumption that the relative prices of uranium and fossil fuel will remain what they are at present for two or three decades ahead. The snag, of course, is that the present price of uranium (about \$50 for a pound of uranium oxide) reflects the decline in the rate at which utilities have been ordering conventional nuclear plants in the past few years. If, as memories of Three Mile Island fade, and the lessons of that accident are properly learned, the utilities turn again to nuclear power as a source of electricity, the price of uranium would shoot up. Is it not, in such circumstances, prudent that there should be data about the operation of some breeder reactor that would allow sensible decisions to be made?

Less cogent arguments have been made by those who say that fast reactors are inherently less safe even than conventional nuclear plants. Anxiety of this kind stems from the observation that breeder reactors contain substantial amounts of fissile material, and that the neutrons involved in the nuclear chain reaction are fast and therefore, on the face of things, likely to complicate the control of fluctuations of reactor output. Neither argument is as persuasive as it seems, however. Weapons designers are always saying that their most serious problem is that of arranging that fissile material can be coaxed into some configuration that will explode, so that the risk that such a configuration will turn up by accident in a breeder core is likely to be small; certainly the risky configurations can with ingenuity be identified in advance; and steps can be taken to avoid them or to prevent the damage they would do. And anxiety about neutron speed should be offset by the relatively low thermal inertia of fuel elements in fast reactors and the high thermal capacity of the molten sodium. None of this suggests that there are no risks in completing Clinch River but merely that theoretical safety studies have probably now gone as far as they can.

The third foundation of the case against Clinch River, of which much will be heard in the weeks ahead, is that all nuclear reactors are an abomination and that fast breeders are a particular abomination because one of their products is plutonium, sometimes described as the "most poisonous substance on earth" and sometimes feared because it might be used for making weapons clandestinely. The arguments presuppose that neither the safety of nuclear plants nor the security of the material they produce can be held within reasonable limits. Yet even the colourful experience of Three Mile Island, essentially a demonstration that it is possible so seriously to damage a nuclear plant that it is virtually useless without scattering dangerous doses of radioactivity in the neighbourhood, cannot be used unambiguously to support the case against Clinch River and what might follow.

US revamps nuclear proliferation policy

Reagan's new plan: reactors for friends

Washington

President Reagan announced last week that he intends to shift the balance of US strategy back to a political rather than a technical approach for restricting the proliferation of nuclear weapons.

In contrast to President Carter, who sought non-proliferation objectives by threatening to withhold access to US nuclear materials and technology, Mr Reagan intends to emphasize "working to improve regional and global stability" and to use the influence which would be generated by the United States' role as a "predictable and reliable partner" in nuclear development.

He proposes to distinguish between different countries in the way in which non-proliferation policies are applied, a move which will please European and Japanese allies but could create problems elsewhere. Mr Reagan also said that his Administration "would not inhibit or set back civil reprocessing and breeder reactor development abroad"; but he added that this condition applied to "nations with advanced nuclear power programs where it does not constitute a proliferation risk".

This was a more moderate statement than some which the President made during his election campaign. The shift seems to have been due not only to a greater awareness of the complexity of non-proliferation, but also to the implications of the Israeli raid on the Iraqi research reactor, which Mr Reagan referred to obliquely in his statement when he talked of the urgency being "highlighted by the ominous events in the Middle East".

But the statement still gives critics of the Administration plenty to chew on. For example, the President announced that he intended strongly to support the efforts of the International Atomic Energy Agency (IAEA) in Vienna to provide an improved international safeguards regime. In particular, he said that the Administration would seek agreement on requiring IAEA safeguards on all nuclear activities in a nonnuclear-weapon state as a condition for any significant new nuclear supply commitment.

Doubts about the efficacy of IAEA safeguards procedures, however, linger on — particularly in the light of evidence provided to the Senate Foreign Relations Committee last month by ex-IAEA inspector Mr Roger Richter.

The Department of Defense, at least,

shares these doubts. In an inter-agency report submitted to the National Security Council, some of which was leaked to the Wall Street Journal last week, the Pentagon expressed doubts about the "weakness of the IAEA as an international institution", complaining of the agency's "lack of an intelligence capability and the limits of its scope and jurisdiction", and warned against "undue reliance on the IAEA by those responsible for national security". Administration officials insist, however, that international efforts to bolster the IAEA, including efforts to develop effective regimes for international plutonium storage and improved cooperation for spent fuel management, must remain central to any global non-proliferation strategy.

One significant deviation from Mr Carter's approach was Mr Reagan's announcement that his Administration intended to use the supply of conventional arms to allies as a way of reducing the motivation to acquire nuclear weapons. This argument has already been used to justify the offer of a five-year military aid package to Pakistan

Mr Reagan also announced that the White House would seek to persuade the Senate to ratify Protocol I of the Treaty for the Prohibition of Nuclear Weapons in Latin America, the so-called Treaty of Tlatelolco. This protocol, already ratified by the United Kingdom and the Netherlands, calls on nations outside the treaty zone to apply the "denuclearization" provision of the treaty to their territories in the zone.

The goal of the Administration's new policy, Mr Reagan said, was "to reestablish a leadership role for the United States in international nuclear affairs". He gave few clues, however, as to how the United States would punish countries which transgressed its guidelines, stating merely that his Administration would view a material violation of the Non-Proliferation Treaty and the Treaty of Tlatelolco as having "profound consequences for international order and United States bilateral relations". Similarly he said that the United States would "view any nuclear explosion by a non-nuclear-weapon state with grave **David Dickson** concern".

UK universities complain to government

A last-minute attempt to persuade the British government to change its mind about the scale on which university budgets are to be reduced was undertaken last week by the Committee of Vice-Chancellors and Principals. A delegation led by Dr Albert Sloman, chairman of the committee and vice-chancellor of the University of Essex, urged on the Secretary of State for Education, Mr Mark Carlisle, that it would cost less to arrange for a slower contraction of the system than to foot the bill for buying out the tenure contracts of academics now likely to lose their jobs. Dr Sloman said that he and his colleagues had been encouraged to hear Mr Carlisle say that he "now understood the problem".

The vice-chancellors' case is simple. University budgets will fall in the next three years by about 15 per cent because of reduced public subventions, the further loss of students from overseas and because the numbers of domestic students are to be reduced. The result is that university staffs will also have to be reduced - the committee's estimate is that 3,000 academics and 4,000 others will have to leave British universities. The direct saving to the government in the next three years will be £400 million, but the cost of buying out tenured contracts could well be £250 million. So the government would save money if the pace of contraction were 2.5 per cent a year, not twice as much.

The estimate of the cost of breaking contracts stems from legal advice the committee has been taking in the past few weeks. Where an academic's contract with

a university assures employment until retiring age (65), premature firing would expose the university concerned to a civil suit. Compensation for breach of contract might range from £40,000 to more than £100,000, according to age, salary and the courts' estimate that the academic concerned would find another job. One complication to come to light is that only 80 per cent of British university teachers are covered by such cast-iron contracts.

Dr Sloman and his colleagues wrung their hands last week over the prospect of endless unseemly legal battles with former academic colleagues. Court cases could take three years to settle. Although technically individual universities would be liable for damages, the vice-chancellors are counting on the government to implement a promise by one of its ministers that no institution would be driven into bankruptcy.

The prospect of mutual assistance within the university system seems, however, to be fading. The pooling of financial reserves (now estimated at less than £75 million) is unlikely to be appealing to the bestendowed universities, usually dealt with leniently in last month's cuts. Schemes for finding jobs for displaced academics at other institutions are likely to founder, according to Dr Sloman, on people's likely calculation that a court settlement would be more advantageous than a job elsewhere. Reducing salaries, or switching to 10-month contracts, would require negotiations with the trade unions for which there is no time.

The actual scale on which redundancies will occur remains to be determined. The committee's estimate of 3,000 academic redundancies is identical with that given by Dr Edward Parkes, chairman of the University Grants Committee, to a parliamentary committee earlier this year. Ministers at the Department of Education and Science are plainly sceptical about such large estimates. But Lord Flowers, vice-chairman of the committee and rector of Imperial College, said last week that not a single university institution in Britain would be able to avoid some redundancies before the next academic year is out.

The committee's calculations also suggest that the consequences of the decreed reduction of student numbers will be more serious than had been foreseen. Total numbers of home students are to be reduced from a peak of 272,000 (expected in the coming academic year) to 249,000 in 1984-85. This, the argument goes, will require that student intakes should be reduced from 78,000 (in the coming year) to 70,000 in 1982-83 and thereafter.

Salford University

Industry advertises

British industry has come to the aid of the University of Salford, the institution singled out by the University Grants Committee (UGC) earlier this month for the largest cut in grant and student numbers. Earlier this week, three leading manufacturing companies joined with the university in announcing the creation of the Campaign to Promote the University of Salford — CAMPUS. To launch the campaign, nearly 200 companies contributed to the cost of an advertisement in three national newspapers yesterday extolling Salford's virtues and calling for further support.

The scale and spontaneity of the support from industry suggests that UGC in deciding where the cuts should fall, may indeed have paid too much attention to grants awarded to universities by research councils and not enough to research funded by industry. The universities that are to suffer the largest cuts in their incomes, Bradford, Aston and Salford, are chiefly technological institutions, noted for their attempts to forge links with local industries. The UGC has told Salford that by 1983-84 its grant will be 44 per cent less than in 1980-81 and that it will have to cut student numbers by about 30 per cent. Although the university's arts faculties will suffer most, science and technology too will be cut drastically.

GEC-Marconi Electronics, Ferranti and Ward & Goldstone Ltd of Salford are the three companies that have taken the initiative in setting up CAMPUS. They say that the University of Salford has provided them with a steady stream of appropriately qualified graduates — GEC has employed 250 in the past seven years — has fostered

successful technology-transfer between university and industry and provides useful refresher courses for experienced technologists and managers. The local authority has also given support, especially to a new science park which is being established near the university campus in an attempt to revitalize manufacturing industry in the north-west of England.

The campaign has two aims: in the short term to convince UGC of the error of its decision and to persuade it to reinstate some of the grant it is taking from the university; and in the long term to foster closer cooperation with industry, in the running of the university. The campaign. however, is not a fund-raising exercise. according to Professor John Ashworth. vice-chancellor designate of the university. Professor Ashworth, who is in the embarrassing position of being chief scientist at the Central Policy Review Staff. the government's think tank, until he takes up the vice-chancellorship in September, hopes that, if the campaign's short-term aim fails, he will be able to call on the industrial supporters of CAMPUS for advice in restructuring the university.

Precisely what action CAMPUS takes in persuading the government and UGC of Salford's merits remains to be seen and will to some extent depend on the response to this week's newspaper advertisements. The other aggrieved technological universities are also being invited to join in the battle.

Judy Redfearn

Carcinogen regulations

Cleansing solution

Washington

Tension between the scientific staff of the US Department of Labor's Occupational Safety and Health Administration (OSHA) and their new political bosses came to a head last week, stimulated by complaints from the European-based International Agency for Research on Cancer (IARC) about the interpretation of data on the carcinogenicity of formaldehyde.

The dispute arose from a letter written by Dr Peter Infante, head of OSHA's Office of Carcinogen Identification and Classification, to Dr John Higginson, director of IARC, which queried the findings of an IARC working group that there were only "limited" data providing evidence of the carcinogenicity of formaldehyde on laboratory animals. Also included was a circular published last year by the National Institute of Occupational Safety and Health (NIOSH) describing formaldehyde as a carcinogen on the basis of various uncompleted studies with rats.

Dr Higginson complained that Dr Infante's letter appeared to "cast aspersions on the scientific integrity and objectivity" of the members of the IARC working group. And shortly afterwards Dr Infante was told by OSHA that he was

being fired for "insubordination" in sending his letter on official notepaper giving what the agency now describes as a purely personal opinion, as it is no longer prepared to endorse the conclusion that formaldehyde is, in fact, a carcinogen, and has withdrawn its co-sponsorship from the NIOSH bulletin.

Dr Infante's case is being seen as a symbol of the new Administration's determination to limit the powers of an agency previously accused of imposing a massive burden of regulation on the private sector. Already the director of NIOSH, Dr Anthony Robbins, has been dismissed by Mr Richard Schweiker, Secretary of Health and Human Services, as a "political activitist", and the Administration has also announced its intention to move NIOSH administrators out of Washington, a step which many agency officials feel is designed to weaken their effectiveness. Last Thursday the head of OSHA, Mr Thorne Auchter, was confronted at an angry hearing by Congressman Albert Gore, chairman of the House Science and Technology Committee's subcommittee on oversight and investigation, who described Dr Infante's firing as unacceptable political pressure on the work of scientists.

On the previous day, several highranking government scientists, including Dr Vincent DeVita, director of the National Cancer Institute, had agreed that data produced last year by the Chemical Industry Institute for Toxicology provided a "sound scientific basis" for suspecting that formaldehyde was carcinogenic in humans.

At the Thursday meeting Mr Gore produced a memorandum, written to Mr Auchter by his special assistant for regulatory affairs, describing the latter's meeting with representatives of the formal-dehyde manufacturers. At the meeting doubts were raised about the data on which the previous administration had based their description of formaldehyde as a carcinogen, doubts which Mr Gore suggested formed the basis of the decision by Mr Auchter to withdraw sponsorship of the circular issued jointly with NIOSH last December.

Dr Infante's immediate boss, Dr Bailus Walker, in his letter of dismissal said that OSHA "lacks confidence in the data" on which the circular, which has no regulatory impact but is meant primarily for information, was based. Dr Walker told Mr Gore that he had dismissed Dr Infante "on the advice" of his boss Mr Auchter.

Mr Auchter agreed that his decision that the carcinogenicity data were inadequate to support the conclusions of the circular, was made without consulting any of the agency's scientists. However, he insisted to Mr Gore that his lack of confidence was based solely on the use of the data for regulatory purposes, as conflicting data also existed.

Dr Infante's dismissal is now the subject

of litigation between himself and the agency, a fact which Mr Auchter initially used as a reason for not providing detailed comments on the case to the investigations committee. However, under pressure from the Congressmen, who presented legal advice that the hearings would not prejudice any later trial, Mr Auchter shifted his stance — and went on to deny that he had ordered Dr Infante's dismissal, directly contradicting the sworn testimony given by Dr Walker.

Dr Walker, who told the subcommittee members that "the data suggested that formaldehyde was a potential carcinogen and should be treated as such", has now resigned from OSHA to take up the position of director of public health for the State of Michigan.

The subcommittee has yet to announce the conclusions of its investigations. Indeed one minority member, Republican Congressman Robert Walker, sharply defended OSHA's actions on the grounds that Dr Infante had broken federal rules in representing his own scientific opinion as that of the agency. Mr Gore, however, was in no doubt "that the formaldehyde industry had "engineered a decision in the agency to change OSHA's view on the scientific data" and that this was behind the decision to fire Dr Infante.

David Dickson

Local DNA guidelines

Boston strikes out

Boston

In what could become a prototype for American cities seeking control of recombinant DNA research, the Boston City Council has passed a law regulating research at universities and commercial companies. The city ordinance follows community activism by residents of the Mission Hill district of Boston, who are concerned about the \$50 million grant by the Hoechst chemical company to the Massachusetts General Hospital and by the leasing of empty hospital space in their neighbourhood to Genetics Institute Inc., the newly formed genetic engineering company.

The city council hearings of the past month have actually been a repeat of events in nearby Cambridge eight years ago, when recombinant DNA research was entirely new. But on this occasion, there was less open conflict between citizens and university officials than in Cambridge.

The new law requires compliance with National Institutes of Health (NIH) standards but there are also further local restrictions which have been added since work began on the original proposal in late May. As well as assuring strict conformity with NIH guidelines, the ordinance demands:

• "Timely response to guideline amendment and permit applications in accordance with good governmental

practice".

- Research proposal not subject to NIH guidelines should receive council-administered permits.
- Broadening and restructuring of the Boston Biohazards Committee an area regulatory organization which will now serve as an advisory board to the commissioner of the Boston Department of Health and provide an annual report to the city council.
- Opening of normally confidential employee health records for "regulatory or public health study purposes".
- Institutions performing recombinant DNA research should monitor the health of their employees and the institutional responsibilities in this area should be "reasonable and related to potential risks".
- Costs of monitoring to be reimbursed to the city by the regulated institutions.

The final version of the ordinance left out the harshest requirement of the original proposal — that institutions should perform regular effluent monitoring and the testing for live organisms in the city sewer system. This was dropped because it is not technologically feasible.

In the past, local universities have agreed that guidelines of some sort would be helpful but have opposed the introduction of laws requiring compliance with official regulations, arguing that universities should set their own standards. But campus officials are generally pleased with the outcome of this debate and confident that they will easily meet the provisions.

The new ordinance will run for five years and is renewable. Several other cities in the Boston area and elsewhere in the United States have begun to review their own proposals for regulation as commercial companies are proliferating.

Michael D. Stein

Aspartame sugar substitute

New court overruled

Washington

It had been described as the first official attempt to resolve a complicated dispute over the safety of a new food additive by using a so-called "science court", with evidence on both sides being presented to a panel of three outside scientists. But last Wednesday the US Food and Drug Administration (FDA) overruled the verdict of its Scientific Public Board of Inquiry, reached after hearings held in January and February last year, and approved the marketing of a new low-calorie sweetener, aspartame.

Permission to market the sweetener had first been requested from FDA by its manufacturers, G.D. Searle, seven years ago. Initially FDA had agreed; but in the light of reports from a scientist at Washington University, St Louis, that the sweetener could produce brain lesions when fed to laboratory animals — and the general

concern that accompanied the decision to ban cyclamates in 1970 — permission was withdrawn the following year pending further studies.

Doubts about the validity of animal studies conducted for Searle to generate the data needed for new drug approval were discounted after two years of independent auditing of the studies. FDA then turned to the brain lesion claims, which were examined by a three-person team headed by Dr Walle J.H. Nauta, professor of neuroanatomy at the Massachusetts Institute of Technology. In their report, issued last October, the scientists said the data shown to them did not support the suggestions that aspartame might kill clusters of brain cells or cause other types of brain damage.

However, the "science court" also raised doubts about whether the reports of brain lesions could be completely discounted on the grounds that the tests had been carried out at doses far higher than those humans would normally experience. It recommended that marketing approval be withheld until further long-term animal tests had been carried out to rule out any possibility that aspartame could result in brain damage.

This conclusion was bitterly contested by Searle, already sitting on a stockpile of 300,000 lb of the sweetener, with a market value of over \$25 million. The company claimed that the three scientists' conclusions made "significant errors" in dealing with issues of tumorigenicity; and that they had "failed to employ biological and statistical principles that would have provided guidance in assessing the potential carcinogencity of a compound".

Searle pointed out that the sweetener is already being marketed in France, Belgium and Luxembourg, and that it has also been approved for use by the Joint Expert Committee on Food Additives of the Food and Agriculture Organization, and the World Health Organization.

The new FDA commissioner, Dr Arthur Hayes, now seems to have accepted Searle's arguments. Following the conclusion of the agency's Bureau of Foods that the "science court's" concerns were unfounded and that the sweetener would be safe even at the "highest conceivable levels" of consumption, he has agreed that it should be approved for use as a sugar-substitute and food additive, although not yet for soft drinks.

David Dickson

Environmental lead

Playing safe

Britain's health and environment departments seem to have won a victory behind the scenes in the government's decision last month to reduce the lead content of petrol from 0.4 to 0.15 g per litre. Other government departments concerned about the financial effects on the car and oil refining industries finally gave

way last May to the arguments that lead from car exhausts could damage the health of children in the inner cities. The environment and transport departments, however, supported the decision chiefly as a way of countering some of the increasingly fierce objections to building new roads.

The decision on lead in petrol came as a surprise just one year after a report commissioned by the health department concluded that there is no proof of a link between low blood-lead levels in children and impaired mental development. That report, prepared by a working party under the chairmanship of Professor P.J. Lawther of St Bartholomew's Hospital. was stimulated by research in Germany and the United States which implicated bloodlead levels as low as 35 µg per dl, now accepted as the maximum permissible by the European Commission. Although the working party concluded that these and other studies were equivocal, it recommended that steps be taken to reduce lead in food and water, seen as major sources, and air, a less significant source.

The Department of Health, however, seems to have persuaded the Department of Energy and the Treasury to act on petrol on the basis of evidence not covered in the Lawther report. A study by Dr R.G. Lansdown of the Great Ormond Street Hospital and Dr William Yule of the University of London Institute of Psychiatry of children in the Greenwich area of London is thought to have shown a correlation between low blood-lead levels commonly found in the British population and a slight impairment in IQ. That study, although made known to government officials, has not yet been published because of difficulties in interpreting data. Dr Yule now expects it to appear in the October issue of Developmental Medicine.

Other evidence which seems to have weighed in the decision includes the findings by the Greater London Council of exceptionally large quantities of lead in dust in some inner city playgrounds, research at ISPRA in Italy which suggests that 25-40 per cent of the total body lead burden may be contributed by lead in petrol and a critique of the Lawther report prepared by Professor D. Bryce-Smith and Dr Robert Stephens for the Conservation Society. The Conservation Society's document complained that the Lawther report did not pay sufficient attention to animal studies and that it underestimated the contribution of airbone lead by failing to allow for the fact that much of the lead in food and dust settles from the air.

The Department of Health seems to have persuaded the government to play safe by ordering the reduction. The search is still on, however, for better evidence of the possible effects of low lead levels on health. Dr Lansdown and Dr Yule are at present engaged on another study of inner city children and are discussing the possibility of a third, more extensive study with the Medical Research Council. Judy Redfearn

UK defence research

Slimming down

The Royal Aircraft Establishment at Farnborough, which with its several outstations is the largest of the in-house research stations run by the British Ministry of Defence, seems also to be the first in line for substantial reorganization. In the past year, the total staff of the establishment has fallen from 4,500 to 3,800. largely as a result of natural wastage (principally retirement) made possible by the top-heavy age distribution at the laboratory, itself a consequence of rapid recruitment during and immediately after the Second World War. But the remaining staff at the laboratory are now anxious that attrition has a lot further to go.

The establishment is the most conspicuous of the defence research establishments considered by the internal government review under Lord Strathcona, whose report has not been formally published but has instead been placed in the library of the House of Commons, where its presence is almost certain to have the most damaging consequences. In respect of Farnborough, the report recommended that further consideration should be given to the need for maintaining three airfields (at Farnborough, Bedford and Boscombe Down), a balloon station (at Cardington), three missile ranges and a variety of wind tunnels that (in Lord Strathcona's view) could well be hived off to private contractors.

At a meeting at Farnborough on 4 March, the director of the establishment, Mr T.H. Kerr, told the staff that the future of these offshoots was being considered by a series of working parties, and that decisions would be made later in the year. The most obvious possibility of change is that missile ranges may in future be managed by private contractors (as is to a large extent already the case at the Welsh firing range at Aberporth). But there are also to be rearrangements of the internal divisions at Farnborough (leading to the loss of relatively senior staff) and the future scale of operations at the establishment has not yet been determined, which is why some members of staff fear that their director's promise (on 4 March) that reductions could be accomplished by retirement, early retirement and other wastage (as well as the transfer of some staff to private contractors) may not be feasible

The chief source of anxiety in the past few weeks, however, is strictly speaking hardly relevant — the steps being taken by private interests in case the Farnborough airfield is eventually put up for sale. It seems to have been decided that, if any of the three airfields is to be sold, the sale of Farnborough would cause the least damage even though the establishment would wish to retain the right to mount the biennial air show of the Society of British Aerospace Companies on the site. The bid to purchase

New Forest flutter

In the name of science, lepidopterists have for many years, chloroformed butterflies to death and displayed them in glass cabinets. Now many of those butterflies are increasingly imperilled by loss of habitat, killing by insecticides and other hazards of the twentieth century. A cautious welcome then, for Britain's very first public "butterfly farm", which opened yesterday (22 July) in the New Forest. It's not being run for the good of science though, simply as a tourist attraction to bring in money to an estate at Ashurst, near Southampton.

There should be more than 1,000 butterlies at Ashurst if the breeding programme is successful, flying freely in a glasshouse covering 6,750 square feet.



Now in Hampshire - the American monarch

Over 50 different species will be on view, mostly colourful imports from the tropics, including the North American monarch, *Heliconius* from South America, swallowtails from South-East Asia, and the Camberwell beauty.

Like all would-be "tourist traps" the farm boasts a gift shop. Books on butterflies will be on sale, of course, but so too will live caterpillars and pupae, presumably to encourage butterfly farming as a hobby. Traditionalists are also catered for, they can buy mounted butterflies.

None of this is likely to do any direct good to the average butterfly in the street, or field. But the "farm" owners reckon that by increasing the public's awareness of what they call "these delicate creatures", life will gradually improve for what's left of Britain's butterfly population.

Charles Wenz

the airfield is based on the hint in the Strathcona Report that the airfield would be suitable as a base for business flying, but will not be considered until all the working parties considering the future of the defence research establishments have had their way.

Last week, at another staff meeting, Mr Kerr had little extra to say. Discussions have begun with potential operators of the parts of the establishment to be hived off, but firm proposals are not expected until early next year. Bidders for these contracts (among which British Aerospace is conspicuous) are being asked to take over existing members of the staff — and unions such as the Institution of Professional Civil Servants are looking for an assurance that their members' terms of employment will not be adversely affected.

French energy policy

The great escape

There were two striking omissions from the roster of nationalization proposals announced by the Prime Minister of France, M. Pierre Mauroy, recently — Framatome and Novatome, the companies which between them account for almost the whole business of constructing conventional and fast breeder reactors in France. The exceptions are remarkable because the Socialist Party had announced before the election that all industries concerned with the nuclear fuel cycle would be nationalized.

M. Mauroy was, however, quite clear in what he said: eleven groups would be nationalized, not one more nor one less. The omission of the nuclear industry is another indication that the new government does not intend seriously to disturb France's growing lead in nuclear technology. Mauroy explained that the companies to be nationalized are included in his list not for doctrinal reasons but to improve their competitive performance as has been done with the car maker Renault. (In fact the new Minister for Industry, M. Pierre Dreyfus, was previously managing director of Renault, so his appointment has somewhat comforted industrial opinion.) Thus the immunity of the nuclear business amounts to a tacit recognition of its independent commerical success.

The grand debate on energy also begins to look less of a confrontation than a few weeks ago. It is expected now to take place in the National Assembly in November — and instead of focusing on nuclear power it will look at all aspects of the energy question, a difficult matter in what will be a packed legislative session. Parliamentary and specialist groups have been set up to investigate separate issues.

Anticipating the debate, M. Mauroy has seen fit to announce the broad outlines of French energy policy. The two priorities will be to develop indigenous fossil fuel production, insofar as that is economic (taking account of world prices) and to pursue a significant nuclear programme.

Mauroy did not mention renewable sources or even conservation, and his remarks on fossil fuel production are taken to imply caution in the further development of France's costly coal compared with the cheaper Australian product. That leaves, of course, nuclear power as the central plank of his energy policy.

On this reading, the government must arrange that the November energy debate will lead to a vote to continue to build new pressurized water reactors. (New starts are temporarily suspended.) The construction of Superphénix, the commerical demonstration fast breeder reactor, continues, and a decision on a second one can be comfortably left for a while.

Robert Walgate

Mongolia's jubilee

Following the lamas

The Mongolian People's Republic this month celebrates the diamond jubilee of its foundation on 11 July 1921. Included, de facto, in the anniversary is the Mongolian Academy of Sciences, since although formally inaugurated only in May 1961, it originated, in the words of Dr B. Shirendyb, president of the academy, from "a small group of scientific workers" who banded together immediately after the establishment of the Republic to raise the scientific standards of the country.

Not that science had been entirely neglected in pre-revolutionary Mongolia. An astronomical observatory had been founded in Ugra (now Ulan Bator) in 1779, and a flourishing medical tradition was preserved in the lamaseries. Nevertheless, in the early years of the new state, the major task was the translation of basic scientific texts from Russian and other European languages. And before even this could be done a more fundamental problem had to be solved of replacing the picturesque but impractical traditional script by a modern alphabet.



Astronomical observatory Mongolian style

Inevitably, present day research in Mongolia is largely directed towards the needs of the economy. During the past five years, the various institutes of the Academy of Sciences carried out 369 research projects, which should ultimately save the economy more than 108 million tugriks (£18 million). Research included the use of methods of nuclear physics in surveying the country's deposits of copper, molybdenum and tungsten, mapping of seismic zones, the extraction of pharmaceuticals from traditional medicinal herbs, and the development of new strains of wheat and breeds of sheep.

International help has not been lacking. The plan to establish a 5 million hectare nature reserve in the Gobi Desert is receiving considerable assistance from the United Nations Environmental Programme, and Comecon countries have some 60 joint research projects in operation with Mongolia. One piece of research, not yet covered by a formal agreement but watched with considerable interest by Mongolian scientists, is the work of Dr Tibor Farkas at Szeged

(Hungary) on the biophysics and biochemistry of frost damage. The Mongolians hope that this work will result in the development of an anti-frost spray for spring wheat, which is at considerable risk in their harsh climate.

Among the facilities that Mongolia has to offer its international collaborators is the Gobi Desert — a valuable hunting ground for Comecon expeditions in search of fossils of dinosaurs and dinosaur eggs.

Vera Rich

Belgian scientific institutions

Arresting decline

Brussels

It has long been felt in Belgium that some of the country's most renowned scientific institutions are falling into decline. Disaffection is so great that last spring the staff of eight major institutions resorted to strikes to draw attention to their grievances. Now at last the government has decided to do something.

A committee of wise men has been given the task of finding ways to unravel the administrative snarks which are held responsible for the problems. The eight institutions under review are the Royal Library of Belgium, the National and Provincial Archives, the Royal Belgian Institute of Natural Sciences, the Royal Museum for Central Africa, the Royal Belgian Observatory, the Royal Belgian Meteorological Institute, the Belgian Institute of Aerospace and the National Centre for the Production and Study of Microbiological Substances.

The problems of these institutions are uniquely Belgian. When the constitution was revised in 1970, it was decided to keep them as national bodies. The conflict between the Flemish and Walloon (French-speaking) communities in Belgium has led to a virtual duplication of all administrations and much else. The education system is completely divided along linguistic lines. As national bodies, the eight institutions in question fall foul of the system and do not benefit from the patronage of either the French or Flemish education ministers.

Every decision has also to be approved by three or four ministries and with a change of government every few months, the national institutions have found it very difficult to get anything done. Most of the directors of the institutions have left in disgust at the administrative confusion.

The committee is due to make its first report within the next three months, but the recommendations are bound to involve more drastic cures than simple administrative pruning. Some of the institutions with more active research departments are sure to be merged with universities. And the worrying size of Belgium's public-sector deficit may prompt the committee members to recommend that parts of the institutions should be axed completely.

Jasper Becker

CORRESPONDE

Radical writings

SIR - Dr Turner's letter (Nature 4 June, p.374) makes the disturbing claim, citing the exchange between Dawkins and myself as evidence, that "the radical scientific opposition to racism requires a denial that there is any genetic variation of any significance from place to place within the human species". I wonder what on Earth he has been reading which provides support for such an assertion? As someone who has been reading, writing and teaching in this area for some years now, I can't remember ever seeing such a claim, and I would be interested if he could produce a single statement from the writings of "radical" biologists since the sociobiology and IQ debates started in the late 1960s which could support his allegation.

The Open University. Milton Keynes, UK

Australian 2,4,5-T

STEVEN ROSE

Sir - Since you published our letter on "Antipodean 2,4,5-T", a sample of the herbicide imported into Australia has been brought to our attention. It had been submitted to the Australian Tariff Board by the importer as a sample of a very large consignment on which lower tariffs had been sought. It was recently analysed by the West Australian Government laboratories and found to consist essentially of the isobutyl ester of 2,4,5-T, and to contain 19 mg per kg of 2,3,7,8-TCDD on an "as received" basis, and 26 mg per kg on a 2,4,5-T basis. (The level of TCDD (2,3,7,8-tetrachlorocyclodibenzo-pdioxin) in 2.4.5-T considered acceptable in Australia at the time was 0.1 mg per kg.)

Evidence given before the Tariff Board inquiry suggests the material may have been produced from fire-damaged potassium trichlorophenolate (KTCP). [A synthetic route to 2,3,7,8-TCCD is through the heating of KTCP^{2,3}.] A letter from the Singapore High Commission (Australia) has confirmed that a fire occurred in the premises of the importer's Singapore company, and pointed out that (1) there were reports of chloracne in the factory workers and (2) after the fire, the neighbouring factory complained of symptoms in its workers. The importer himself described this "fire damaged" material as of "good value"4, and so one assumes that it entered commerce in Australia.

All of this makes more pressing our concern for Australia's epidemiological inquiry into Vietnam veterans' claims. Use of 2,4,5-T with such high levels of TCDD can prejudice the controls in this study. It will also have implications for ordinary civilian usage.

We hope that one result of our work will be that the Australian authorities will increase their surveillance of chemical imports.

PETER HALL BEN SELINGER

Australian National University, Canberra, Australia

- Hall, P., Selinger, B., Field, B. & Kerr, C. Nature 290, 8
- Langer, H.C. & Brady, T.P. Thermal Analysis 2, 273 (1974).
- Langer, H.C. Brady, T.P. & Briggs, P.R. Envir. Hith Perspect. 5, 3 (1973).
- 4.5-T Products and 2,4,5-Trichlorophenol and its Salts (D.S.A.) Official Transcript of Proceedings. Commonwealth of Australia, 31 August, 1972; pp. 73, 74.

Conservation areas

Sir — It would seem injudicious for Dr Ratcliffe to claim1, as his guarantee of rightness, the wisdom of Parliament when a minister of the Crown can say2, after receiving the advice of the Nature Conservancy Council (NCC) "I welcome the international recognition that it is totally unacceptable for species to be allowed to disappear", though this is quite in keeping with the NCC's concern to safeguard Sites of Special Scientific Interest (SSSIs) "in perpetuity". If the NCC has not got the financial resources to provide invariable, immediate and adequate compensation to the owners of SSSIs, how will their perpetual preservation policy prevent glacial readvance over the Black Wood of Rannoch, or pluvial destruction of the Pennine limestone pavements, or, perhaps, provide fall-out shelters for Dartford warblers?

I suppose that one must expect if one has the temerity to express an unfashionable point of view that one will be misunderstood accidentally and deliberately^{3,4}, so may I make it clear that I am not advocating the wilful destruction of anything, organism or habitat, but I do feel that anyone, acting as a scientist, needs a better excuse that a personal predilection for the rare, the exotic, or the status quo before he asks people to forgo the benefits of cultivating their gardens or puts others to the national expense of compensating them for their abstinence. I had been led to believe that science was an organized body of knowledge concerned with the physical world as we find it, static or dynamic as the case may be, that scientists were by their training perhaps better equipped than most to make reliable predictions of the likely outcome of the processes which they observe, but that as scientists they have no claim to superior knowledge or wisdom in prescribing what goals or courses of action are desirable on moral, political or economic grounds. It is against this assumption of authority, which is more than hinted at in Dr Ratcliffe's last sentence, that I am railing.

COLIN MUIR Department of Zoology, The University, St Andrews, UK

- . Ratcliffe, D.A. Nature 290, 539 (1981)
- Heseltine, M. *Hansard* 3, col. 526 (1981).
 Disney, R.H.I. *Nature* 290, 432 (1981).
 Heyworth, M.F. & Wainscott, J.S. *Nature* 290, 440 (1981).

Israeli raid

Sir - I was unpleasantly surprised to read the leading article in Nature entitled "Making Israel atone for Tamuz" (18 June, p.523). I and many others of your readers are of the view that Nature is a scientific journal, which justifiably includes articles on scientific or educational policy in addition to the research material which it publishes. However, this was an article of a purely political nature, written about a very sensitive issue without any backing of specialized knowledge. It was in my view an unwise decision to publish such an article in Nature and a dangerous precedent.

It is, of course, impossible to enter into a full discussion with you on the reasons for Israel's decision to bomb the nuclear plant in

Iraq, but there are certain facts which have been ignored in this article, which should be brought to your attention.

- (1) There is a great deal of evidence that Iraq has ambitions to acquire a nuclear weapon and it has built up its military power in the last few years to an alarming magnitude. As this country is at war with Israel, having consistently refused to enter into any peace negotiations, this must be a serious threat to the security of Israel.
- (2) To ask Israel to accept the report of the UN International Atomic Energy Agency is expecting a lot, in view of the attitude of the United Nations and its agencies towards Israel in the past. If Israel is to survive it must depend on its own intelligence assessments. This is exactly what it did in this case, and it acted before it was too late.
- (3) The article ignores the fact that Israel has never refused to enter into peace negotiations with its Arab neighbours. Except in the case of Egypt these countries have not only refused but quite openly proclaimed their ambition to destroy the State of Israel; Iraq has been one of the leading advocates of this policy. Under these circumstances how can one expect Israel to enter into a "Non-Proliferation Treaty"?

ANNE BELOFF-CHAIN Imperial College of Science and Technology. London, UK

Forbes, not Wallace

SIR — In his review of a recent symposium on biogeography, Colin Patterson regards A. R. Wallace as the inventor of "biogeography in the accepted sense", and gives the impression that he thinks very little of the scientific competence of Charles Darwin in this context (Nature, News and Views 25 June, p.612).

I refrained from joining in the previous arguments in your columns about cladism, evolution and Darwin, but in this instance I must protest that in enthroning Wallace and demoting Darwin, Dr Patterson is overplaying his hand and ignoring the earlier literature. Darwin was well aware that the continents were not immutable (see the sketch of 1842 and the essay of 1844), and that previous epochs may have had land connections different from those of the present. It is incorrect to claim that evolutionary hypotheses based on unchanging continents dominated biogeographical thinking up to the 1960s, when Wegener's views were finally accepted by orthodox geophysicists. Many of the published biological arguments of the period were about the extent and timing of the land and sea connections, not whether they

Darwin was also aware of contemporary studies of geographical distribution, such as the excellent review of Edward Forbes, not mentioned by Dr Patterson, published in the first volume of the Memoirs of the Geological Survey, 1848. In this article Forbes clearly stated an hypothesis for a previous land connection between the Iberian peninsula and Ireland. If anyone is to be selected as the father of biogeography it should be Forbes.

A. J. SOUTHWARD

Marine Biological Association, Plymouth, UK

NEWS AND VIEWS

Ocean currents and wind: how well can the connection be made?

from Charles C. Eriksen

A recent issue of the Journal of Geophysical Research (86, C3, 20 March 1981) contains two articles which serve to demonstrate the wide gulf between observations and models of wind-driven ocean response. The paucity of reliable upper ocean measurements and suspension of Ekman dynamics make the situation even worse near the equator. One article, by S.G.H. Philander and R.C. Pacanowski, describes the results of a numerical simulation of the response of a stratified equatorial ocean to periodic wind forcing at periods from days to years. The other, by R.A. Weller, describes current profiles made at mid-latitude from a drifting research platform. While Philander and Pacanowski make rather detailed predictions of oceanic response, Weller finds that Ekman theory, a cornerstone of dynamical oceanography (W.V. Ekman in 1905 predicted current profiles which spiral and decay with depth through a balance of frictional and rotational forces in response to wind stress at the surface), fails to account for the rather weak decay of shear away from the surface which he observed.

The problems of measuring in the upper ocean are formidable. It is only recently that instruments have been developed which succeed in removing the contaminating effects of surface waves on current measurements. This success is achieved by utilizing linear sensors which can deal with currents which are not confined to the horizontal plane. The time scales of wind forcing and ocean response make sequential measurements at one or more fixed locations an attractive method of examining ocean physics. These eulerian descriptions of the flow are often collected from current meters tethered at fixed depths along a deep sea mooring. Weller used newly developed current meters in a profiling mode, raising and lowering them by winch from the uniquely stable Research Platform Flip (a giant spar buoy resembling a submarine tipped on end, which pitches and rolls hardly at all). Since Flip drifted modestly, the measurements are not eulerian, but their excellent accuracy allows records of current shear to be compared with time-dependent Ekman

models. Although the observed changes in phase with depth are modelled by simple Ekman dynamics at most frequencies, the amplitude structure is not. The discrepancy ultimately seems to be nonlinear. Turbulence produces apparent diffusivities which greatly exceed molecular values. The spatial structure of eddy viscosity (turbulent diffusion of momentum) is poorly known, as is the role of larger-scale nonlinearities in the flow. These two unknowns keep upper ocean response to wind stress in the class of 'fascinating unsolved problems' in oceanography and promise to do so for some time in the future.

Near the equator, Ekman theory breaks down because the horizontal Coriolis forces associated with the Earth's rotation vanish. There is no satisfactory theory for the planetary boundary layer at low latitudes; most theories use a body force formulation (stress distributed a priori in a slab of water) to circumvent this problem. This arbitrary linkage is used to provide a forcing mechanism, to which the response by large-scale ocean dynamics is sought. Numerical models are often the only means by which the complicated effects of density stratification, friction, nonlinear effects and ocean boundaries can be considered together. In the equatorial oceans it is clear that all of these effects are important. Everyone seeks simple force balances, and discarding one or more of these effects in favour of the others is a popular method of equatorial theorists. Unfortunately, largely for technical reasons, there is relatively little guidance from observation for the correct choice in a given situation. Numerical studies also require data taken at discrete intervals to keep computations manageable (even the largest computing facilities can be easily overburdened by the size and density of calculations demanded by modest models of ocean circulation). The resulting simulations are not altogether realistic, but at least describe the mechanisms at work in different flow regimes. Philander and Pacanowski state various results about how the ocean responds to wind forcing, but it should be remembered that these are the results of a model study of a numerical ocean which one cannot be sure behaves exactly as the real oceans do.

The results Philander and Pacanowski report are an example of what can be said about oceanic response from an incorporation of the general effects, but not the details, of the mechanism by which wind energy is transferred to the ocean. They highlight the unique character of equatorial oceans — that it is possible to have a Kelvin wave trapped to the equator which can carry energy eastwards, in addition to equatorial analogues of the long Rossby waves at mid-latitude which carry energy to the west. Philander and Pacanowski identify three ranges of period: (1) at the short periods (a few days) strong currents are not generated, but rather a spectrum of equatorial waves; (2) at intermediate periods (weeks, months) upper ocean currents are generated primarily which reflect off the basin boundaries in a complicated fashion; and (3) at very long periods (greater than several months to a year or more, depending on the basin size) steady-state response is achieved. The nonlinear nature of the problem causes Rossby waves to be modified by the mean currents at low frequencies. Also, rays along which energy travels have shallow inclinations at long period, so that the deep ocean can only contain wave energy which is the result of one or more reflections off the basin ends. There are very few measurements by which any of the predictions of the model can be tested; however, the general contrast between observations in a small basin and a large one (Atlantic versus Pacific) tends to confirm the predictions of the model. It is a big job to observe enough of the ocean to really test these models in even the most rudimentary way. At this point large-scale theories are far more sophisticated than data. Observations are needed to verify the assumptions upon which the models rest, as well as the general model results.

Charles C. Eriksen is in the Department of Earth and Planetary Sciences at the Massachusetts Institute of Technology.

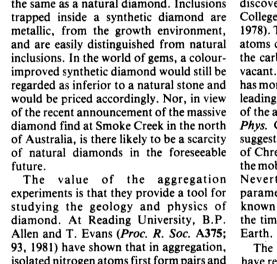
Migrating nitrogen atoms in diamond

from Gordon Davies

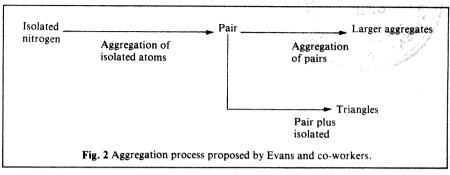
By crystallographic standards gem quality diamonds are highly impure. Most of them contain nitrogen at about one part in a thousand — some 10²⁰ atoms of nitrogen per cubic centimetre of diamond. Significant colouring is usually produced in a crystal when it contains about 1017 impurity atoms per cubic centimetre, but natural gem diamonds are usually colourless because the nitrogen atoms have aggregated into pairs, each pair replacing two carbon atoms. The paired form does not absorb visible light as its absorption bands lie in the ultraviolet part of the spectrum. However, if even a small fraction of the nitrogen is present in the simplest form, as isolated nitrogen atoms with each atom replacing one carbon atom, then an unattractive brown colour is produced. Most synthetic diamond is of this type and is unsuitable for use as gem stones.

In nature many diamonds are also found in which the nitrogen aggregation has gone further, producing aggregates of three or more nitrogen atoms (see Fig. 1). Diamonds like these are often of considerable commercial value as gem stones, for in a triangular arrangement the nitrogen can absorb near-ultra violet radiation and emit the energy in the visible part of the spectrum, enhancing the brightness of the diamond in a whiter-thanwhite effect.

In the early 1970s Soviet science journals reported that synthetic diamonds grown at higher temperatures and pressures than usual had a small amount of their nitrogen in the aggregated forms typical of natural diamonds, although most of the nitrogen was still scattered through the lattice as isolated atoms. A few years later, at the General Electric Corporate Research and Development Laboratories in Schenectady, New York, R.M. Chrenko, R.E. Tuft and H.M. Strong observed substantial aggregation of the nitrogen in some of their own synthesized diamonds when the diamonds were heated to about 2,000K for 30 minutes. In one diamond they converted three-quarters of the isolated nitrogen atoms into aggregated forms. The technique was published in Nature (270; 141, 1977) and protected by patent (British Patent specification 1578987).



isolated nitrogen atoms first form pairs and then migrate to produce the larger aggregates (see Fig. 2). The longer the diamond is held at a high temperature, the further the aggregation proceeds, so the state of aggregation in any diamond gives some idea of the length of time it has been heated. The temperature at which natural diamonds grew can be measured independently from the chemistry of their inclusions; for all diamond sources a temperature of about 1,450 K is indicated (J.J. Gurney and B. Harte Phil. Trans. R. Soc.



The possibility of improving the colour of synthetic diamonds may appear to have commercial implications for the gem industry. In practice, however, the cost of growing gem-sized diamonds is prohibitive, and to convert any nitrogen in the diamond requires a further heat treatment at the aggregation temperature of 2,000 K. The processed diamond would still not be the same as a natural diamond. Inclusions

A297; 273, 1980). Extrapolating the

laboratory results of Chrenko et al. down to this low temperature, Allen and Evans estimate that those rare natural diamonds containing only isolated nitrogen atoms must have been ejected to the surface of the Earth within 50 years of their growth — a very short time scale geologically.

The accuracy of this estimate is currently unknown because of a complication discovered by A.T. Collins at King's College, London (J. Phys. C11; L417, 1978). The speed at which isolated nitrogen atoms can migrate is increased if some of the carbon atom sites in the diamond are vacant. A nitrogen atom next to a vacancy has more room to manoeuvre in the crystal, leading sometimes to a 50-fold acceleration of the aggregation process (A.T. Collins J. Phys. C13; 2641, 1980). Collins has also suggested that the original measurements of Chrenko et al. may have overestimated the mobility of the isolated nitrogen atoms. Nevertheless, when the migration parameters of the nitrogen atoms are known better it will be possible to fix the time a diamond has spent deep in the

The nitrogen aggregation experiments have recently cleared up one long-standing problem of diamond research. Many natural diamonds contain twodimensional defects, with diameters of the order of tens of nanometres, lying in their cube planes. These 'platelets' only occur when the diamond contains a large concentration of nitrogen. During the 1960s it was generally assumed that all the nitrogen was tied up in the platelets. Then E.V. Sobolev, V.I. Lisoivan and S.V. Lenskaya (Sov. Phys. Dokl. 12; 665, 1968) showed that there was no simple correlation between the number of platelets in a diamond and the concentration of nitrogen it contained. During the 1970s it was found that the optical properties of diamonds could be satisfactorily explained if the platelets were simply ignored, and the nitrogen assumed to exist only in small aggregates like the pairs of nitrogen atoms. But now, T. Evans, Zengdu Qi and J. Maguire (J. Phys. Gordon Davies is in the Wheatstone Physics

Laboratory, University of London King's College.

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Fig. 1 Highly schematic diagrams of the smallest nitrogen structures in diamond: a, the isolated nitrogen atom, b, the nitrogen pair, c, the nitrogen triangle. In the nitrogen triangle, the N atoms share one common C atom as the nearest neighbour; they are not bonded directly together.

C14; L379, 1981) have produced platelets in a synthetic diamond solely by heating at 2,700 K for 4 hours and at 2,800 K for 2 hours. Initially, the diamond contained about 10^{20} cm⁻³ isolated nitrogen atoms. After the heat treatment some 10^{15} cm⁻³ platelets with diameters of 20–50 nm were produced — equivalent to about 2×10^{19} cm⁻³ defect atoms — as well as the other aggregated forms of nitrogen which accounted for some 10^{20} cm⁻³ nitrogen

atoms. The experiments can only be understood if the platelets are made of nitrogen. The apparent discrepancy in the numbers above is expected since the distribution of nitrogen in synthetic diamonds is inhomogeneous.

The picture emerging for the 1980s of nitrogen in diamond is that it is present in both the platelets and in the atomic-sized structures. Key experiments are required to complete the picture: accurate calibrations

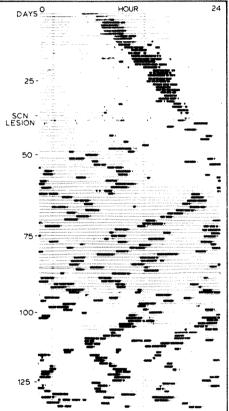
have to be made so that the concentration of nitrogen in each structure may be measured easily; the thermodynamics of each stage of the aggregation have to be found; and ¹⁵N isotope shifts in the optical features of the platelets could establish more about the role of nitrogen in the platelets. Although much still has to be done, the migration of nitrogen in diamond is turning out to be an extremely profitable line of study.

Are the suprachiasmatic nuclei the location of the biological clock in mammals?

from Fred W. Turek

Over the past twenty-five years, largely due to the efforts of Colin Pittendrigh and Jürgen Aschoff, it has been demonstrated that the generation of circadian (that is, approximately 24-hour) rhythms in animals depends on an internal biological clock. The endogenous oscillator is normally synchronized by environmental factors, principally the photoperiod, to the 24-hour rotation of the Earth upon its axis. Hundreds of circadian rhythms have been described and the formal properties of circadian systems analysed. Recently, attention has switched to the location of the biological clock(s) and the mechanisms by which the clock is coupled to the many rhythms it drives. Hundreds of experiments in scores of laboratories around the world have been carried out in an attempt to elucidate the neural elements involved. However, it is only within the past few years that the neuroscience community has begun to apply the entire armamentarium of neural techniques to the study of circadian rhythms. These initial studies have already yielded a number of important findings and have laid the groundwork for future work involving the search for the circadian clock in animals.

In mammals, the hypothalamic suprachiasmatic nuclei (SCN) have become the centre of attention. Compelling evidence that this region of the hypothalamus plays an important part in circadian organization was first provided by Stephan and Zucker¹ and Moore and Eichler² when they discovered that destruction of the SCN in rats altered both the entrainment by light/dark cycles and generation of circadian rhythms. Since these pioneering studies, many researchers have observed that bilateral lesions of the SCN in rodents disrupt a variety of circadian rhythms including drinking, eating, locomotor behaviour, plasma corticosterone, pineal N-acetyltransferase activity, sleep, heart rate and body temperature. In addition, SCN lesions disrupt oestrous cyclicity and photoperiodic time measurement, two parameters which depend on the circadian system for temporal information3.



Continuous record of the wheel-running activity of a male hamster maintained in constant light. Successive 24-hr days are plotted from top to bottom. On day 37 of this record, the animal received a bilateral lesion of the SCN which resulted in a clear disruption of the normal circadian rhythm of locomotor activity (from G.E. Pickard and F.W. Turek, unpublished results).

Since it is now quite clear that multicellular organisms are made up of a population of circadian oscillators, the search for the biological clock is really the search for that clock or system of clocks which coordinates the various internal oscillators with each other and the outside environment. Evidence for the existence of a population of oscillators comes from many different experiments including some that involve the destruction of the SCN in rodents. Early studies indicated

that lesions of the SCN resulted in the complete loss of entrainment and the abolishment of free-running circadian rhythms but more extensive studies by Rusak4 in the golden hamster suggested that a variety of independent rhythmic oscillators exist in the absence of the SCN. The figure shows the dramatic effect that lesions of the SCN can have on the circadian rhythm of locomotor activity in the hamster. On some days (for example, days 87-96) no rhythmic pattern is obvious, while on other days bimodal (about 12 hr: for example, days 100-111) and trimodal (about 8 hr: for example, days 113-120) activity patterns are generated. Rusak postulated that such patterns indicate that circadian oscillators continue to function in these animals, but the SCN are necessary to integrate these oscillators into a single circadian framework. Further evidence that at least some circadian rhythms persist in mammals after destruction of the SCN comes from work on squirrel monkeys. Although the circadian rhythms of behavioural activity, feeding and drinking are abolished following bilateral destruction of the SCN, the rhythm of body temperature persists⁵

Attempts have been made to locate the endogenous biological clock by determining the pathways which connect the environmental factors, principally light, to the entrained circadian system. A direct retino-hypothalamic tract (RHT) which terminates in the SCN has been observed in many mammals6 and it has been suggested that this is the primary pathway by which light information reaches the circadian system. However, this has been difficult to prove conclusively because the close proximity of the SCN to the optic chiasm in rodents has not made it possible to sever the RHT without destruction of the SCN. Indirect evidence of a major role for the RHT is the observation that rodents can entrain to a light/dark cycle when all known retinal inputs to the brain, except the RHT, have been destroyed. Nevertheless, disruption of the primary optic tract, leaving the RHT intact, can alter the entrainment pattern of the locomotor activity rhythm in hamsters7. The relative importance of the RHT and other retinal inputs to the brain for the entrainment of the circadian system by light still remains to be determined.

Through the use of recently developed neural anatomical tracing and staining techniques, new details about the morphology and the afferent and efferent connections of the SCN have emerged8. The SCN is a complex nucleus that has a number of recognizable subdivisions and within each division there is a heterogeneous population of neurones. Morphological studies of the SCN suggest that there is a great deal of intercellular communication within one nucleus as well as between the nuclei⁸⁻¹⁰. Inputs to the SCN arise from the retinae, ventral lateral geniculate nuclei and midbrain raphe nuclei, and a number of other inputs may exist¹⁰. Interestingly, the number of retinal ganglion cells projecting to the SCN is quite small and certain classes of ganglion cell are more likely to project to the SCN than others11. Relatively little is known about the efferent projections from the SCN but. in general, it appears they leave dorsally and caudally and many terminate among cells in various hypothalamic nuclei¹².

Direct evidence for the ability of the SCN to generate circadian rhythms has been supplied by Inouye and Kawamura¹³. They found clear evidence of circadian rhythmicity in neural activity in a variety of different brain regions when they recorded multiple unit activity with extracellular electrodes. When a Halasz knife was used to create a hypothalamic island containing the SCN, they found that circadian rhythmicity in spontaneous neural activity was abolished in all brain regions outside the island but persisted inside the island. This suggests that the SCN region is capable of generating circadian rhythms without input from elsewhere and that the rhythmicity in other brain regions is dependent upon input from the SCN. Circadian fluctuations in glucose consumption and protein synthesis 14,15

have also been observed in the SCN, although it is not known whether the rhythms are intrinsic to the SCN region itself.

A number of laboratories are attempting to record neural activity from the SCN in hypothalamic slices maintained in vitro. In a preliminary paper, Groos16 reported that single units from the rostral hypothalamus (including the SCN) maintained in vitro exhibited clear circadian rhythms in their firing rate and suggested that neural rhythms were intrinsic to the SCN.

Very few studies have examined the neurochemistry of circadian systems in mammals. Zatz17 has demonstrated that the infusion of carbachol, a cholinergic agonist, adjacent to the SCN could mimic the effects of light on the circadian rhythm of rat pineal N-acetyltransferase activity. These results suggest that acetylcholine may be involved in the photoentrainment of mammalian circadian systems and point out the potential usefulness of the neuropharmacological approach in determining the mechanisms by which circadian rhythms are entrained and selfgenerated.

Most of our understanding of the role of the SCN in the generation of circadian rhythms has been derived from investigations with small rodents, particularly rats and hamsters. However, recent studies involving lesions of the SCN region in lizards, birds and primates^{5,18} indicate that this region of the brain plays a central part in the circadian organization of many, if not all, vertebrate species.

Whether the SCN represent the master biological clock, or a major component of a complex circadian system, in mammals remains an unanswered question. However, whatever the precise role of the SCN in the generation of circadian rhythms turns out to be, one thing is clear; the SCN have provided neuroscientists with a starting point for the eventual elucidation of the neural basis of circadian rhythmicity in mammals.

Fred W. Turek is in the Department of Neurobiology and Physiology, Northwestern University, Evanston, US.

Towards the numerical magnetosphere

from Martin Brown

THE SOLAR WIND is a tenuous stream of hot protons and electrons originating in the solar corona and flowing approximately radially outwards from the Sun into the Solar System at speeds of \sim 400 km s⁻¹. The Earth's dipolar magnetic field presents an obstacle to this flow and excludes it from a cavity known as the magnetosphere. Because the speed of the solar wind is greater than all hydromagnetic wave propagation speeds in the solar wind medium, the perturbing influence of the Earth on the flow reaches only to the order of ten Earth radii upstream, where a standing shock is found. Downstream from the Earth, the magnetosphere is extended in a tail to great distances.

The large quantity of data available from satellite and ground-based observations of electromagnetic fields, flow velocities and particle number densities around the Earth, both in the magnetosphere and the undisturbed solar wind, has encouraged theoretical research into magnetospheric phenomena. There is major interest in the calculation of the steady-state flow of the solar wind past the Earth and its dynamic response to changes in the solar wind. The complex geometry and the need to consider interactions between electric currents and electromagnetic fields make a complete analytical solution inaccessible, but two recent publications (Leboeuf et al. Geophys. Res. Lett. 8; 257, 1981; Lyon et al. Phys. Rev. Lett. 46; 1038, 1981) show that techniques of numerical simulation developed for research in aerodynamics and plasma physics may be very useful. Although the application of numerical methods to the global structure and behaviour of the magnetosphere is not a new idea, previous work suffered from lack of computing power and from inadequate simulation techniques which allowed only a simplified version of the problem to be considered: the shape of the magnetosphere was assumed and a flow around it calculated (Spreiter et al. Planet. Space Sci. 17; 233, 1969). Larger computers now make more ambitious models possible, and attempts to simulate the time-dependent behaviour of the magnetosphere in two and even three dimensions, treating fields and currents in a proper self-consistent way, are very fashionable.

Two-dimensional numerical models are quite common in many fields and produce respectable results, but three-dimensional simulations are still quite rare since they tax to the limit the memory size and speed of operation of the most powerful computers available. In spite of this, notable results have been achieved with three-dimensional computer codes, especially in the fields of controlled weather prediction,

Martin Brown is in the Blackett Laboratory, Imperial College, London.

^{1.} Stephan, F.K. & Zucker, I. Proc. natn. Acad. Sci. U.S.A. 69, 1583 (1972).

Moore, R.Y. & Eichler, V.B. Brain Res. 42, 201 (1972).

Stetson, M.H. & Watson-Whitmyre, M. Science 191, 197

Rusak, B. Fedn Proc. 38, 2589 (1979).

Fuller, C.A. et al. Am. J. Physiol. (in the press). Moore, R.Y. in Biological Rhythms and their Central Mechanisms (eds Suda, M., Hayaishi, O. & Nakagawa, H.) 343 (Elsevier, Amsterdam, 1979).

Rusak, B. J. comp. Physiol. 118, 165 (1977). van den Pol, A.N. J. comp. Neurol. 191, 661 (1980). Guldner, F.H. & Wolff, J.R. Expl. Brain Res. 32, 77

^{10.} Silverman, A.J. & Pickard, G.E. Neurosci. Abstr. 6, 266

^{11.} Pickard, G.E. Brain Res. 183, 458 (1980)

Swanson, L.W. & Cowan, W.M. J. comp. Neurol. 160, 1 (1975).

Inouye, S.T. & Kawamura, H. Proc. natn. Acad. Sci. U.S.A. 76, 5962 (1979).

Schwartz, W.J. & Gainer, H. Science 197, 1089 (1977)

van den Pol, A.N. Am. J. Physiol. 240, R16-R22 (1981).

Groos, G. Chronobiologia 6, 103 (1979).

Zatz, M. Fedn Proc. 38, 2596 (1979). Takahashi, J.S. & Menaker, M. Fedn Proc. 38, 2583

thermonuclear fusion and aircraft design. The present restriction on memory size means that rather few mesh points can be used in each coordinate direction of the finite difference models. For example, in the simulation of Leboeuf et al. a mesh of size $32 \times 16 \times 8$ and spacing about one Earth radius was used, allowing only poor spatial resolution. A two-dimensional simulation (Lyon et al.) in the plane perpendicular to the path of the Earth's orbit around the Sun gives better resolution, but since the motion of particles and magnetic field lines around the sides of the Earth is important, a distorted picture emerges without resort to ad hoc procedures for attempting to include these effects.

Several recognizable magnetospheric features are reproduced in these models; density maxima are found at the nose and over the polar caps, and a low-density cavity exists behind the Earth's dipole field. The cross-sectional shape of the simulated magnetosphere also has similarities with that deduced from satellite measurements. Magnetic field line reconnection, an important process by which field lines can break and rejoin to neighbouring lines through resistive current flows, occurs in both models. Reconnection takes place in the nose of the magnetosphere where the interplanetary magnetic field (IMF), arriving with the solar wind, connects with the Earth's field, and in the long tail where the magnetic field configuration, directed away from the Earth in the southern half and towards the Earth in the northern half, is maintained by a perpendicular current sheet separating these regions. Field lines from the northern and southern halves near the current sheet can break and join each other leaving a neutral line downstream from the Earth. A so-called 'open' magnetosphere results from reconnection (Dungey Phys. Rev. Lett. 6; 47, 1961) since the IMF connects to the internal field of the magnetosphere, allowing entry of solar wind particles down the field lines. Although the general topology of these magnetic field line structures is reproduced in simulations, the rate of reconnection is much greater than it should be because of dissipative effects inherent in both methods which gives rise to 'numerical' diffusion of field lines. This causes the neutral line in the tail to be placed much nearer the Earth than it is in reality.

Some dynamic phenomena of interest appear in the models. Fluttering of the boundary of the simulated magnetosphere (Leboeuf et al.), which is also seen by spacecraft, appears to be the result of the well known Kelvin-Helmholtz instability. The two-dimensional code (Lyon et al.) attempts to model a geomagnetic substorm (a poorly understood, large-scale event in the magnetosphere powered by the solar wind) by switching on a southward-pointing IMF in the incoming solar wind. This causes rapid reconnection in the tail of

the model, the neutral line approaching the Earth and then receding in a way very similar to that seen in the magnetosphere and predicted by theory.

The numerical magnetosphere is, at present, a rather distant cousin of the real one, though they have many common

general features. These simulations can best be thought of as feasibility demonstrations; real advances will only come using much finer meshes on the next generation of large computers, and with the development of numerical schemes with less dissipation.

The evolution of cooperation

from Robert M. May

Most people are familiar with the paradox of 'The Prisoner's Dilemma', which provides an elegant metaphor for the problems of achieving mutual cooperation among members of a group. It has indeed been said (Grofman, in *Frontiers of Economics* University Publications, Virginia, 1975) that more than 2,000 papers have been written on the paradox and its philosophical implications.

In its basic form, the Prisoner's Dilemma involves two individuals, each of whom can choose either to cooperate or to defect in each of a series of discrete interactions. For these encounters, an illustrative pay-off matrix has the following character: if both players cooperate, each gains 3 units of whatever currency is at stake; if one cooperates and the other defects, the defector gains 5 units and the cooperator gets 0; if both defect, both gain 1 unit. It is apparent that, on any isolated encounter, the best strategy is to defect: if the opponent also defects, you score 1 rather than the 0 that you would have scored had you cooperated; if he cooperates, you score 5 rather than the 3 that cooperation would have gained for you. Thus, irrespective of the other player's choice, it pays to defect. But, if both players defect, both score 1 unit rather than the 3 units they could both have scored had they cooperated. Hence the

Clearly, the players' strategic decisions will depend on the likelihood of future encounters. If the individuals know they are destined never to meet again, defection is the only rational choice. By extension, if the total number of interactions is precisely known in advance, defection seems to remain the unbeatable strategy (defection is optimal on the last encounter, and therefore also on the next-to-last, and so on, back to the first).

However, if the series of encounters is to stretch indefinitely into the future, or, more generally and more realistically, if there is always a finite probability, w, that after the current interaction the two protagonists will meet again, the strategic choices become more complex.

Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

Smale (Econometrica 48; 1617, 1980) has recently brought the formal apparatus of the theory of dynamical systems to bear on the problem, in the limiting case when there is no finite horizon and no discounting of the future. In these models "There is always a tomorrow in our plans, and it is as important as today. . . . There is a history, a beginning of history, but no end". On the assumption that decisions may be based on some kind of summary or average of past outcomes, Smale shows that there is a class of 'good strategies' that are optimal and stable, corresponding to 'Nash equilibrium points'. These good strategies are essentially cooperative ones. Although Smale's analysis is formal and abstract, it clearly demonstrates that cooperative strategies can arise, provided some plausible general conditions are met.

A more empirical approach to the problem of finding a 'best' strategy is reported by Axelrod and Hamilton (Science 211; 1390, 1980), in a paper whose title I have stolen for this News and Views article. In two computer tournaments, Axelrod solicited strategies for playing the Prisoner's Dilemma game (with the specific pay-off matrix defined above) from game theorists in economics, sociology, political science, mathematics, physics and evolutionary biology. The first tournament involved a total of 15 entries, which were paired against each other in a round robin tournament, with each contest lasting 200 moves. The 15 strategies varied from the very elaborate ("An example is one which on each move models the behaviour of the other player as a Markov process, and then uses a Bayesian inference to select what seems the best choice for the long run"), to one in which each choice was totally random. The winning strategy was a simple one entered by Anatol Rapoport (of the Institute for Advanced Study at Vienna). Called TIT FOR TAT, it cooperated on the first move, and thereafter did whatever its opponent did on the previous move. Axelrod's second, larger tournament had 62 entries from six countries, and the pairwise contests lasted an average of about 200 moves (specifically, there was a probability w = 0.99654 that any given move would not be the last). Again, Rapoport's TIT FOR

TAT emerged clearly triumphant.

In retrospect the success of TIT FOR TAT can be attributed to three features: it was never the first to defect, it immediately retaliated when provoked, and it was forgiving after just one act of retaliation.

TIT FOR TAT's emergence as the 'best' strategy from the maelstrom of contenders in Axelrod's tournaments depends explicitly on the protagonists meeting again and again. Pursuing this aspect of the problem in an analytical way, Axelrod and Hamilton show that (with the pay-off matrix defined above) TIT FOR TAT will always win provided the probability, w, for a given pair of players to meet again exceeds 2/3. More generally, if the reward for mutual cooperation is R units, for mutual defection is P units, and for cooperation-defection is T to the defector and S to the cooperator, then the strategy of TIT FOR TAT will dominate all others provided that the probability, w, of a subsequent encounter exceeds

$$(T-R)/(R-S)$$

Notice that, in this general case, we must have T > R > P > S for the Prisoner's Dilemma to exist; also we require that R > (S + T)/2 (otherwise alternation of cooperation and defection is superior to sustained mutual cooperation).

These heuristic and theoretical studies of the Prisoner's Dilemma are of interest in their own right. Axelrod and Hamilton, however, go further to make a convincing case that this model sheds light on the way cooperation may evolve by darwinian selection in the natural world, among groups of individuals who are not necessarily closely related.

Axelrod and Hamilton emphasize that a formal theory for the evolution of cooperation needs to answer three questions. First, how can a cooperative strategy get an initial foothold in an environment which is predominately noncooperative? Second, what type of strategy can thrive in a varied environment composed of other individuals using a wide diversity of more or of less sophisticated strategies? Third, "under what conditions can such a strategy, once fully established, resist invasion by mutant strategies (such as 'cheating' by pure defectors)?" The studies of TIT FOR TAT answer these questions about initial viability, robustness and stability. Provided the probability that interaction between two individuals will continue is sufficiently great, cooperation based on reciprocity can indeed get started in an asocial world, can flourish in a variegated environment and can defend itself once fully established.

In sketching tentative biological applications of their ideas, Axelrod and Hamilton note that there are two main requirements for cooperation to evolve by this broad route. The first is that "an individual must not be able to get away with defecting without the other individuals being able to retaliate effectively.

The response requires that the defecting individual not be lost in an anonymous sea of others. Higher organisms avoid this problem by their well developed ablity to recognize many different individuals of their species, but lower organisms must rely on mechanisms that drastically limit the number of different individuals or colonies with which they can interact effectively". The second requirement is that the probability w for the same two individuals to meet again must be sufficiently high.

One way to help keep the interactants together is to use some fixed place of meeting. Thus aquatic cleaner mutualisms (in which a small fish or a crustacean removes and eats ectoparasites from the body, or even the inside of the mouth, of a larger fish which is its potential predator) "occur in coastal and reef situations where animals live in fixed home ranges or territories"; they seem to be unknown in the free-mixing circumstances of the open sea. Pursuing this line, the authors also argue that ant colonies participate in many cooperative mutualisms, whereas honeybee colonies — which are much less permanent in place of abode - have no known cooperative symbionts but many exploitative parasites.

An important simplification in the work of Axelrod and Hamilton, and in Axelrod's computer tournaments, is that all pay-offs are counted equally. The future is not discounted. In many

biological applications, however, future gains (measured in terms of reproductive effort or other coinage related to darwinian 'fitness') may be worth much less than current gains. One offspring in the hand can literally be worth two in the future. I think Axelrod and Hamilton's analysis can be widened to include such discounting of future gains, at least in simple cases, by formally redefining the probability of future encounters, w, to include an appropriate discount factor. If this is so, it adds a further requirement that must be satisfied before cooperation can evolve: not only must there be a sufficiently high probability that any two protagonists will encounter each other again, but also the gains from such future encounters must not be too heavily discounted. This consideration is an additional restriction on the biological circumstances under which cooperation may be able to evolve along the lines envisioned by Axelrod and Hamilton.

In a survey of organisms ranging from bacteria to primates, Axelrod and Hamilton indicate many examples where aspects of territoriality, mating systems or disease may be related to the evolution of cooperation. Intriguing though these speculations are, I think the real importance of their paper is in providing a rigorous, formal example of a mechanism whereby cooperation based on reciprocity may evolve.

New twists to DNA and DNAcarcinogen interactions

from Stephen Neidle

THE discovery of left-handed DNA structures (the Z forms) has undoubtedly provided a major impetus to the reawakening of interest both in nucleic acid structure generally and in wider questions of nucleic acid recognition. Such recognition, whether by proteins (see D.R. Davies News and Views 290; 736, 1981), or by smaller molecules such as drugs, mutagens and carcinogens, must to some extent be specific to the sequence of the nucleic acid residues involved. It is now apparent from the crystal structure analysis1 of the dodecanucleotide DNA fragment d(CGCGAATTCGCG) that subtle, yet distinctive, sequence-dependent structural features occur at differing points on a classical right-handed B-DNA-type double-helical nucleic acid. These are seen

sequence which has the potential of existing in the very different Z-DNA left-handed form. It is thus in principle interesting to ask whether such aberrant regions of structure are involved in recognition processes.

A central question concerns the con-

in an extreme form in an alternating dG-dC

A central question concerns the coexistence of left- and right-handed helices within the same trait of DNA. Recently, it has been reported that oligomeric (dG-dC) fragments of known size have been successfully inserted into DNA restriction fragments2. Both circular dichroism and phosphorus (31P) NMR measurements showed that these alternating sequences behaved in a manner typical of the $B \rightarrow Z$ transition induced by increases in salt concentration. Since the non-alternating sequences in the fragments studied did not show such behaviour, it is clear that both forms of DNA can co-exist, with a roughly eleven-base pair interface between them. The visualisation of this intermediate area

Stephen Neidle is in the Cancer Research Campaign Biomolecular Structure Research Group, at the Department of Biophysics, King's College London. will surely provide fertile ground for the model-builders, at least until relevant crystal structures are obtained. Even though the result implies that a considerable length of (dG-dC) oligomer is required for full Z character to be shown in between these interfacial regions, it is not unreasonable to suppose that rather shorter (dG-dC) traits will have some abnormal structural character.

The salt-induced conformational transition of DNA (B - Z) is the basis of all experimental probes of left-handed DNA in solution. However, it remains true that until recently, it has been no more than a strong presumption (albeit a logical one) that the high-salt form in solution does indeed correspond to the left-handed structures observed in the crystalline solid state. Evidence is now accumulating that this assumption is a valid one. Thus, Sarma and his associates3 have shown that the observed proton NMR chemical shift data for high-salt poly(dG-dC). poly(dG-dC) corresponds very well with the spectral data calculated theoretically from a Z-DNA helical structure, and not at all with that from B-DNA. Furthermore, it is evident that this close agreement almost certainly excludes other more recherché DNA structures, at least in this context. Interesting structural questions have been posed by the observation4 that the alternating copolymer, when fully methylated at the cytosine 5 position, undergoes a B-Z transition at a roughly physiological salt concentration which is much less than that required for the transition in the unmethylated polymer. Since it has often been suggested that C5 methylation in eukaryotes is related to control of gene expression, it is indeed tempting to speculate that here, at last, may be a role for Z-DNA. Indeed one keenly awaits firm evidence that any biological function actually does involve a switch of DNA helicity from B→Z. The recent production of antibodies specific for the Z form⁵ may well prove to be a powerful tool for providing such data.

It was apparent soon after the discovery of Z-DNA that certain atoms, such as the C-8 of guanine residues, are rather more exposed than in B-DNA6. The C-8 site is favoured in binding of the tricyclic aromatic hydrocarbon derivative acetylaminofluorene (AFF), a muchstudied hepatic carcinogen. In view of this, and the knowledge that at least some mutational hot-spots (for example in

'Ames-test' histidine genes of Salmonella strains) contain clusters of alternating (dGdC) residues, there has recently been much interest in the possibility that Z-DNA is involved in carcinogenic processes.

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Interaction of poly(dG-dC). poly(dGdC) with the active species N-acetoxy-AAF results in random modification7-9 of the polynucleotide, which then shows Z-like character even at low salt concentrations. No helix disruption seems to have occurred as the polynucleotide is not susceptible to S, nuclease which attacks single-stranded regions. The physical model that emerges is of AAF attaching to guanine residues which then for purely steric reasons have to be in the syn conformation of Z-DNA itself. On the other hand, with nonalternating (essentially random) sequences AAF binding involves some local destabilisation of the double helix, such as is suggested by a base displacement model9. This has the syn guanine residue no longer paired but swung out and displaced from the double helix. One suspects that these two types of AAF-binding models are actually closely related in structural terms. It will be of some interest to see how they differ biologically, such as in their susceptibility to excision repair.

Diphtheria toxin: which route into the cell?

from Simon van Heyningen

A single molecule of diphtheria toxin can kill a eukaryotic cell. Several other bacterial protein toxins also have comparable activity and all are large molecules which have to penetrate the cell membrane before they can act. Diphtheria toxin was the first of these to be understood, at least in outline, but several important details have only recently been published.

Diphtheria toxin (molecular weight 60,000) is made up of two polypeptide chains, fragment A (21,000) and fragment B (39,000), linked by a disulphide bond. The toxin first binds to cells through the B fragment. This leads to the entry into the cell of fragment A - which then folds to an active conformation, and catalyses a reaction in which the ADP-ribose of NAD is transferred to elongating factor 2 (EF2), an essential protein component of ribosomal protein synthesis. Since ADPribosylated EF2 is inactive, protein synthesis stops and the cell dies. It is because fragment A is an enzyme that the toxin is so active.

Fragment A is highly specific. Its only significant substrate is a single amino acid residue in EF2 whose structure has recently been determined by NMR1. It is a complex derivative of histidine named 'diphthamide', 2-(3-carboxyamido-3-[trimethylammoniol propyl)histidine. The specificity of the toxin is not, however, just for diphthamide, but for something more complicated: peptides containing diphthamide isolated from tryptic digests of EF2 are not ADP-ribosylated.

Since the toxin ADP-ribosylates the EF2 of all eukaryotes (and even of the archaebacteria), diphthamide must be highly conserved. The amino acid sequence around it also shows little species variation. It is hard to believe that so complicated a modification has evolved to provide a substrate for diphtheria toxin, so one might expect diphthamide to be required for the action of EF2. Surprisingly, however, mutants that lack diphthamide (and are insensitive to toxin) show no defect in protein synthesis. These mutants can be divided into three complementation groups, in agreement with the number of enzymic modifications which the structure of diphthamide suggests would be needed to make it from histidine. Diphthamide is presumably also the substrate for ADPribosylation by the toxin of Pseudomonas aeruginosa, which works in a very similar way, but not for cholera or E. coli toxins which are less specific and ADP-ribosylate different proteins.

Before fragment A can have its effect on EF2 it first has to pass into the cell. Entry begins with the binding of fragment B to a receptor on the outside of the cell membrane. The nature of this receptor was unknown until recently (although it had been mapped on the human chromosome), but is has now been identified as a glycoprotein that can be isolated from guinea-pig cells by immunoprecipitation². It is not a true receptor in the endocrinological sense, since binding to it does not in itself have any effect. Rather, it is a prerequisite for entry that the toxin birds firmly to the cell. Quite different ways of doing this are effective; derivatives of fragment A with other cell-surface ligands such as antibodies or even subunit B of cholera toxin can still enter the cell.

Once the toxin is bound there are two different ways in which the A fragment could enter the cell. It could cross the

Simon van Heyningen is in the Department of Biochemistry, University of Edinburgh Medical School.

^{1.} Drew, H.R. et al. Proc. natn. Acad. Sci. U.S.A. 78,

Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A. & Wells, R.D. *Nature* 290, 672 (1981).
 Mitra, C.K., Sarma, M.H. & Sarma, R.H. *Biochemistry*

^{20, 2036 (1981).} 4. Behe, M. & Felsenfeld, G. Proc. natn. Acad. Sci. U.S.A.

^{5.} Lafer, E.M. et al. Proc. natn. Acad. Sci. U.S.A. 78, 3546

Wang, A.H.-J. et al. Nature 282, 680 (1979).

Sage, E. & Leng, M. Proc. natn. Acad. Sci. U.S.A. 77, 4597 (1980).

Idem Nucleic Acids Res. 9, 1241 (1981).
Santella, R.M., Grunberger, D., Weinsteing, I.B. & Rich, A. Proc. natn. Acad. Sci. U.S.A. 78, 1451 (1981).

membrane directly (either by itself or with a carrier), or it could be endocytosed, in which case the problem of crossing the membrane into the cytosol would still remain. For this and other reasons, endocytosis has not been a favoured hypothesis, but some new experiments have made it seem much more likely³.

Uptake of toxin is greatly increased at low pH. When cells are exposed to toxin at pH 4.5 for ten minutes, the rate of decrease in protein synthesis is the same as it would have been using a thousand times as much toxin at neutral pH. As the pH is low in the lysosomes, the results immediately suggest that that is where toxin might normally enter the cell. There is other evidence that this is what happens. When the pH inside the lysosomes is increased by incubating the cells with ammonium chloride, the toxin is very much less active (although its binding to the cell surface is not decreased). However, this protective effect of ammonium chloride is abolished if the cells are exposed to low pH for even as little as 20 seconds. Experiments using antisera suggest that, at neutral pH, toxin is trapped in intracellular vesicles, but, at low pH, it can penetrate the membrane directly; lysosomes are no longer involved and so ammonium chloride has no effect.

What may normally happen is that the toxin is taken up by endocytosis into intracellular vesicles which then fuse with lysosomes. Most of the toxin will be destroyed by the lysosomal enzymes, but at the low pH in the lysosomes, a few molecules of fragment A will escape into the cytoplasm.

How does fragment A penetrate membranes at low pH? Recent experiments using artificial lipid bilayers may provide a clue4. Diphtheria toxin and a mutant (crm 45) that lacks the receptor-binding region of fragment B can bind to these layers and form transmembrane channels through them. These channels are formed best when one side of the membrane is at low pH and the other is neutral, and their width (perhaps about 18 Å diameter though there is some disagreement) is probably large enough for fragment A to pass through provided it unfolds to a fully extended form. Fragment A is a very stable protein which could easily refold to a native conformation afterwards.

The formation of these channels probably involves more than one molecule of fragment B transversing the membrane. Fragment B is well able to do this: it has a

hydrophobic domain that could dissolve in the core of the membrane lipids, and a hydrophilic domain that could interact with the polar head groups⁵.

It seems unlikely that the mechanisms used by bacterial toxins for entering cells

and affecting their metabolism are unique. Rather, they may also be used in other, as yet unidentified, ways. Study of these curious proteins should help the understanding of the normal properties of cells.

The actin-myosin interface

from Howard White

UNDERSTANDING the molecular mechanism of muscle contraction will almost certainly require a detailed knowledge of the structure of, and the interaction between, the primary contractile proteins, actin and myosin. A simple model in which each myosin-S1 binds to a site completely contained with a single actin subunit has been generally assumed from the binding stoichiometry of one myosin-S1 per actin subunit. The observation made several years ago by Chantler and Gratzer (Biochemistry, 15, 2219; 1976) that myosin-S1 binds to monomeric actin covantly bound to sepharose strengthened the case for a simple one-to-one interaction between each myosin head and actin filament subunit.

In this issue of *Nature* (p.301) Mornet *et al.* report that a water soluble carbodiimide crosslinks the 95K dalton heavy chain of myosin-S1 to two actin subunits and conclude, that the binding site for each myosin-S1 to filamentous actin spans two actin subunits or is at least close enough to form zero-length crosslinks with two actin subunits. Although crosslinking experiments of this type have a rather perilous history, the authors have devised an extensive series of controls that considerably strengthen their argument.

First, the crosslinking reaction is quite specific. Actin activated with carbodiimide is crosslinked to myosin-S1 but myosin-S1 activated with carbodiimide is not crosslinked to actin. The crosslinking reaction is prevented by either pyrophosphate or MgATP, which specifically dissociates myosin-S1 from actin. Second, the composition of the crosslinked species, two actin subunits and one myosin-S1 heavy chain, initially suggested by a molecular weight of 180K dalton on SDS gels was verified by a molar ratio of two 3 H-actin to ¹⁴C-S1 in the crosslinked polypeptide. Third, the possibility that S1 binding to actin enabled intermolecular crosslinks to be formed between actin subunits was eliminated by the following experiments. Trypsin specifically cleaves the heavy chain of myosin-S1 bound to f-actin into two peptides of 75K and 22K daltons; cleavage of carbodiimide crosslinked actomyosin-S1 produces two peptides with molecular weight of 120K and 64K daltons. The proteolytic fragments of the

crosslinked actomyosin-S1 were identified as 22K (S1)-actin and 75K (S1)-actin by specific fluorescent labeling of the actin and myosin-S1 before crosslinking and cleavage.

Hence, two different regions of the myosin-S1 heavy chain appear to be cross-linked to separate actin subunits. The authors propose a model in which the myosin-S1 binds to a site comprising two actin subunits on opposite strands of the actin helix. However, their data and recent three-dimensional reconstructions obtained from electron micrographs of negatively stained actomysin-S1 filaments (Taylor and Amos J. molec. Biol. 147, 297; 1981) are equally compatible with the myosin-S1 being bound to two actin subunits on the same strand of the actin filament.

The enzymatic properties of the crosslinked actomyosin may provide a new tool to help unravel the increasingly complex mechanism of actomyosin ATP hydrolysis. Crosslinked actomyosin-S1 hyrolyzes MgATP at a rate comparable to the rate of hydrolysis by actomyosin-S1 in solution extrapolated to infinite actin concentration. There is, however, one notable difference — the large dependence of rate upon ionic strength that is so characteristic of actomyosin-S1 in solution (primarily due to the ionic strength dependence of the equilibrium of $M \cdot ADP \cdot P_i + actin \ge acto \cdot M \cdot ADP \cdot P_i$ which precedes the rate-limiting step) is absent. Crosslinked actomyosin-S1 thus appears to provide a covalent model for the so-called non-dissociating pathway of actomyosin ATP hydrolysis that could previously be observed only at a very low, non-physiological ionic strengths.

One may well ask how all of this fits the notion of rotation of the myosin heads bound to actin — the postulated work-producing step of most of the popular models of contraction today. The molecular basis of muscle contraction is indeed far from being solved and continues to provide both the structuralists and biochemists with more riddles to decipher.

Van Ness, B.G., Howard, J.B. & Bodley, J.W. J. biol. Chem. 255, 10710 and 10717 (1980).

Proia, R.L., Hart, D.A., Holmes, R.K., Holmes, K.V. & Eidels, L. Proc. natl Acad. Sci. U.S.A. 76, 685 (1979).

Sandvig, K. & Olsnes, S. J. Cell Biol. 87, 828 (1980); and Draper, R.K. & Simon M.I. J. Cell Biol. 87, 849 (1980).

Donovan, J.J., Simon, M.I., Draper, R.K. & Montal, M. Proc. natl Acad. Sci. U.S.A. 78, 172 (1981); and Kagan, B.L., Finkelstein, R.A. & Colombini, M. Proc. natl. Acad. Sci. U.S.A., in the press.

natl. Acad. Sci. U.S.A., in the press.

5. Lambotte, P., Falmagne, P., Capiau, C., Zanen J.,
Ruysschaert, J.-M. & Dirkx, J. J. Cell Biol. 87, 837
(1980).

Howard White is assistant Professor of Biochemistry at the University of Arizona in Tucson.

ARTICLES

Magma chamber profiles from the Bay of Islands ophiolite complex

J. F. Casey*‡ & J. A. Karson†

*Department of Geological Sciences, State University of New York at Albany, Albany, New York 12222, USA †Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Models of the oceanic lithosphere derived from constraints imposed by the geology of ophiolite complexes require a magma chamber to produce the cumulate and other plutonic portions of the oceanic crust and upper mantle. Evidence from the Bay of Islands ophiolite complex indicates that layered plutonic rocks have not formed solely due to differential crystal settling to the horizontal floor of a magma chamber, but have formed largely by heterogeneous nucleation and in situ crystallization along gently inclined as well as steeply inclined bounding surfaces of a large magma reservoir. Coherent, well defined and systematically oriented, large-scale, arcuate patterns described by mesoscopic igneous layering have been identified in the plutonic sections of two ophiolite massifs in the Bay of Islands complex. These layering patterns seem to outline the shape of a large, continuously evolving, steady-state magma chamber that extended along a ridge axis and ended at a transform fault intersection.

EVIDENCE from marine geology, geophysics and theoretical considerations suggests that steady-state magma chambers may exist beneath fast-spreading accreting plate boundaries 1-6 while only small ephemeral chambers may occur along slow-spreading plate boundaries⁷. Previous models for the evolution of the oceanic crust and upper mantle have been built around the concept of a steady-state chamber and generalized observations from ophiolite complexes^{6,8-11}. Ophiolite complexes provide cross-sectional views of large-scale, metamorphic-plutonicvolcanic layered sequences widely believed to be on-land exposures of ancient oceanic lithosphere 11,12. There is general agreement that volcanic rocks and underlying sheeted diabase dykes are formed by continuous crustal extension and magmatism at an oceanic spreading centre. The evolution of the underlying stratiform plutonic assemblage, however, has been modelled in several different ways. All models must deal with the problem of producing a subhorizontally layered assemblage from a continuously widening subvertical crack. All previous steady-state models assume the formation of cumulates occurs only along subhorizontal surfaces, but this requires extremely large magma chambers^{9,11} uhless tectonic rotations occur⁸. Most interpretations of ophiolites suggest that cumulate layering should be restored to a subhorizontal orientation parallel to the large-scale stratiform structure of the complex 11,13,14. Evidence from the plutonic sections of the Bay of Islands ophiolite complex, however, indicates that 'cumulates' have formed along steeply inclined magma chamber walls and that the final geometry of the fine-scale igneous layering is much more complex than that assumed in most previous models or than is apparent from reconstructions of dismembered ophiolites.

Bay of Islands ophiolite complex

The Bay of Islands ophilite complex (Fig. 1) is located along the west coast of the island of Newfoundland, Canada. The ophiloite is exposed in four large massifs (each ~ 20 km across) which are considered to be the dissected remnants of a once continuous allochthonous sheet of early Ordovician oceanic crust and upper mantle. The tectonic setting and rationale for the interpretation of the complex as oceanic lithosphere are discussed elsewhere 15-20. The large-scale stratiform structure of the ophiloite complex (Fig. 2) includes (from base to top): metamorphic tectonic periodotite, ultramafic and mafic plutonic rocks,

†Present address: Geology Department, University of Houston, Houston, Texas 77004, USA.

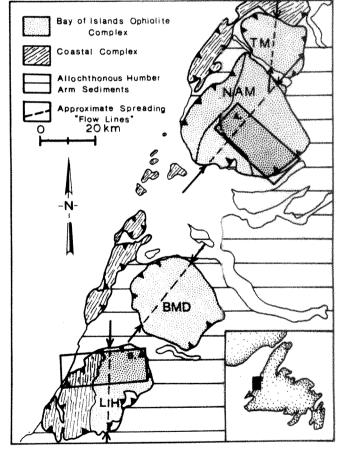


Fig. 1 Generalized geological setting of the Bay of Islands ophiolite complex. Inset shows location in western Newfoundland. Boxes a and b show location of sketch maps in Fig. 3. Massifs: TM, Table Mountain; NAM, North American Mountain; BMD, Blow-Me-Down Mountain; and LH, Lewis Hills. Arrows and dashed lines indicate trace of inferred seafloor spreading flow lines.

sheeted diabase dykes, and basaltic volcanic rocks. The Bay of Islands ophiolite clearly conforms to the current working definition of an ophilite complex¹¹ and includes wide outcrop areas suitable for detailed studies. Although the uppermost units have in some places been removed by erosion, and some

sections are structurally dismembered, uninterrupted sections interpreted as exposures of former upper mantle to sea floor, are well preserved.

The Bay of Islands complex may be divided into two petrogenetically distinct components (Fig. 2), a residual upper mantle assemblage depleted by subcrustal partial fusion and extraction of basaltic liquids^{8,21,22}, and a 'magmatic' component, the assemblage derived by differentiation of those basaltic extracts. The magmatic component can be divided into two large-scale structural assemblages²³. One is an upper intrusive-extrusive carapace of mafic volcanics and sheeted diabase dykes interpreted as the continuously extended and rapidly cooled lid of a magma chamber. The second is a plutonic group believed to have formed by fractionation of basaltic melt in a crustal-level magma chamber. This group includes (from base to top): (1) layered ultramafic cumulates, mainly dunite, wehrlite, clinopyroxenite and chromitite; (2) interlayered ultramafic and gabbroic cumulates (the transition zone), mainly dunite, wehrlite, troctolite, olivine-gabbro and anorthosite; (3) layered gabbroic cumulates, mainly troctolite, olivine-gabbro and gabbro; and (4) isotropic gabbroic rocks, mainly gabbro and metagabbro. This type of plutonic association has been observed in many ophiolite complexes and, by analogy to continental stratiform intrusive bodies, is interpreted in terms of magma chamber processes 11.3 We believe that the internal structure of the plutonic group of the magmatic component can provide some useful constraints on the dimensions and shape of the magma chamber(s) from which the complex formed.

It is assumed in reconstructing the massifs, which are now folded on a large scale, that major, lithostratigraphic-unit contacts had a subhorizontal orientation before obduction and that the mean orientation of diabase dykes was parallel or subparallel with the ridge axial plane at the time of formation. In general, two distinct types of crustal cross-sections are preserved in surface exposures of the complex. One type is reconstructed

as an approximately vertical section through the Bay of Islands complex and is oriented parallel to a spreading flow line (that is, normal to the palaeo-spreading centre and parallel to fracture zones). This type of section is exposed in map view of the northern three massifs (Figs 1 and 3a) due to tilting of major lithostratigraphic units about obduction-related, clinal fold axes oriented approximately parallel to the spreading direction²⁵. The second type of section is preserved in surface exposures of the southernmost massif, the Lewis Hills (Figs 1 and 3b). The Lewis Hills section exposes the intersection of the Bay of Islands complex with a subvertical zone containing a highly deformed and metamorphosed ophiolitic assemblage, the coastal complex, which is interpreted as oceanic crust and upper mantle with a history of transform fault tectonics predating its juxtaposition with the Bay of Islands complex^{20,26}. The outcrop pattern of the Lewis Hills massif is controlled by a large scale, obduction-related syncline; its axis plunging gently to the northnorthwest approximately parallel with the inferred spreading direction and the trend of the coastal complex. The western part of the massif lies in the core of this syncline and exposes the subvertical, highly-deformed coastal complex. The eastern side of the massif is tilted to the west and exposes the deeper structural levels of the plutonic section and the upper levels of the harzburgite section of the Bay of Islands complex.

Internal structure of the plutonic group

Contrary to assumptions commonly made in the reconstruction of dismembered ophiolite sections, the mesoscopic-scale cumulate layering is generally not parallel to the contacts between the large-scale lithologic units as first noted by Dewey and Kidd⁸. Broad arcuate patterns, defined by cumulate layering oriented up to 90° oblique to lithologic unit contacts, are observed within the plutonic group. Although in detail these arcuate patterns

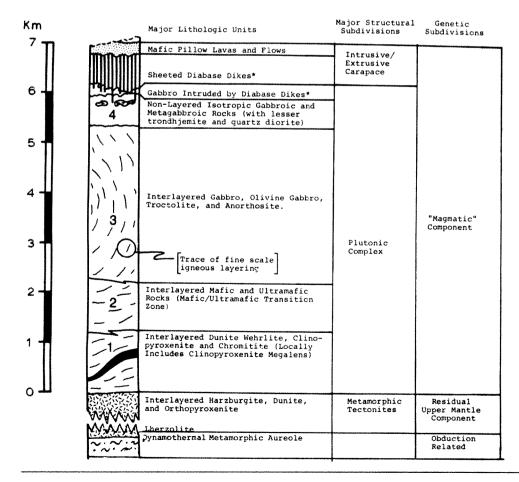


Fig. 2 Generalized columnar section through the Bay of Islands ophiolite complex and interpretations of major lithologic units. The magmatic component is to scale; the residual component is condensed (actual thickness approaches 4-5 km).

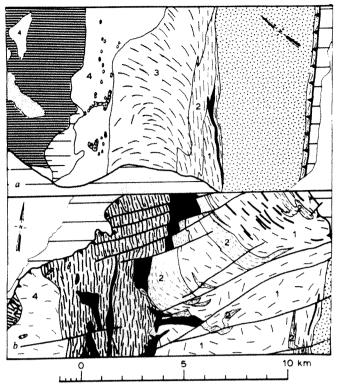


Fig. 3 Geological sketch maps of two areas in the Bay of Islands ophiolite complex (see Fig. 1). a, Southern half of the North Arm Mountain massif. Shading as in Figs 1 and 2, ruled lines on the diabase dyke units are oriented in the approximate direction of the mean dyke trend. b, Central part of the Lewis Hills massif. Light grey shading and bold squiggles indicates highly deformed rocks of the coastal complex including highly strained ultramafics, mertagabbros, amphibolites, and granulites; dark grey shading indicates intrusive periodotite bodies of the coastal complex; bold dashed lines are chromitite megalens units. Both maps are oriented with the mean trend of diabase dykes (the palaeo-ridge axis trend) parallel with the longer edges of each map.

appear to be quite irregular, the large-scale relationships are clear. In two of the massifs, North Arm Mountain and the Lewis Hills (Fig. 3), detailed mapping has revealed especially welldefined arcuate structures described by fine-scale igneous layering. The patterns identified in these two massifs, however, have very different geometric relationships with respect to the inferred spreading direction during formation of the complex. (The textural terminology and classifications systems developed for rocks believed to have originated by crystal settling^{27,28} can be applied to many coarse-grained plutonic rocks regardless of origin. We do not interpret layered plutonic rocks of the Bay of Islands ophiolite as cumulates in the genetic sense, but use the terms cumulate, cumulus, primocryst, intercumulus and postcumulus because they are descriptive regardless of the rocks origin and because a satisfactory, non-genetic terminology is not well developed. For further discussions see refs 29, 30.)

The southern half of the North Arm Mountain massif²⁵ (Fig. 3a) exhibits the best defined example of this arcuate structure in the Bay of Islands complex. We emphasize, however, that similar discordant relationships between cumulate layering and unit contacts have been mapped in all of the other massifs. In the North Arm Mountain massif a nearly complete ophiolite sequence is preserved. The present-day erosional surface which truncates the sequence is nearly parallel to the inferred seafloor spreading flow lines, that is, normal to the mean trend of diabase dykes in the sheeted dyke unit. Fine-scale igneous (cumulate) layering attitudes within the plutonic group change gradually and continuously upward from subparallel to the major lithologic unit contacts (that is palaeohorizontal planes) in the basal ultramafic and transition zone units to orientations highly oblique to these contacts in the gabbroic units. At the upper

contact of the layered gabbroic rocks (Unit 3, Fig. 3a), layering attitudes are $\sim 90^{\circ}$ or even apparently overturned with respect to the major lithologic unit contacts. In a reconstructed section the discordant fine-scale igneous layering consistently strikes subparallel to the sheeted dyke direction while defining broad arcuate patterns as depicted in Figs 3 and 4. The axis of this arc or bend in the layering lies approximately in the plane of the major lithologic unit contacts and also in the plane of the mean dyke orientations indicating that, as exposed, the pattern essentially defines a cross-section of a half cylinder whose long axis originally lay in a subhorizontal orientation within the ridge axial plane. The convex side of the arc points towards the spreading direction which is inferred from one-way chilling statistics from the sheeted dyke unit and the sense of shear deduced along the coastal complex transform 20,26 .

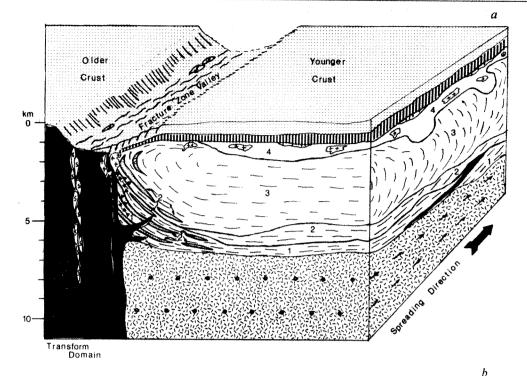
A single clinopyroxenite-rich 'megalens' paralleling fine-scale layering occurs within the ultramafic cumulates on North Arm Mountain. This megalens varies in thickness from 0 to 300 m (Fig. 3a) and consists of aggregates of small lenses or layers of dominantly clinopyroxenite, with lesser amounts of interlayered dunite, wehrlite and chromitite. This megalens can be traced laterally for over 4.0 km as it cuts obliquely across the ultramafic cumulate section. Where the megalens intersects the lower contact of the transition zone, clinopyroxenites become interlayered with gabbroic rocks. These gabbroic layers increase in abundance with height in the transition zone at the expense of clinopyroxenite layers until the megalens cannot be distinguished.

An important relationship within the plutonic group concerns the relative orientations of fine-scale igneous layering, megalenses, the contacts between major lithologic subdivisions of the plutonic assemblage, and the direction of cryptic chemical variations. In continental stratiform intrusions, to a first approximation, fine-scale igneous layering is controlled by and lies parallel to the various major rock units. It also remains orthogonal to the direction of cryptic chemical variations. Fine-scale layering and megalenses in the Bay of Islands complex plutonic group, however, consistently maintain an oblique relationship to the contacts beween the major lithologically defined subdivisions of the plutonic group and in some cases have trends parallel with the direction of cryptic chemical variations²².

Similar, but as yet less well-defined arcuate structures occur elsewhere in the Bay of Islands complex on the Blow-Me-Down Mountain and Table Mountain massifs where highly oblique layering relationships are also present. In the Lewis Hills massif^{26,32}, a different, but well-defined arcuate structure is present (Fig. 3b). Cumulate layering and (layered cyclic) megalens units up to 1.0 km thick are mutually parallel throughout the plutonic group. In the eastern half of the massif these features lie subparallel to the basal contact of the magmatic suite along that contact and in the central part of the massif layering is subhorizontal. To the west, however, fine-scale igneous layers vary continuously in orientation through approximately 90° approaching subvertical adjacent to the coastal complex. These layers commonly include local concentrations of angular detached blocks of layered gabbro. This geometry and these intrusive relationships suggests lapping of the plutonic group against the subvertical contact with high-grade metamorphic rocks of the coastal complex. The structure preserved in the Lewis Hills massif probably formed as relatively young crust of the Bay of Islands complex was accreted to older deformed crust of the coastal complex at a ridge/transform intersection^{20,26}. The axis of the large-scale arcuate structure defined in this area lies parallel to the inferred trend of the fracture zone and normal to the attitudes of undeformed diabase dykes in the massif (that is, approximately perpendicular to the axis defined for the northern massifs).

Interpretation of the internal structure of the plutonic group

Although the large-scale lithologic unit thicknesses and details of the internal structure of the plutonic group vary considerably



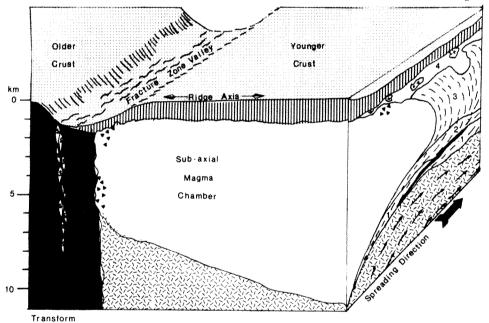


Fig. 4 a, Block diagram summarizing the reconstructed preobduction, post-accretion geology geometrical relationships within the Bay of Islands and coastal complexes. The front face of the block diagram is oriented perpendicular to the spreading direction and crosses an inactive transform domain (the coastal complex). The other face of the diagram is oriented parallel to the spreading direction. b, Block diagram depicting a schematic model for the formation of the geology depicted in a. Orientation of diagram is similar, except that the front face is coplanar with the ridge axial plane and it crosses the transform domain at the point at which it becomes inactive. The arcuate geometry of the fine-scale igneous layering and megalenses is the product of heterogeneous nucleation, in situ crystallization and layer formation along the bounding surfaces of the steady-state magma chamber. Shading as in Figs. 1-3. S. serpentinite: ▲ xenoliths; small arrows, flow paths in the upper mantle.

across the Bay of Islands complex²³, these differences seem to represent variations on a fairly simple theme. In the two types of arcuate structures described above, mesoscopic and sometimes even larger-scale cumulate layering are clearly not parallel to the contacts between the large-scale lithologic units. By analogy with the inferred horizontally-layered seismic structure of contemporary oceanic crust these contacts probably originated in a subhorizontal orientation. Whereas the deepest structural levels of the Bay of Islands plutonic group show signs of extensive subsolidus deformation, we emphasize that the bulk of this group has not been penetratively deformed^{23,25,26}. Therefore, we believe that the first-order geometry of layering as described above must be the result of igneous processes because we have accounted for all obduction or post-obduction structures in the reconstructions.

Domain

Numerous studies of igneous plutons with cumulate layering indicate that cumulate textures and layering commonly can form along subvertical walls of magma chambers as well as sub-

horizontal floors ^{29,30,33-36}. Integrated field and theoretical studies of cumulate rocks suggest that crystals nucleate and grow mainly along the margins of large magma chambers rather than homogeneously throughout the magma followed by vertical settling²⁹. Thus, cumulate layering may mimic the shape of the walls of such magma chambers as, we believe, is the case in the Bay of Islands complex. Any model for the origin of the igneous layering requires formation along the magma/wall (or floor) interface and therefore these layers must define isochrons. The fact these igneous layers as well as megalens units generally cut across major lithologic unit contacts and sometimes parallel the general direction of cryptic chemical variation within the plutonic group of the Bay of Islands complex implies pronounced and probably systematic physical and/or chemical heterogeneities along the bounding surface of the magma chamber.

Certain detailed aspects of the plutonic rocks, which are pertinent to the discussion of the origin of the fine-scale igneous layering in the Bay of Islands complex, also support the conten-

tion made above on the grounds of layer discordancy that much of the plutonic section formed by in situ crystallization processes at the bounding surface of the magma chamber. Fine-scale igneous layering in the plutonic section generally ranges in thickness from less than a centimetre to a few metres. Two types of layering are dominant. The first type, designated 'uniform layering', is characterized by uniform modal proportions and physical and textural properties. The second type, designated 'stratified layering', is characterized by size and/or modal stratification. The most common examples of stratified layers in the complex are those exhibiting modal stratification, but lacking size stratification. Size-stratified layers are present only locally and layers exhibiting both size and modal stratification are rare. Many of the stratified layers exhibit reversed stratification and some single layers exhibit both normal and reversed stratification. Where studied in detail²⁵, however, most layers (>80%) in the Bay of Islands plutonic sections are uniform in character. Consequently, the dominant sequence of layer types consists of successive uniform layers with a general lack or total absence of interlayered stratified layers. These sequences of solely uniform fine-scale igneous layers cannot be explained by the 'intermittent current hypothesis' proposed by Wager and others^{27,37} which is a model of layer formation by differential crystal settling in conditions of continuous crystallization within a magma chamber, because such a model demands that layer sequences be characterized by either alternating uniform and stratified layers, or successive stratified layers. Because of the abundance of sequences exhibiting successive uniform layers without intervening stratified layers, we believe the crystal settling mechanism of layer differentiation proposed by these workers was probably not important in the Bay of Islands complex. Goode³⁸ proposed an alternative mechanism involving differential crystal settling, not in conditions of continuous crystal nucleation, but in conditions of discontinuous nucleation. This model can, in theory, explain the existence of reversely stratified layers and can account for successively repeated uniform layers having a single cumulus (that is, primocryst) phase. Successively repeated uniform layers having more than one primocryst phase are, however, abundant in the transition zone and layered gabbroic units of the complex and they are often bounded by sharp form contacts²⁸ which involve no change in mineralogy, but simple changes in grain size, texture, or modal proportions. This type of layer sequence is not predicted or readily explained by this alternative model of layer development by differential crystal settling. We suggest that the sequences of layer types observed in the plutonic section of the Bay of Islands may be more appropriately interpreted in terms of in situ crystallization models rather than differential crystal settling models.

Comb layering^{34,39}, inch-scale layering⁴⁰ and intercumulus layering³⁸ are locally abundant in the plutonic section and constitute layer types which are interpreted to form by in situ crystallization processes^{28,29,36,38}. Colloform growth structures similar to those described within the marginal border group of the Skaergaard intrusion^{28,36} and layers exhibiting discontinuous protrusions which resemble reef-like growth structures and commonly contain elongate dendritic crystals oriented perpendicular to the regional strike of more normal layering are also commonly observed and can be interpreted in a similar fashion. Chromitites exhibiting chain textures occur in the ultramafic unit of the plutonic section and may imply in situ crystallization and/or heterogeneous nucleation²⁹. Oikocrystic textures characteristic of some wehrlitic and feldspathic dunites and some gabbroic rocks are difficult to interpret as the result of crystal settling and accumulation processes followed by in situ post-cumulus oikocrystic growth²⁵, rather, the lack of a selfsupporting crystal framework of supposed, smaller primocryst phases included within the larger, supposed post-cumulus oikocrysts may indicate a wholly in situ origin for these rocks. Considered with evidence on the discordancy of the fine-scale igneous layering, we interpret these data to indicate that the bulk of the plutonic section has formed as the result of nucleation and growth of crystals at the bounding surface of the magma chamber. Heterogeneous nucleation of crystals at the margins of the chamber may, in part, be the result of the very low degrees of super-cooling expected in the interiors of extremely large magma chambers at mid-ocean ridges.

Lateral continuity to thickness ratios of fine-scale layers are very small in comparison to most continental layered mafic intrusion^{28,40} and even the thickest layers can seldom be followed for more than 50 m along strike. While lithologically distinct megalens units within the plutonic sections can be followed for significant distances along strike (as much as 4 km), their constituent fine-scale layers are also lens-like pinching out in either direction along strike over as much as 50 m or as little as 1 m. If these layered plutonic rocks form due to in situ crystallization, the lack of lateral continuity must be interpreted as the result of fairly significant lateral changes in physico-chemical conditions on a scale approximated by the lateral continuity of the layering. This may indicate that the edges of the magma chamber were periodically or continuously characterized by a fairly stagnant boundary layer36 in which diffusion-controlled differentiation processes might be operative.

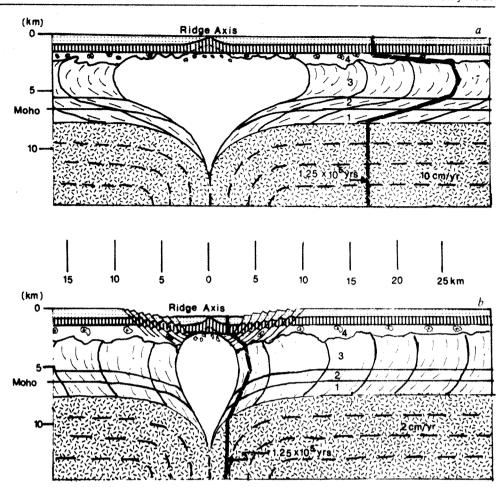
In the northern three massifs the arcuate shapes defined by cumulate layering are interpreted as profiles generated successively along one side of an elongate cylindrical magma chamber. We prefer to interpret this structure as the product of progressive growth of the plutonic assemblage as it rode first upwards and then laterally away from the spreading centre axis above a depleted-upper-mantle conveyor belt (Fig. 4). The suggested structure (Fig. 5) is very similar to a first approximation to the "infinite onion" model first proposed by Cann⁶. In fact, the details of the reconstructed profiles in the Bay of Islands complex are even more onion-like than those of Cann's theoretical model, however, the genetic distinctions made by Cann between cumulate, layered gabbro and isotropic, plated gabbro do not seem to apply here.

The magma chamber profiles and major lithologic units of the plutonic section observed in the Bay of Islands complex seem to be continuous and systematic and to lack large-scale truncations. This strongly suggests that a steady-state magma chamber was present. The longest axis of the chamber was parallel to the ridge axis; its ultimate length being limited by its truncation at fracture zone intersections. The minimum height of the magma chamber can be estimated from the thickness of the plutonic group to be ~6 km. While allowing for possible rotation of unit contacts in the structurally deepest regions due to the motion of the underlying residual upper mantle, reasonable estimates of maximum magma chamber height based on the North Arm Mountain structure would range from 6 to 8 km if measured at the precise ridge axis. Assuming bilateral symmetry, the width of the chamber could have been as much as 14-16 km based on the length of the clinopyroxenite megalens and the trajectory of fine-scale igneous layering in the overlying transition zone and gabbroic unit. Such a large magma chamber is not inconsistent with theoretical thermal calculations^{5,42} and seismic results^{2,3,14} for mid-ocean ridges with fast spreading

The magma chamber profiles described above appear to be quite variable as layering trends are often very irregular locally. Even in map areas adjacent to those described here similar large-scale arcuate structures are not well defined. These areas are characterized by highly variable discordant layer orientations and may reflect considerable unevenness in the morphology of the chamber walls in time and space. Some of these irregularities might evolve due to magma current erosion and/or the growth of reef-like protrusions formed by localized rapid growth at points along the walls²⁵. Localized fracturing and cooling of the crust by hydrothermal circulation might also cause cold spots along chamber walls where crystallization might occur at an accelerated pace.

In the Lewis Hills massif, an arcuate profile oriented approximately perpendicular to those in the northern massifs exists. This structure is interpreted as the result of vertical pinching-out

Fig. 5 Schematic models for the formation of oceanic lithosphere depicted by cross sections oriented perpendicular to a fast (10 cm yr half spreading rate) spreading centre (a) and a slow (2 cm yr spreading centre (b). Shading as in Fig. 2. a is based on the geology and approximate unit thick nesses observed for the North Arm Mountain section. intrusive/extrusive carapace of pillow lava, diabase and some underplated gabbro floating over magma chambers of different widths and different shapes are depicted. Chambers are situated over the diverging limbs of depleted upper mantle. Bold dashed lines indicate 1.25×10^5 yr isochrons. Bold solid lines depict the shapes of the steady-state magma chamber at various times in the past as determined from the arcuate patterns of fine-scale igneous layering (thin dashed lines). Note that the fine-scale igneous layering (or time lines) strike obliquely across the major lithologic subdivisions of the plutonic section reflecting chemical zontation of the magma chamber. Flow lines for the upper mantle and overlying crust are depicted by dashed lines in the residual mantle unit. Sea floor topography depends on the equilibrium size of the



magma chamber⁴⁵ which is ultimately determined by the spreading rate^{5,42}. The final fine-scale igneous layering geometry is determined by the size and shape of the former magma chamber.

of the magma chamber where it was truncated at a ridge-ridge transform fault. The structure seems to indicate that the lower surface of the magma chamber shallowed considerably at this intersection while the seafloor presumably deepened. Assuming no major change in the level of the top of the harzburgite unit, a relatively thin overlying gabbroic unit is inferred (Fig. 4b). Overall the structure suggests decreasing height of the magma chamber as the fracture zone intersection is approached. Lateral dimensional changes cannot be clearly resolved in the preserved layering geometry. Very irregular layering, abundant gabbro xenoliths, and periodotite plutons obscure the details of the layering in the central part of the Lewis Hills massif along the preserved magma chamber truncation. The increase in relative thickness of ultramafic cumulates in addition to the shallowing of the chamber floor could result in progressive shallowing of the Moho near fracture zones as documented by recent marine seismic refraction studies⁴³.

Model of plate accretion

A mid-ocean ridge environment represents a site of mantle upwelling as well as mantle divergence⁴⁴ in which the mantle flow direction makes a right angle turn from vertical beneath the ridge to horizontal on its flanks. In such a case the 'frozen in' mantle flow lines will be only moderately inclined to be horizontal on the ridge flanks. When viewed on a large scale this upwelling can be modelled in terms of divergent flow of a viscous fluid⁴⁵. As in any such viscous flow, the mantle flow rising and diverging beneath an oceanic ridge follows smooth curved paths. A sharp right angle turn of the flow lines is not possible because it would require infinite acceleration at the inflection point.

Nelson⁴⁵ has suggested that the surface of the upwelling mantle must, in effect, define an axial valley of some finite width and has modelled the variable topography along mid-ocean ridges in terms of mantle divergence and the equilibrium size of the sub-axial magma chamber.

The structural data from the tectonites of the upper harz-burgite section and the lowermost plutonic section of the Bay of Islands complex are consistent with the mantle upwelling model. The tectonite lineation direction, interpreted to lie close to the plastic flow direction, is subparallel to palaeohorizontal surfaces and subperpendicular to the mean dyke orientation. Both the foliation and lineation have been 'frozen' into a subhorizontal position which lies close to the predicted flow direction in the mantle ^{46–48}.

The contact between the rocks of the plutonic group and the residual harzburgite of the ophiolite which seems to be sub-horizontal represent an unconformity between rocks of magmatic origin and depleted upper mantle origin. The existence of this sharp contact requires the surface of residual mantle to intersect the base of the crustal level magma chamber at some time⁸. The most likely place for the creation of this contact is near the axis of divergence where the 'surface' of the upwelling residual mantle is, in essence, continually being created.

Because the lowermost plutonic section is affected by the same high temperature ductile deformation that affects the residual harzburgite, internal deformation must occur here while accretion of the plutonic section onto the surface of the upwelling mantle proceeds. The flow lines in the overlying plutonic rocks will grossly parallel the flow lines of the surface of the upwelling mantle as they are progressively accreted, while the carapace of the magma chamber which probably has a bulk

density equal to or less than the magma below²⁷ simply 'floats' on top of it. The carapace of the chamber, and therefore the sea floor, will not necessarily mirror the surface of the upwelling mantle until the entire thickness of the crust has solidified at the outermost extremity of the magma chamber. The equilibrium size of the magma chamber, largely dictated by spreading rate^{5,42}, will determine the ridge topography⁴⁵ (Fig. 5).

From the point of formation of the surface of the mantle (the axis of divergence) the plutonic section will gradually be accreted and thicken largely by heterogeneous nucleation and in situ crystallization at the bounding surface of the magma chamber, until at the outer margin of the steady state chamber the crust is completely solidified. The layer pattern preserved in plutonic section will outline the former surfaces of the chamber and therefore represent isochrons. Identification of a pattern will depend on regularity in the shape of the chamber, and if delineated, will conform to the size and shape of the magma chamber. The shape and size defined by the layering pattern is unlikely to be the same in every ophiolite and in the simplest case the equilibrium size of the chamber ultimately depends on spreading rate^{5,42}. Therefore, an estimate of relative spreading rate within ophilolites may be made using the chamber dimensions defined by these profiles. Where spreading rates are very slow or where non-steady-state episodic spreading occurs magma chambers may become so narrow that they periodically solidify completely. Large-scale truncations of the layered

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- van Andel, Tj. H. & Ballard, R. D. J. geophys. Res. 84, 5390-5406 (1979).
 Orcutt, J. A., Kennett, B., Dorman, L. & Prothero, W. A. Nature 250, 475-476 (1975).
 Rosendahl, B. R. et al. J. geophys. Res. 81, 5294-5304 (1976).
 Herron, T. J., Stoffa, P. L. & Buhl, P. Geophys. Res. Lett. 11, 989-992 (1980).
 Sleep, N. H. J. geophys. Res. 80, 4037-4042 (1975).
 Cann, J. R. Geophys. J. R. astr. Soc. 39, 169-187 (1974).
 Nisbet, E. G. & Fowler, C. M. R. Geophys. J. R. astr Soc. 54, 631-660 (1978).
 Dewey, J. F. & Kidd, W. S. F. Bull. geol. Soc. Am. 88, 960-968 (1977).
 Greenbaum, D. Nature phys. Sci. 238, 18-21 (1972).
 Hopson, C. A. & Pallister, J. S. Abstr. of Papers, Int. Ophiolite Symp. Cyprus Geol. Surv. Dept. Nicosia. 37 (1972).
- Nicosia, 37 (1979). 11. Geotimes 17, 24-25 (1972).

- Coleman, R. G. Ophiolites 230 (Springer, New York, 1977).
 Salisbury, M. H. & Christensen, N. I. J. geophys. Res. 83, 805-817 (1978).
 Juteau, T., Nicolas, A., Dubessy, J., Fruchard, J. C. & Bouches, J. L. Bull. geol. Soc. Am. 88, 1740-1748 (1977).
- Williams, H. Can. J. Earth Sci. 12, 1874-1894 (1975).
 Church, W. R. & Stevens, R. K. J. geophys. Res. 76, 1460-1466 (1971).
 Dewey, D. F. & Bird, J. M. J. geophys. Res. 76, 3179-3206 (1971).
 Malpas, J. Phil. Trans. R. Soc. A 288, 525-546 (1978).

- Jacobson, S. B. W. & Wasserburg, G. J. J. geophys. Res. 84, 7429-7445 (1979).
 Karson, J. A. & Dewey, J. F. Bull. geol. Soc. Am. 89, 1037-1049 (1978).
- Malpas, J., & Strong, D. F. Geochim. cosmochim. Acta 39, 1045-1060 (1975).
 Irvine, T. N. & Findlay, T. C. Earth Phys. Br. Dept Energy, Mines, Resour. Can. 42, 97-128
- 23. Casey, J. F., Dewey, J. F., Fox, PO. J., Karson, J. A. & Rosencrantz, E. in The Sea Vol. 3 (ed Emiliani, C.) (Wiley, New York, in the press).

structure and lithologic units would be expected to result in such cases. We see no such evidence at present in the Bay of Islands complex. Given this and the observed dimensions inferred from the layering profiles, a relatively fast spreading history is inferred. High temperature ductile deformation of the lower plutonic section in some cases may obscure the original angle of inter-

Regular well-preserved and ordered structures such as those described here may be rare in ophiolite complexes. However, identical patterns have been described for the Semail ophiolite49. On the other hand, if such relations have been overlooked or distorted by forcing all cumulate layering into a reconstructed subhorizontal orientation, as is commonly required, serious errors in layer thickness estimates could result. This might partially account for small apparent thicknesses of ophiolites relative to the seismic thickness of the oceanic crust12

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- 24. Jackson, E. D., Green, H. W. II & Moores, E. M. Bull. geal. Soc. Am. 86, 390-398 (1975).
- Casey, J. F. thesis, State Univ. New York, Albany (1980); Karson, J. A. thesis, State Univ. New York, Albany (1977)
- Wager, L. R. & Brown, G. M. Layered Igneous Rocks (Oliver & Boyd, Edinburgh, 1968). Jackson, E. D. Fortschr. Miner. 48, 128-174 (1971).

- Campbell, I. H. Lithos 11, 311-321 (1978). McCall, G. J. H. & Peers, R. Geol. Rdsch. 60, 1174-1263 (1971).
- Rosencrantz, E. thesis, State Univ. New York, Albany (1980). Karson, J. A. Geol. Surv., Can. Open File Rep. 628 (1979).

- Karson, J. A. Creol, Surv., Can. Open Pile Rep. 0.28 (1978).
 McClay, K. R. & Campbell, I. H. Geol. Mag. 113, 129-139 (1976).
 Taubeneck, W. H. & Poldervaart, A. Bull. geol. Soc. Am. 71, 1295-1322 (1960).
 Irvine, T. N. Geol. Soc. Am. Mem. 138, 240 p (1974).
 McBirney, A. R. & Noyes, R. M. J. Petrol. 20, 487-554 (1979).

- Wager, L. R. & Deer, W. A. Medd. Grønland 105, 4, (1939). Goode, A. D. T. J. Petrol. 77, 379-397 (1976).
- Donaldson, C. H. Min. Mag. 41, 323-336 (1977)
- Hess, H. H. Geol. Soc. Am. Mem. 80, 230 p (1960).
- Irvine, T. N. in The Evolution of the Igneous Rocks (ed. Yqder, H. S.) (Princeton University Press, 1979).

- Kusznir, N. J. Geophys. J.R. astr. Soc. 61, 167-181 (1989).

 Detrick, R. S. & Purdy, G. M. J. geophys. Res. 85, 3759-3777 (1980).

 Langstreth, M. G., LePichon, X. & Ewing, M. J. geophys. Res. 71, 5321-5355 (1966).

 Nelson, K. D. Geophys. J.R. astr. Soc. (in the press).
- Nicolas, A., Bouchez, J. L. & Boudier, F. Tectonphysics 14, 143-191 (1972).
 Nicolas, A. T. & Poirier, J. P. Crystalline Plasticity and Solid State Flow in Metamorphic
- Rocks (Wiley, New York, 1976).
 48. Christensen, N. I. & Salisbury, M. H. J. geophys. Res. 84, 4601-4610 (1979).
- Glennie, K. W., Hughes-Clark, M. W., Moody-Stuart, M., Pilaar, W. F. H. & Reinhardt, B. M. Geol. Myb. Gen. Ver. Verh. 31, 1-423 (1974).

Structure of the actin-myosin interface

Dominique Mornet, Raoul Bertrand, Pierre Pantel, Etienne Audemard & Ridha Kassah

Centre de Recherches de Biochimie Macromoleculaire du CNRS, Route de Mende (BP 5051), 34033 Montpellier Cédex, France

The topography of the rigor complex between F-actin and myosin heads (S1) has been investigated by carbodiimide zero-length cross-linking. The results demonstrate for the first time that the 95,000-molecular weight (95K) heavy chain of the myosin head enters into van der Waals contact with two neighbouring actin monomers; one is bound to the 50K domain and the other to the 20K domain of the myosin chain. The covalent F-actin-S1 complex can be isolated; it shows a vastly elevated Mg²⁺-ATPase. Each pair of actin subunits in the thin filament seems to act as a functional unit for specific binding of a myosin head and stimulation of its Mg²⁺-ATPase activity.

MUSCULAR contraction and cell motility are thought to operate by a cyclic displacement of myosin heads (S1 'crossbridges') along the actin filaments. The mechanical force is

generated at the myosin-actin interface and is coupled to Mg2+-ATP hydrolysis catalysed by the actin-myosin complex. The mechanism of the interaction is poorly understood.

The aims of the work reported here were to identify the participating elements and elucidate the main structural features

^{*} To whom correspondence should be addressed.

of the actin-myosin head interaction region and to establish the relationship of this van der Waals complex to actin activation of the myosin ATPase activity and cross-bridge function. Using a chemical cross-linking reaction as a molecular probe, we have investigated the mode of attachment of S1 heavy chain to F-actin in a variety of experimental conditions, and have identified the covalently associated actin-S1 entities formed. We have also determined the enzymatic properties of the myosin heads consequent on their irreversible attachment to F-actin in defined conditions.

Experimental strategy

Our experiments were based on three critical parameters. (1) Cross-linking reactions were performed on rigor complexes of F-actin using the following: native chymotryptic S1 (A1 + A2), S1 (A1), S1 (A2)1 and the three tryptically fragmented S1 derivatives²⁻⁴, the heavy chain of which is a complex of two or three fragments: (27K-50K-20K) (molecular weights 27,000-50,000-20,000)-S1, (27K-70K)-S1 and (75K-22K)-S1. These proteolytic S1 derivatives result from restricted tryptic hydrolysis of S1 in the absence and presence respectively of F-actin, which specifically modulates the breakdown of a 2K segment linking 50K and 20K fragments and confers protection against loss of actin-S1 Mg²⁺-ATPase³. All these derivatives interact reversibly with actin. The fragmented nature of their heavy chain allows direct identification of the parts of the chain which are engaged in cross-linking with actin. In preliminary experiments, cross-linking reactions were also carried out using heavy meromyosin (HMM)¹

(2) Before cross-linking, rigor actin-S1 complexes were prepared, in which either the actin or the S1 (or its derivatives) were labelled with the fluorescent dye N-(iodoacetyl)-N'-(1-sulpho-5-naphthyl)ethylenediamine (1,5-IAEDANS), the actin at cysteine 373 and the S1 at SH₁ thiol group³. This

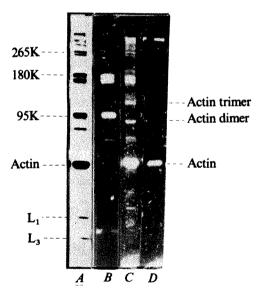


Fig. 1 SDS-gel electrophoretic analysis of actin-S1-containing crosslinked products. F-actin purified according to Spudich and Watt¹⁸ was weshed and equilibrated by sedimentation in 100 mM 2-N-morpholinoethanesulphonic acid (MES), pH 6.0, then immediately incubated (2.5 mg ml⁻¹) with 15 mM EDC (added as a solid) in the MES buffer for 2 min at 20 °C. A sample aliquot was mixed with 5-10 volumes of \$1 solution in the same buffer (final molar ratio of actin/S1 = 2). After 10 min at 20 °C, samples (=35 µg S1) were submitted to SDS-polyacrylamide slab gel electrophoresis (5-18% gradient acrylamide) using a 50 mM Tris/100 mM boric acid buffer system3. Gels were viewed using long-wave UV light and stained with Coomassie blue. Before use, F-actin or S1 was made fluorescent with 1,5-IAEDANS according to Takashi¹⁹ and Duke et al.²⁰, respectively. A, protein bands stained with Coomassie blue after reaction of S1 with EDCactivated actin; this pattern is unchanged when labelled S1 or labelled actin is used. B. fluorescent bands produced by reaction of IAEDANS-S1 with activated F-actin. C, fluorescent species produced by reaction of S1 with activated IAEDANS-actin. D, pattern of fluorescence in the EDC-activated IAEDANS-actin control. No change in S1 pattern was observed when the cross-linking reaction was processed in the absence of actin.

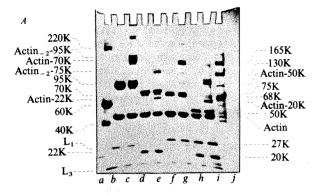
procedure allowed direct identification of actin and 20K peptide-containing material (SH₁ is located in the 20K unit⁵) in the observed cross-linked products. The molar ratios of actin/S1 present in cross-linked species were estimated quantitatively. This was done by measuring the ³H/¹⁴C ratio in the species resulting from cross-linking reactions performed on complexes of actin and S1, labelled at the sites mentioned above with ³H-iodoacetic acid and ¹⁴C-iodoacetamide, respectively.

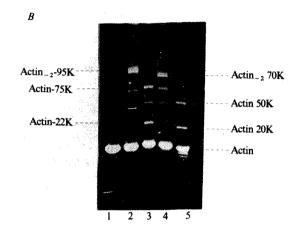
(3) Cross-linking reactions were catalysed with 1-ethyl-3 (3-dimethylaminopropyl)carbodiimide (EDC), a water-soluble carbodiimide that forms zero-length covalent bonds between amino and carboxyl groups in the contact area between two proteins. Most experiments were done using our highly versatile two-step procedure. First, actin was treated with EDC (activation phase), then an aliquot of the activated protein was mixed with a solution of S1, to give at least a fivefold dilution of the actin (condensation phase). This second step ensures a simultaneous quenching of the initial EDC reaction and covalent association of the two proteins. This procedure avoids most of the disadvantages of the usual one-step method; it suppresses intramolecular cross-linking when fragmented S1 or doubleheaded HMM are used, it allows the two phases of the reaction to be carried out separately in specific optimal conditions, and it also permits the controlled EDC-activation of either actin or S1 at will, and thus affords information on the compositions of both proteins at the interface.

Specific binding of 95K heavy chain to two vicinal actin subunits

Analysis of the products of reaction of EDC-activated actin with S1 (A1+A2) by gradient SDS-polyacrylamide slab gel electrophoresis gave the protein band pattern shown in Fig. 1A. It is characterized by the presence of two new components, both present as doublets. Estimations on calibrated 5-18% acrylamide gels indicated that the major species had Mrs of \sim 180,000 (185,000 and 175,000 for the components of the doublet), while that of the other, minor species was 265,000 (260,000 and 270,000). The distribution of the fluorescence in the protein bands formed from EDC-treated rigor complexes containing either fluorescent actin or fluorescent S1 demonstrated unequivocally that the two protein doublets were covalently cross-linked adducts of actin and S1, as label from either source was incorporated (Fig. 1B, C). They were sometimes accompanied by traces of two components which were fluorescent only when fluorescent actin was used and which were found in the EDC-actin control (Fig. 1D). Their electrophoretic mobility is consistent with their assignment as intramolecularly cross-linked actin dimers and trimers. The exact origin of the actin-S1 protein doublets is unknown but it does not arise from the heterogeneity associated with the presence of heads bearing different alkali light chains, as similar results were obtained with purified S1 (A1) and S1 (A2).

While the minor 265K product is sometimes absent, the major 180K derivative is always abundant in a wide range of experimental conditions (pH 6-7.5 at 0-20 °C and ionic strength 0.01-0.1 M in non-nucleophilic buffer). In optimal conditions ~30% of S1 becomes covalently bound to F-actin as determined by sedimentation (see below). However, no cross-linking occurs when activated actin is reacted with S1 in the presence of -pyrophosphate or Mg²⁺-ATP (5 mM), which are known to dissociate the actin-myosin complex, and more importantly, no covalent association can be obtained between native actin and EDC-activated S1 (although the reagent does react appreciably with \$1 carboxyl groups in the standard conditions of the activation reaction). The absence of covalent links between activated S1 and actin is not due to any effect of EDC on the reversible association between S1 and actin, as the 180K product is readily formed when the cross-linking reaction is done using the conventional one-step procedure. Therefore the 180K complex seems to be the result of a very specific crosslinking process which requires the formation of a bimolecular





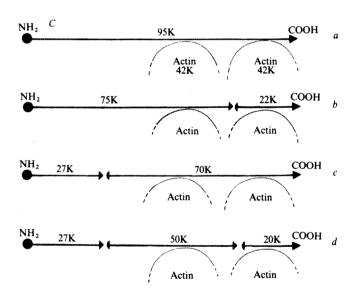


Fig. 2 Mapping of the heavy chain segments bound to the actin subunits by cross-linking the trypsin-modified S1 derivatives to fluorescent F-actin. The tryptically fragmented S1 species were prepared as described elsewhere and condensed to EDC-activated IAEDANS-actin as described in Fig. 1 legend; the same procedure was followed for native S1. Samples were analysed by electrophoresis on 5-18% acrylamide slab gels. A, protein banding pattern after staining with Coomassie blue. Lanes a and j, protein markers: ferritine (M, 220,000), y subunit of Escherichia coli RNA polymerase (165,000), β -galactosidase (130,000), bovine serum albumin (68,000), catalase (60,000), aldolase (40,000). Lanes b and c, native S1+ labelled actin before and after cross-linking. Lanes d and e, labelled actin + (75K-22K)-S1 before and after cross-linking. Lanes f and g, labelled actin+(27K-70K)-S1 before and after cross-linking; h and i, labelled actin + (27K-50K-20K)-S1 before and after cross-linking. B, location on gel of fluorescent actin-containing cross-linked products. Lane 1, fluorescent actin control; 2, fluorescent actin cross-linked to native S1; 3, fluorescent actin cross-linked to (75K-22K)-S1; 4, fluorescent actin cross-linked to (27K-70K)-S1; 5, fluorescent actin cross-linked to (27K-50K-20K)-S1. C, schematic diagram illustrating the relationship of the two actin subunits to the segments of myosin head heavy chain. a, Native S1; b-d, typically fragmented S1 derivatives.

Table 1 Estimation of the actin/S1 molar ratio present in the cross-linked 180K product

Experiments	³ H-actin/ ¹⁴ C-S1 (molar ratio)
1	2.5
2	1.6
3	2.3
4	1,4
5	2.6
6	2.4
7	1.5
	and the second s

 3 H-carboxymethyl actin (prepared according to Takashi et al. 23) (~ 2.0 3 H per mol protein, 18 mCi mmol $^{-1}$) was activated with EDC and reacted with 14 C-carboxyamidomethyl S1 (obtained by the same precedure: ~ 1.5 14 C per mol protein, 45 mCi mmol $^{-1}$) as described in Fig. 1 legend. Samples ($\sim 100~\mu g$ S1) were subjected to SDS-polyacrylamide gel electrophoresis (4-18% acrylamide gradient). After staining with Coomassie blue, the 180K protein doublet was isolated as a gel slice; two slices were combined and treated with 1 ml of Soluene 100 at 40 $^{\circ}$ C for 4 h. Radioactivity was measured in 10 ml of scintillator using a Packard 3003 counter. Contribution of 3 H to 14 C counts was subtracted using 3 H $^{-14}$ C calibration mixtures. The data shown are from different cross-linking experiments.

complex in which EDC-activable carboxyl groups on the actin are in contact with nucleophilic, probably amino groups on the myosin heads. This conclusion agrees with the suggestion made by Bárány and Bárány⁶ that the actin-myosin complex is a polyelectrolytic system in which the actin behaves as a polyanion and the myosin as a polycation. In addition, the ionic strength dependence of the rate constant of association of actin with S1 (ref. 7) indicates an ion-paired charge interaction at the recognition site.

The apparent mass of the 180K species containing both S1 and actin suggested that it must be generated by the association of two actin monomers with a single 95K heavy chain. The first direct evidence for this composition was obtained by determining the ³H/¹⁴C ratios of the 180K protein doublet formed by cross-linking ³H-actin to ¹⁴C-S1. The results showed an average ratio of ~2 which indicates a stoichiometry of two actin subunits per head (Table 1). Peptide mapping of the cross-linked species formed by complexes of fluorescent actin with the three tryptically fragmented S1 derivatives (Fig. 2A, B) not only confirmed this result but also led to the important conclusion that the two actin subunits are not associated by intermolecular EDCinduced cross-links but rather are separately and specifically bound to two different segments of the 95K heavy chain, that is, 50K and 20K fragments. Thus (75K-22K)-S1 gives rise to two new fluorescent cross-linked products, actin-75K peptide (M_r = 120,000; 120K) and actin-22K peptide ($M_r = 64,000$) (Fig. 2A, lanes d and e; B, lane 3). When (27K-70K)-S1 is used, the two actin subunits are both bound to the intact 70K segment to form the new cross-linked species (actin₂-70K peptide ($M_r = 155$ -160,000), Fig. 2A, lanes f and g; B, lane 4). The two actinbinding domains present in (27K-50K-20K)-S1 can be characterized by their ability to cross-link to the pair of actin monomers, yielding actin-50K peptide ($M_r = 94,000$) and actin-20K peptide ($M_r = 62,000$) (Fig. 2A, lanes h and i; B, lane 5). Interestingly, only the actin₂-70K peptide complex, containing two actin monomers, yields a doublet on the gel; all the other species, including actin-75K peptide, contain a single actin subunit and migrate as single bands. This suggests that the observed electrophoretic heterogeneity may be due to the presence of the pair of actin molecules. We have confirmed the distribution of the actin pair between the various heavy chain segments (see Fig. 2C) by repeating the above observations with fluorescent fragmented S1. Moreover, when the reaction mixture of EDC-activated fluorescent actin-S1 was treated with trypsin², a large amount of actin₂-70K fragment was liberated, together with smaller proportions of actin-20K peptide and actin-50K peptide, which indicates that the actin subunits are dispersed in a similar manner on intact S1 and its fragments. We have failed to observe a cross-linked species corresponding to

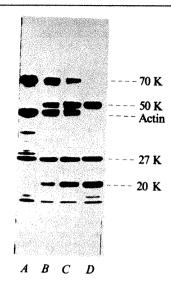


Fig. 3 Relationship between S1 binding to actin and the tryptic sensitivity of the 95K heavy chain (at low ionic strength, excluding any effect of salt on the S1-actin binding affinity). Tryptic conversion of actin-bound S1 into actin-activable (27K-70K)-S1 and actin-nonactivable (27K-50K-20K)-S1 derivatives. Samples A, B and C were analysed by electrophoresis as described in Fig. 1 legend; S1 fragmented in the absence of actin was used as control (lane D). Molar ratios of actin/S1 present in the digestion mixture were A = 2, B = 1 and C = 0.5.

the direct association between a single actin monomer and the intact or fragmented 95K heavy chain.

A cross-linking study was carried out at different actin/S1 molar ratios (0.5-10) to investigate conditions which favour the formation of the 265K derivative; this showed that the amount of the 180K component alone increases with increasing concentration of actin, reaching a maximal value at an actin/S1 molar ratio near 2. In contrast, yield of the cross-linked 265K component remains rather low even at an actin/S1 ratio of 1, although further studies (see below) showed that all S1 molecules are bound to actin at this ratio. Thus we assume that rigor attachment of an actin pair to the first S1 molecule imposes a different geometry on the binding of the second S1 molecule. This difference emerges from the capacity of the activated actin carboxylates to cross-link the first S1 compared with the much less efficient attachment of the second S1 at the topologically equivalent second site. This is indicative of the steric nonequivalence of the two heads in the complex.

Strong support for this scheme comes from a parallel study undertaken to define the relationship between the tryptic sensitivity of the 50K-20K junction of S1 heavy chain and the relative amount of actin needed to protect it. The actin-S1 ATPase activity, which depends on the structural integrity of the 50K-20K join³, is insensitive to trypsin at actin/S1 molar ratios ≥2. Below a ratio of 2 it decreases, and at a ratio of 1, when all S1 molecules are bound to actin as assessed by turbidimetry and sedimentation⁸, ~50% of this enzymatic activity is lost. The gel electrophoretic pattern of the tryptic digest of an equimolar mixture of actin and S1 (Fig. 3) clearly indicates the formation of two types of S1 derivative in equimolar amounts: (27K-70K)-S1 (ATPase activity activable by actin) and (27K-50K-20K)-S1 (ATPase activity not actin activable). At the critical molar ratio of actin/S1 = 2, only the former active derivative is present. Thus trypsin is able to discriminate between the two kinds of S1 bound to the same actin dimer unit, these being the 'trypsinsensitive' and 'trypsin-insensitive' species (Fig. 5). We obtained similar results with both S1 and myosin in other ionic conditions³. In this regard, the tryptic susceptibility of the 50K-20K junction of S1 heavy chain seems to be an excellent probe of the mode of attachment of the head to actin. It indicates also that the

protective action of F-actin towards the 50K-20K join^{2,3} is not due to the simple binding of S1 to actin but rather requires a specific actin-head recognition interface generated by the association of an actin dimer with a single S1 molecule in the absence of nucleotide. This mode of actin-head rigor interaction probably also occurs in vivo, as suggested by the findings of Lovell and Harrington⁹ on the tryptic susceptibility of rabbit skeletal myofibrils in physiological rigor conditions.

Covalent F-actin-S1 complex

Because the actin- and nucleotide-binding sites in myosin are coupled10, we have examined the effect of covalent association of S1 with actin on its Mg2+-ATPase activity. We found that the Mg²⁺-ATPase activity was greatly increased, and Fig. 4A, B shows that the extent of the enhancement depends on the time courses of the activation of the actin by EDC and of the attachment of S1 to the activated actin. The extent of Mg2 ATPase activation is apparently related to the amount of 180K product formed; it increases with increasing actin concentration and reaches a plateau at an actin/S1 molar ratio near 2. Conditions (see above) which suppress the 180K species also eliminate the ATPase activation. Control experiments have shown that P. liberation is not induced by the small amount of free actin accompanying S1 in the ATPase assay; in addition, this ATPase, in contrast to that of the reversible actin-S1 complex, is not affected by increased ionic strength. Finally, a parallel decrease in K⁺-ATPase of the EDC-activated actin-S1 mixture accompanies Mg²⁺-ATPase stimulation (Fig. 4B). We conclude that only the fraction of S1 that is covalently bound to actin is responsible for this new enzymatic behaviour and is able to catalyse massive hydrolysis of Mg²⁺-ATP. This is demonstrated by ultracentrifugal fractionation of the EDC reaction mixture in the presence of Mg²⁺-pyrophosphate into soluble and insoluble S1-containing fractions. The supernatant contains only residual S1 (Fig. 4C, lane 2) with unmodified enzymatic activity, but the pellet consists of irreversibly actin-bound S1 (Fig. 4C, lane 3) and shows elevated Mg2+-ATPase, the K+-ATPase being close to zero. The former activity remains unchanged when assayed in the presence of an added excess of free actin.

On the basis of the amount of S1 covalently linked to actin, estimated spectrophotometrically from the concentration of the enzyme in the supernatant⁸, we have calculated the specific Mg^{2^+} -ATPase activity to be $22\pm2~\mu\mathrm{mol}~\mathrm{P_i}$ per min per mg actin-bound S1, which corresponds to a turnover rate of $40~\mathrm{s}^{-1}$. When compared with the initial value of $0.02~\mu\mathrm{mol}~\mathrm{P_i}$ per min per mg actin-bound S1 (turnover rate = $0.036~\mathrm{s}^{-1}$ in agreement with reported values¹), this represents at least a 1,000-fold increase in the ATPase; the turnover rate of the covalent actin-S1 complex is strikingly similar to the V_{max} of the usual actin-activated ATPase of S1 (A1 + A2) extrapolated to infinite actin concentration¹. We refer to this unprecedented acceleration of Mg^{2^+} -ATP hydrolysis catalysed directly in vitro by actin-bound S1 as 'superactivation', to differentiate it from any other activation process observed for this myosin activity in vitro

Conclusions and implications

On the basis of these results we conclude that each actin monomer has two heavy chain-binding regions, specific for the 50K and 20K elements, respectively. The myosin head-binding unit of the thin filament in rigor conditions would thus be a contiguous actin pair. Consequently each functional pair of neighbouring actin monomers must have two pairs of head sites allowing the stoichiometric binding of a pair of heads (Fig. 5). This is supported by the occurrence of the 265K species which comprises actin and S1 and whose apparent mass agrees with its identity as the product of a pair of 95K heavy chains cross-linked with a pair of actin molecules.

Our results agree with the model recently proposed by Namba et al. 11 for the thin filament structure of crab striated muscle

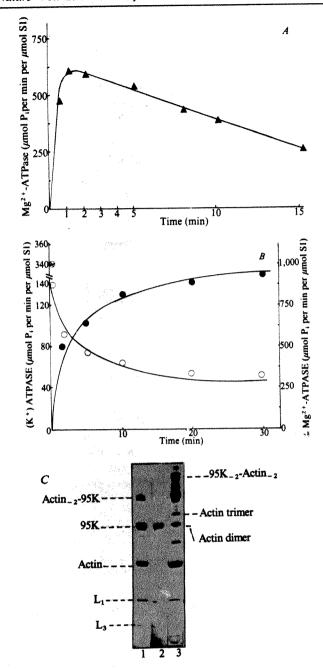


Fig. 4 Superactivation of the $\mathrm{Mg^{2^+}}$ -ATPase of S1 covalently bound to EDC-activated F-actin. A, dependence of the $\mathrm{Mg^{2^+}}$ -ATPase stimulation on the time course of actin activation by EDC. F-actin (2.5 mg ml⁻¹) in 100 mM MES buffer, pH 6.0, was reacted at 20 °C with 15 mM EDC (added as a solid). At the times indicated, a protein aliquot was withdrawn and mixed with 10 volumes of \$1 solution in the same buffer (final molar ratio of actin to S1 = 2); after 10 min of condensation reaction at 20 °C, samples (20 μg S1) were assayed for Mg²⁺-ATPase²¹. B, dependence of the Mg²⁺-ATPase stimulation and K+-ATPase inhibition on the time course of S1 condensation to EDC-activated actin. F-actin was first activated with EDC for 2 min, then mixed with S1 in conditions described above for A. At different times in the condensation reaction, S1 samples, 20 µg and 25 µg, were taken in parallel and assayed for ${\rm Mg}^{2+}$ -ATPase and ${\rm K}^+$ -ATPase, respectively 21 . \blacksquare , Acceleration of ${\rm Mg}^{2+}$ -ATPase activity, and \bigcirc , loss of ${\rm K}^+$ -ATPase activity of S1 covalently bound to actin. As reversible binding of S1 to actin inhibits the K^+ -ATPase at zero time of the cross-linking reaction²², the K^+ -ATPase was assayed in the presence of actin (actin/S1 ratio = 2); the value found was 60% lower than that given by free S1 (⊗). C, selective separation of the covalent actin-\$1 complex. After a 10-min period of condensation between activated actin and S1, the reaction medium was adjusted to 10 mM sodium pyrophosphate, 100 mM KCl, 5 mM MgCl₂ and 50 mM HEPES pH 7.5; after centrifugation at 140,000g for 45 min at 4 °C, the pellet was gently resuspended in the initial volume of a solution containing 10 mM KC 1.5 mM MgCl₂ and 50 mM Tris-HCl pH 8.0. It was subjected, together with the supernatant, to electrophoresis on gradient acrylamide gel as described in Fig. 1 legend. Lane 1, total reaction medium; lane 2, supernatant containing unreacted dissociable S1; lane 3, pellet containing nondissociable S1, covalently bound to the actin filament as major actin 2-S1, and minor actin 2-S12 species.

analysed by X-ray diffraction, which showed myosin heads attached to pairs of adjacent actin monomers. Actin-20K peptide and actin-50K peptide are now being isolated for elucidation of the points of contact between actin and the two heavy chain segments. Experiments in progress have also indicated EDC-induced cross-linking of actin dimers to the heavy chains of HMM, with concomitant superactivation of the Mg²⁺-ATPase. These data, together with those for the parent myosin, will be reported in detail elsewhere; the use of myosin is of particular interest because it contains molar amounts of LC2 light chain, the presence of which is reported to change the shape of the attached heads¹².

The covalent actin-S1 complex hydrolyses Mg²⁺-ATP at a very high rate because of the irreversible occupation of the actin site on S1. This provides the first direct experimental evidence for the possible non-dissociating pathway of ATP hydrolysis by actin-S1^{13,14}. Cross-linking of actin to S1 does not impair the catalytic properties of the enzyme but, on the contrary, preserves the active conformation of the ATPase site resulting from the interaction of actin with S1. The covalent actin-S1 material offers a valuable tool for investigating the kinetic parameters of ATP hydrolysis by actin-bound S1 and for determining the nature of the conformational changes transmitted to the ATPase site when the actin site is bound.

The ability of S1 covalently complexed to actin to hydrolyse ATP at an effective rate as high as that which normally occurs in the presence of infinite actin concentration should be considered in relation with the report of Bárány and Burt¹⁵ which indicates that in vivo contraction is associated with 1,500 -5,000-fold activation of myosin ATPase, which has never been seen in any in vitro system. Our covalent actin-S1 derivative may represent the first assembly in vitro of interacting actin-head complex resembling that found in the native cell.

The rigor association of S1 heavy chain with an actin dimer seems to be closely related to the molecular mechanism of stimulation of myosin Mg²⁺-ATPase by F-actin. Estes and Gershman¹⁶ have shown that neither G-actin nor F-actin monomers can activate the ATPase; they have concluded that the specific activating ability of F-actin is possibly formed or is stabilized by the association of two adjacent subunits. In addition, Fasold *et al.*¹⁷ have reported that isolated, non-polymerizable actin dimers generated by chemical cross-linking of F-actin bind stoichiometrically to S1 and activate the ATPase

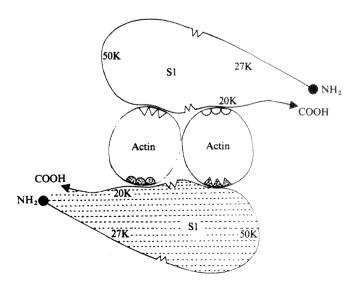


Fig. 5 Schematic diagram for stoichiometric binding of S1 to two actin monomers which are on two strands of the actin helix: hatched S1 is only poorly cross-linked, trypsin-sensitive S1; open S1 is strongly cross-linkable, trypsin-insensitive S1 (tryptic sensitivity is implicitly related to the splitting of the 50K-20K join).

25% relative to F-actin. Our results strongly support all these observations and further suggest that the proper positioning of the actin pair on the 50K and 20K segments of the heavy chain is a prerequisite for, at least, the initiation of the ATPase activa-

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- Weeds, A. G. & Taylor, R. S. Nature 257, 54-56 (1975).
 Mornet, D., Pantel, P., Audemard, E. & Kassab, R. Biochem. biophys. Res. Commun. 89. 925-932 (1979).
- Mornet, D., Bertrand, R., Pantel, P., Audemard, E. & Kassab, R. Biochemistry 20, 2110-2120 (1981).
- 4. Mornet, D., Pantel, P., Bertrand, R., Audemard, E. & Kassab, R. FEBS Lett. 123, 54-58
- Gallagher, M. & Elzinga, M. Fedn Proc. 39, 2168 (1980).
- Bárány, M. & Bárány, K. Biochim. biophys. Acta 41, 204-216 (1960).
 White, H. D. & Taylor, E. W. Biochemistry 15, 5818-5826 (1976).

- 8. Margossian, S. S. & Lowey, S. J. molec. Biol. 74, 313-330 (1973).
 9. Lovell, S. J. & Harrington, W. F. Fedn Proc. 39, 1935 (1980).
 10. Marston, S. B., Tregear, R. T., Rodger, C. D. & Clarke, M. L. J. molec. Biol. 128, 111-126

tion process.

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- Namba, K., Wakabayashi, K. & Mitsui, T. J. molec. Biol. 138, 1-26 (1980).
 Craig, R. et al. J. molec. Biol. 140, 35-55 (1980).
 Taylor, E. W. CRC crit. Rev. Biochem. 6, 103-164 (1979).

- 14. Stein, L. A., Schwarz, R. P., Chock, P. B. & Eisenberg, E. Biochemistry 18, 3895-3909

- Bárány, M. & Burt, C. T. Fedn Proc. 38, 338 (1979).
 Estes, E. J. & Gershman, L. C. Biochemistry 17, 2495-2499 (1978).
 Fasold, H., Baumert, H. & Bender, N. Biochem. Soc. Trans. 3, 935-936 (1975).
- Spudich, J. A. & Watt, S. J. biol. Chem. 246, 4866-4871 (1971).
 Takashi, R. Biochemistry 18, 5164-5169 (1979).
- 20. Duke, J., Takashi, R., Ue, K. & Morales, M. F. Proc. natn. Acad. Sci. U.S.A. 73, 302-306
- 21. Mornet, D., Pantel, P., Audemard, E. & Kassab, R. Eur. J. Biochem. 100, 421-431 (1979). Cooke, R. & Franks, K. Biochemistry 19, 2265-2269 (1980)
- Takashi, R., Duke, J., Ue, K. & Morales, M. F. Archs Biochem. Biophys. 175, 279-283

Gene conversion between duplicated genetic elements in yeast

Jennifer A. Jackson & Gerald R. Fink

Section of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, New York 14853, USA

The mitotic recombination behaviour of a duplication of the his4 region on chromosome III in the yeast Saccharomyces cerevisiae was studied. The major recombination event between the duplicated segments is gene conversion unassociated with reciprocal recombination. The rad52-1 mutation preferentially decreases mitotic gene conversion. These results suggest that mitotic gene conversion may occur by a different pathway from that occurring in meiosis. This mitotic gene conversion may be important in yeast mating type interconversion and the maintenance of sequence homogeneity in families of repeated eukaryotic genes.

GENETIC recombination is the result of an exchange between homologous regions of DNA and can occur between two segments located on homologous chromosomes, or between repeated segments of DNA on the same chromosome. Two types of homologous recombination have been observed in yeast and other organisms; reciprocal and non-reciprocal exchange, or gene conversion (reviewed in refs 1-3). In yeast, meiotic gene conversion is associated with reciprocal recombination4; as many as half the conversion events are accompanied by reciprocal recombination of flanking markers, and this conversion-associated recombination is frequent enough to account for all the genetic exchange observed in yeast meiosis. Current models for meiotic recombination postulate an exchange of DNA strands between homologous DNA duplexes^{1-3,5,6}. If there are base sequence differences between the two duplexes, the resulting structure contains a local region of heteroduplex. Gene conversion is believed to be due to the correction of the heteroduplex region. These models postulate that resolution of the heteroduplex results in recombination of flanking markers in

Increasing evidence suggests that mitotic recombination may occur by different pathways from those of meiotic recombination. In mitosis, gene conversion is not as closely associated with reciprocal exchange^{7,8}. Roman⁷ found that UV-light irradiation enhanced mitotic reciprocal recombination more than gene conversion, and that nitrosoguanidine enhanced gene conversion preferentially. In addition, Boram and Roman have isolated an allele of rad18 that enhances spontaneous mitotic gene conversion, but not reciprocal recombination. A further difference between mitotic and meiotic recombination in yeast is seen in the effects of other mutations. The reml-1 mutation enhances both types of exchange in mitosis, but has no effect on meiotic recombination 10,11. In contrast, the rad52-1 mutation abolishes meiotic recombination, but only decreases mitotic exchange 12-14. Thus, in mitosis, gene conversion and reciprocal recombination can be separated from each other. These considerations suggest that there may be mechanistic differences between meiotic and mitotic recombination.

We have used hybrid plasmids containing yeast DNA linked to an Escherichia coli plasmid to study mitotic recombination. During transformation these hybrid plasmids integrate by a homologous recombination event and create a direct duplication of the yeast DNA separated by plasmid sequences¹⁵. We have constructed a direct duplication of a 24-kilobase (kb) BamHI fragment on chromosome III which contains the his4 gene. By genetic techniques, different mutations were introduced into each of the two copies of the his4 gene, creating a duplication with the structure his4A pBR313 his4C. Mitotic recombination between the two his4 mutant genes can yield a HIS4+ recombinant. Whether the event which generated the recombinant was gene conversion or reciprocal recombination can be determined by analysis of the recombinants. A reciprocal

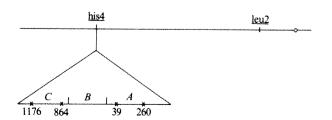
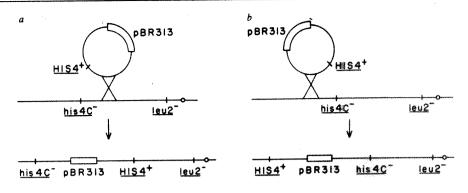


Fig. 1 Map of chromosome III. The his4 region is shown subdivided into A, B and C. The relevant his4A and his4C mutations are shown.

Fig. 2 Formation of duplication strains by transformation. The plasmid YIP300, which consists of a BamHI fragment carrying the HIS4⁺ gene inserted into pBR313, is shown to integrate into the yeast chromosome III by a homologous recombination event. The recombination event can occur centromereproximal or centromere-distal with respect to the his4C mutation on chromosome III. a, Orientation of the two his4 genes if the exchange event occurs proximal to the centromere; b, orientation of the two his4 genes if the exchange event occurs distal to the centromere. The line represents yeast DNA and the box represents pBR313 sequences.



recombination event gives rise to a HIS4⁺ segregant which has lost the duplication and the intervening plasmid sequences (see Fig. 4). A gene conversion event between the two elements of the duplication yields a HIS4⁺ segregant which has retained the duplication and the intervening plasmid sequences. Thus, a large number of mitotic recombinants can be scored as gene convertants or reciprocal recombinants by colony hybridization using plasmid DNA as a probe¹⁶. Using strains carrying duplications of the his4 region, we have shown that gene conversion, not associated with reciprocal recombination, is the major event giving rise to HIS4⁺ segregants in mitosis. Our studies of these strains also show that the rad52-1 mutation preferentially decreases mitotic gene conversion.

Construction of the duplication strains

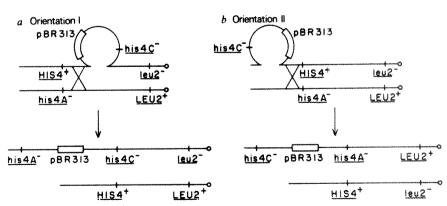
Yeast strains duplicated for the his4 gene were constructed by transformation¹⁵. The donor DNA was plasmid YIP300 which contains ~24-kb BamHI fragment of yeast DNA inserted into the BamHI site of the vector pBR313¹⁷. The HIS4⁺ gene is located near the middle of this BamHI fragment. The recipient was a haploid yeast strain containing two his4C mutations (his4-864 and his4-1176) and the leu2-3 mutation on chromosome III (Fig. 1). Transformation of this stable Hisstrain to HIS4⁺ occurs by the integration of the YIP300 plasmid by homologous recombination. Integration of this plasmid creates a duplication of the his4 region in one of two possible orientations. The orientation of the HIS4⁺, pBR313 and his4⁻ sequences with respect to leu2 depends on the site of integration

relative to the his4C mutations (Fig. 2). The HIS4⁺ transformants in either orientation are unstable—this instability results from mitotic recombination between elements of the duplication that give rise to his4⁻ segregants, and was also observed in yeast strains carrying a duplication of the leu2 region created by transformation¹⁵.

We devised a positive selection for recombination events in the duplicated region using strains in which each of the two copies of the his4 gene carry different mutations that can recombine to yield a HIS4⁺ segregant. These strains were constructed by crossing individual HIS4⁺ leu2⁻ transformants with a LEU2⁺ strain carrying two mutations in the his4A region: his4-260 and his4-39. A His duplication strain with the structure his4A⁻ pBR313 his4C⁻ (his4-260, his4-39, pBR313, his4-1176, his4-864) can arise from this cross by meiotic recombination event (Fig. 3). Crosses of individual transformants with a his4A⁻ LEU2⁺ strain give rise to recombinant His duplication strains of two orientations (Fig. 3). Both orientation I (his4A⁻ pBR313 his4A⁻ LEU2⁺) frequently give rise to HIS4⁺ segregants by mitotic recombination events.

When the HIS4⁺ transformants are crossed by the his4A⁻ strain, one parent has the standard arrangement on chromosome III and the other has an insertion of pBR313 flanked by a direct duplication of his4. Tetrad analysis of these crosses indicates that neither the duplication nor the pBR313 sequences affect meiotic recombination in the his4-leu2 interval. Analysis of 101 tetrads yielded a map distance between his4 and leu2 of 16.8 centimorgans, which is similar to the his4-leu2

Fig. 3 Formation of his4 duplication strains of orientations I and II. Transformation with YIP300 yielded duplications of the his4 alleles in two orientations (Fig. 2). Individual His transformants of both orientations were crossed by a LEU2 strain carrying two mutations in the his4A region (his4-260 and his4-39). Four types of His progeny resulted from this cross; three of these are completely stable and carry a single his4 region. Colony hybridization using plasmid DNA as the probe shows that they have no plasmid sequences. Stable His progeny are: (1) a non-recombinant which contains the two his4A mutations (his4-260 and his4-39); (2) a recombinant which contains the two his4C mutations (his4-1176 and his4-864);



and (3) a recombinant carrying all four mutations (his4-260, his4-39, his4-1176 and his4-864). The fourth type is a recombinant which contains a duplication of the BamHI fragment. The formation of this recombinant is shown. One element of the duplication contains the his4A mutations and the other contains the his4C mutations. The orientation of these mutations in the duplication depends on the site of integration of the YIP300 plasmid into the chromosome (see Fig. 2). The Leu phenotype of these recombinants is indicative of the orientation of the alleles in the duplication. The His recombinants carrying a duplication can be easily detected because they frequently give rise to His segregants by mitotic recombination events occurring between the two his4 regions, whereas the other His progeny are completely stable. Colony hybridization using plasmid DNA as a probe shows that the unstable His progeny retain the pBR313 sequences. Crosses of these strains with wild type (HIS4+) give rise to recombinant His progeny containing one or the other component of the duplication. The segregation of each of the components of the duplication in these crosses confirmed the structure in the duplications. a, The recombination event giving rise to a his4 duplication strain of orientation II. The thin line represents yeast DNA and the box represents pBR313 sequences.

Table 1 Frequency of HIS4+ formation in duplication strains

Orie	ntation I his4A pBR3	Orient	ation II his4C pBF	R313 his4A			
Strain	Culture no.	HIS4 ⁺ frequency per cell (×10 ⁻⁵)	Mean	Strain	Culture no.	HIS4 ⁺ frequency per cell (×10 ⁻⁵)	Mean
7217-30D RAD+	1	43		7084-48A RAD+	1	16	
	2	12			2	6.3	
	3	15			3	8.3	
7313-9 <i>B RAD</i> +	1	43			4	3.7	
	2	5		7083-18A RAD+	1	10	
	3	12	21.7 ± 17.7		2	6.8	
7503-8C rad52-1	1	1.8			3	5.8	8.1 ± 3.7
	2	3.8		96-40B rad52-1	1	3.8	
	3	1.2			2	1.9	
	4	1.0			3	2.4	
	5	3.9			4	2.8	
71-43 rad52-1	1	1.3			5	2.1	
	2	3.3		95-13D rad52-1	1	2.1	
	3	1.0	2.2 ± 1.1		2	2.4	2.5 ± 0.6

A single colony of the strain to be tested was inoculated into 5 ml of liquid YEPD medium⁵¹ and grown to a concentration of $\sim 10^7$ cells per ml. Cultures were washed, diluted and plated on to five YEPD agar plates for a total cell count and on to five minimal agar plates to estimate the frequency of His⁺ colonies. The plates were incubated at 30 °C for 3 days before counting. The frequency of His⁺ cells was determined by dividing the number of His⁺ cells per ml by the total number of cells per ml.

distance (17.4 centimorgans¹⁸) found in crosses where both parents had the standard arrangement.

Mitotic recombination in haploids and diploids

Mitotic recombination occurs more frequently between two his4 genes located on a single chromosome III in a haploid than does recombination between his4 genes located on different chromosome IIIs in a diploid. The spontaneous mitotic recombination behaviour of duplications was studied using haploid strains in which one element of the duplication contained the his4A mutations and the other contained the his4C mutations. The frequency of HIS4+ formation was measured in these his4 duplications when the alleles were in both orientation I and orientation II. HIS4+ formation was also measured in heteroallelic diploids (his4A-/his4C-) of normal chromosome structure. Heteroallelic diploids of mating type a/a and α/α were studied in addition to an a/α diploid. Formation of HIS4 recombinants occurred with a frequency of 0.6×10^{-5} in a/α diploids, 0.1×10^{-5} in a/a diploids and 0.2×10^{-5} in α/α diploids. The haploid his4 duplication strain of orientation I gave rise to prototrophs at a frequency of 21.7×10^{-5} . Orientation II duplications gave rise to prototrophs at a frequency of 8.1×10^{-5} (Table 1). (The difference in frequency between orientations I and II is not statistically significant (P > 0.90). Thus, duplications recombine at a frequency which is at least an order of magnitude greater than that measured in diploids.

In meiosis, a diploid containing a duplication on each

homologue (his4C⁻ pBR313 his4C⁻/his4A⁻ pBR313 his4C⁻) yields His⁺ recombinants in 1.6% of the tetrads (2/123). Our finding that the frequency of recombination in mitosis is rare compared with that in meiosis agrees with all previous studies on recombination in yeast^{4,7-11}.

Gene conversion is the major recombination event

To study the mitotic recombination events which occur between elements of a duplication, the HIS4+ recombinants arising from haploid duplication strains were analysed by colony hybridization using pBR313 DNA as the probe. The presence or absence of pBR313 is indicative of the event which gave rise to the HIS4+ recombinant. Gene conversion unassociated with reciprocal recombination will give a HIS4+ recombinant which retains the two copies of the his4 region and the pBR313 sequences (pBR313⁺). A HIS4⁺ recombinant with a single copy of the his4 region and no pBR313 sequences (pBR313-) can be generated by two types of event. The first is a simple reciprocal recombination event between the mutant alleles (see Fig. 4). Alternatively, gene conversion of one of the His alleles to His. followed by reciprocal recombination will give rise to a Hist pBR313 recombinant. These two events cannot be distinguished from each other, but as both involve reciprocal exchange they will be classified together as reciprocal recombination

Analysis of HIS4⁺ recombinants arising from duplications in orientation I showed that 15 (12%) had lost the pBR313

Table 2 Frequency of gene conversion and reciprocal recombination among HIS4⁺ recombinants

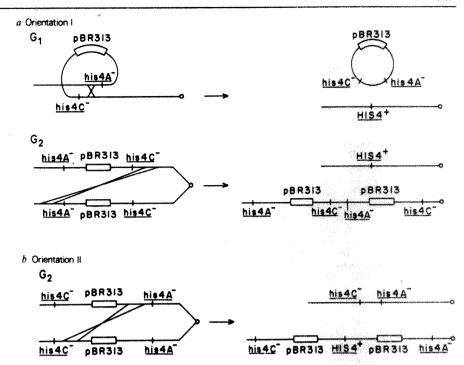
		Colony	hybridization				
Strain		Mean frequency recombination $(\times 10^{-5})$	No. of colonies tested	% His ⁺ pBR313 ⁺	% His ⁺ pBR313 ⁻	Mean frequency gene conversion (×10 ⁻⁵)	Mean frequency reciprocal recombination (×10 ⁻⁵)
Orientation I							
7217-30D	Rad ⁺	23.3	127	88	12	20.5	2.8
7503-8C	rad52-1	2.3	192	3	97	0.07	2.2
Orientation II							
7084-48A	Rad ⁺	8.6	199	97*	3	6.2	2.3
96-40B	rad52-1	2.6	146	59†	41	0.18	2.4

Frequency of recombination was determined by dividing the number of prototrophs per ml by number of cells per ml. Mean frequency of recombination is the arithmetic average of frequencies shown in Table 1. Mean frequency of gene conversion was calculated by multiplying % gene conversion, as determined by colony hybridization and Southern analysis, by mean frequency recombination. Mean frequency of reciprocal recombination was calculated by multiplying % reciprocal recombination, as determined by colony hybridization and Southern analysis, by mean frequency recombination.

^{*} Southern hybridization analysis of eight independent His⁺ recombinants showed that 1/4 were triplications and 3/4 were duplications of the his4 region (see Fig. 5).

[†] Southern hybridization analysis of eight independent His⁺ recombinants showed that 7/8 were triplications and 1/8 were duplications of the his4 region (see Fig. 5).

Fig. 4 Formation of HIS4⁺ prototrophs by reciprocal recombination. a, Reciprocal recombination in orientation I. In G1, before DNA replication, an intra-strand event will yield a HIS4+ recombinant with a single copy of the his4 region, and a circular molecule carrying both his4 mutations and pBR313. In G2, after DNA replication, an inter-strand event will yield a HIS4* recombinant with a single copy of the his4 region, and a his4 recombinant with three copies of the his4 region and two of pBR313. b, Reciprocal recombination in orientation II. In G2, an inter-strand event will yield a HIS4+ recombinant with three copies of the his4 region and two copies of pBR313. The his4 recombinant has a single copy of the his4 region. In G1, a simple reciprocal recombination event cannot yield a HIS4+ recombinant. The thin line represents yeast DNA and a box represents pBR313 sequences.



sequences and 112 (88%) had retained them (Table 2). Southern analysis of total yeast DNA using the BamHI fragment of his4 DNA as a probe showed that the His⁺ pBR313⁻ recombinants have a single copy of the his4 region. This analysis indicates that the recombinants which lose the pBR313 sequences are the result of reciprocal recombination. Confirmation that the major class of recombinants, His⁺ pBR313⁺, arose by gene conversion comes from two lines of evidence. First, Southern analysis using the BamHI fragment of his4 DNA as a probe showed that these recombinants retain two copies of the his4 region. Second, when any individual His⁺ pBR313⁺ recombinant was crossed by an untransformed HIS4⁺ strain, either his4A⁻ or his4C⁻ progeny were recovered. This result indicates that in orientation I most recombinants result from gene conversion.

Most HIS4⁺ recombinants which were produced by his4⁻ duplications in orientation II were also due to gene conversion. There are three types of HIS4+ recombinants which result from this orientation. The first type is one which retains the two copies of the his4 region and one copy of the pBR313 sequences—this type of HIS4⁺ recombinant results from a gene conversion event unassociated with reciprocal exchange. The second type is a HIS4+ recombinant which carries a triplication of the his4 region and two copies of the pBR313 sequences. This type can be generated by an uneven reciprocal recombination event between sister chromatids (Fig. 4). The third type has a single copy of the his4 region and no plasmid sequences. This type could result from the conversion of one of the mutant alleles to His*, followed by a reciprocal recombination event, or alternatively from triple crossovers. Both types 2 and 3 are classified as reciprocal recombinants because their formation involved a reciprocal exchange.

Analysis of 199 $HIS4^+$ recombinants arising from a strain carrying the duplication in orientation II showed that 193 (97%) had retained pBR313 and 6 (3%) had lost pBR313 (Table 2). To determine which fraction of the His⁺ pBR313⁺ recombinants is due to gene conversion, Southern analysis using plasmid DNA as a probe (Fig. 5) was performed on eight independent His⁺ pBR313⁺ recombinants. This experiment showed that six of the eight recombinants had two copies of the his4 region and two had three copies. Assuming that these independent isolates are representative of the whole population, we conclude that ~25% of the $HIS4^+$ recombinants from orientation II strains are due to reciprocal recombination and 75% are due to gene conversion.

Our finding that most of the HIS4⁺ recombinants from the

duplication strains are gene convertants agrees with previous studies on recombination in yeast. Intragenic recombination both in mitosis and meiosis is usually a result of gene conversion rather than reciprocal recombination ^{19,20}. It has been shown that up to half of the meiotic conversion events are associated with reciprocal recombination⁴. In contrast, the major mitotic recombination event observed in duplications is gene conversion, unassociated with reciprocal exchange. Our evidence for this conclusion is based on the retention of the duplication and plasmid sequences in most HIS4⁺ recombinants. This result agrees with studies of mitotic recombination in diploids^{7,8}, and suggests that mitotic gene conversion may proceed by a mechanism which is different from that in meiosis.

These observations on duplications of the his4 locus apply to other regions of the yeast genome. Duplications of the leu2 gene, with the structure leu2-101 colE1 leu2-3 leu2-112, behave identically to the his4 duplications. About 95% of the LEU2+ recombinants are gene convertants. In a duplication with the structure LEU2+ ColE1 leu2-3 leu2-112, 17 Leu segregants were generated by a recombination event which separated leu2-3 from leu2-112. All these segregants retained the vector sequences (eight were leu2-112 ColE1 leu2-3 leu2-112 and nine were leu2-3 ColE1 leu2-3 leu2-112) (C. Styles and G. R. Fink, unpublished data). Thus, a high frequency of gene conversion occurs in prototrophic or auxotrophic recombinants as long as the event is intragenic.

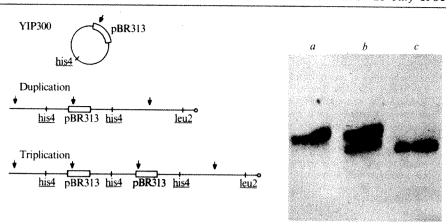
the event is intragenic.

Previous work²¹⁻²³ has shown that spontaneous and induced intragenic mitotic recombination can occur before DNA synthesis, at the two-strand stage. The reciprocal recombination which we observe in the duplication strains suggests that mitotic recombination is not restricted to one stage of the cell cycle. The fact that duplication strains in orientation II give rise to prototrophs which carry a triplication of the his4 region (Fig. 4) argues that, in a haploid, mitotic recombination can occur after DNA synthesis. This is the first evidence of sister-strand exchange in chromosomal regions outside the rDNA cistrons^{24,25}. It is unclear what proportion of the mitotic recombination events occur at this stage, because the other classes of recombinants could be produced either before DNA replication by an intrastrand recombination event or after DNA synthesis by an inter-strand event.

Gene conversion is decreased by rad52-1

The rad52-1 mutation has been reported to abolish meiotic recombination and to reduce mitotic recombination 12-14. We

Fig. 5 Quantitation of his4 regions. The diagram shows the configuration of the plasmid YIP300, a strain carrying a duplication of the his4 region and a strain carrying a triplication of this region. The thin line represents yeast DNA; the box represents pBR313 sequences and arrows represent SmaI endonuclease sites. Also shown is an autoradiogram of the hybridization profile of Smal-digested DNA from a a duplication: h a triplication and c, YIP300 using labelled plasmid as a probe. The two Smal fragments carrying pBR313 sequences in the duplication migrate together on an agarose gel. These two fragments are found in a triplication that has, in addition, a smaller Smal



fragment carrying pBR313 sequences which is the same size as the YIP300 plasmid. Nuclear DNA was isolated from the yeast strains by the method of Cryer et al. 46. Plasmid YIP300 was isolated as described by Hirt 47. After digestion with Smal the fragments were separated by agarose gel electrophoresis on a horizontal 0.5% agarose slab gel run at 100 V for 18 h. DNA was transferred to nitrocellulose filters 48 which were hybridized to a DNA probe 32p-labelled by nick translation 49. The probe was pBR322, a plasmid derived from pBR31350.

found that the rad52-1 mutation lowers the frequency of HIS4+ formation for his4 duplications of both orientations I and II. Further analysis of the HIS4⁺ recombinants produced in a rad 52-1 background indicate that gene conversion is affected preferentially. For orientation I, rad52-1 lowered the frequency of HIS4+ formation ~10-fold (Table 1). When HIS4+ recombinants were examined by colony hybridization using plasmid DNA as a probe, 186 (97%) had lost the pBR313 sequences (Table 2). In comparison, only 12% of the HIS4⁺ recombinants produced by the RAD52+ strains in orientation I had lost pBR313. The loss of the plasmid sequences indicates that a reciprocal recombination event had occurred (Fig. 4). When the overall frequency of HIS4+ formation in orientation I strains is separated into reciprocal recombination and gene conversion components, the component representing the reciprocal recombination frequency is essentially the same in the rad52-1 and RAD52+ backgrounds (Table 2). Thus, the 10-fold decrease in HIS4+ formation in rad52-1 strains is due to a greater than 200-fold decrease in gene conversion events.

The rad52-1 mutation lowered the frequency of HIS4+ formation in duplication strains of orientation II threefold. Colony hybridization using plasmid DNA as the probe showed that 145 (59%) of the HIS4+ recombinants had retained pBR313 and 101 (41%) had lost this sequence (Table 2). The proportion of His+ pBR313+ recombinants which were due to reciprocal recombination was determined by Southern hybridization analysis of whole yeast DNA from eight independent isolates (Fig. 5). This analysis showed that seven of the eight His+ pBR313+ recombinants carried a triplication of the his4 region, and therefore were a result of a reciprocal recombination event. The other His+ pBR313+ recombinant carried a duplication of the his4 region. As the eight strains are independent isolates, we assume that they are representative of the entire His⁺ pBR313⁺ population. On this assumption, 88% of the His+ pBR313+ and all the His+ pBR313 recombinants, or 93% of the total HIS4+ recombinants arising in a rad52-1, orientation II background are due to reciprocal recombination. When the overall frequency of HIS4⁺ formation is separated into reciprocal recombination and gene conversion components, the component representing the reciprocal recombination frequency is the same as that of the Rad52⁺, orientation II strain, and gene conversion has been decreased ~30-fold

There is a difference in the extent to which rad52 decreases the frequency of gene conversion in the strains of orientations I and II (Table 2). Others have reported that the effect of rad52 on mitotic recombination varies from 6- to 200-fold depending on the genetic interval studied $^{12-14}$. The differences we observed may be due to the orientation of the his4 alleles or to differences in the genetic backgrounds of the strains used here.

Studies on the effect of the rad52-1 mutation in diploid strains have shown that this allele affects mitosis and meiosis differently¹²⁻¹⁴. Meiotic recombination is completely abolished and mitotic recombination decreased. We have shown that in strains carrying the rad52-1 mutation the frequency of gene conversion not associated with reciprocal exchange is decreased dramatically, whereas the frequency of reciprocal recombinants is not affected (Table 2). The His⁺ prototrophs classed as reciprocal recombinants could be generated by gene conversion followed by reciprocal exchange. If this association of gene conversion with reciprocal recombination is frequent, then rad52 only affects mitotic gene conversion that is not associated with reciprocal exchange.

Discussion

We have shown that the major mitotic recombination event observed in duplications is gene conversion unassociated with reciprocal exchange. This result is consistent with the results of previous studies on mitotic recombination between single-copy genes in heteroallelic diploids^{7,8}. In addition, we observe that the rad52-1 mutation, which decreases mitotic exchange in diploids^{12,14}, preferentially decreases gene conversion in our system. These findings, taken together with earlier studies, suggest that gene conversion and reciprocal recombination occur by different pathways in mitosis.

Gene conversion between repeated DNA sequences may be involved in biologically important phenomena in yeast. For example, mating type interconversion is a complex event which seems to have some features in common with gene conversion within gene duplications^{26,27}. Mating type in yeast is controlled by two alleles of the MAT locus, MATa and MATa. Homothallic strains switch from one mating type to the other at a high frequency^{28,29}. This switching of mating type requires two other loci, HML and HMR³⁰⁻³². HML and HMR are unexpressed copies of the α and a information which can donate this information to the MAT locus^{33,34}. The replacement of MAT information by HML or HMR does not lead to a change at the donor locus. This aspect of homothallic switching is similar to gene conversion within duplications. One copy of the duplication acts as a donor of genetic information and the other as recipient. The donor sequences are not altered during the event. In contrast to mitotic gene conversion, mating type interconversion is a directed event; a information replaces MA $T\alpha$ and α information replaces MATa^{33,34}. Studies of cloned mating type genes show that the MAT, HML and HMR loci are direct repeats of common DNA sequences on chromosome III35,3 Thus, mating type loci are like the non-tandem direct repeats of the his4 gene which we have studied. Unlike the his4 gene duplications, where the two regions are completely homologous, each mating type locus contains one of two non-homologous

sequences (an 850-bp α-specific sequence or a 700-bp aspecific sequence) flanked by sequences homologous to all three loci³⁶. In spite of the differences between mating type interconversion and mitotic gene conversion, both involve the transfer of genetic information without rearrangement of the donor sequences. Moreover, the suggestion that the two processes occur by a similar event is supported by the finding that homothallic strains carrying the rad52-1 mutation are defective in mating type interconversion13.

Sequence homogeneity between highly repeated DNA sequences is thought to be maintained by mitotic recombination. In yeast, rDNA is composed of ~100 repeats of a 9-kb unit per haploid genome³⁷. Genetic and biochemical evidence suggests that the rDNA repeat units are tandemly arrayed, and within any individual strain these repeat units are homogeneous³⁸ Unequal sister-strand reciprocal recombination has been shown to occur in the rDNA cistrons in both mitosis and meiosis of

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- Hotchkiss, R. D. A. Rev. Microbiol. 28, 445-468 (1974).
- Radding, C, M. A. Rev. Biochem. 47, 847-880 (1978). Stahl, F. W. Genetic Recombination (Freeman, San Francisco, 1979)

- Hurst, D. D., Fogel, S. & Mortimer, R. K. Proc. natn. Acad. Sci. U.S.A. 69, 101-105 (1972). Holliday, R. Genet. Res. 5, 282-304 (1964). Meselson, M. S. & Radding, C. M. Proc. natn. Acad. Sci. U.S.A. 72, 358-361 (1975).
- Roman, H. Cold Spring Harb. Symp. quant. Biol. 21, 175-185 (1956); in Genetics Lectures Vol. 2 (ed. Bogart, R.) 43-59 (Oregon State University Press, Corvallis, 1971).
- Roman, H. & Jacob, F. Cold Spring Harb. Symp. quant. Biol. 23, 155-160 (1958)
- Boram, W. R. & Roman, H. Proc. natn. Acad. Sci. U.S.A. 73, 2828-2832 (1976)
- Golin, J. E. & Esposito, M. S. Molec: gen. Genet. 150, 127-135 (1977).
 Malone, R. E., Golin, J. E. & Esposito, M. S. Curr. Genet. 1, 241-248 (1980).
- 12. Game, J. C., Zambe, T. J., Braun, R. J., Resnick, M. & Roth, R. M. Genetics 91, 51-68 (1980).
- Malone, R. E. & Esposito, R. E. Proc. natn. Acad. Sci. U.S.A. 77, 503-507 (1980).
 Prakash, S., Prakash, L., Burke, W. & Montelone, B. A. Genetics 94, 31-50 (1980)
- 15. Hinnen, A., Hicks, J. B. & Fink, G. R. Proc. natn. Acad. Sci. U.S.A. 75, 1929-1933 (1978).
- 16. Ilgen, C., Farabaugh, P. J., Hinnen, A., Walsh, J. M. & Fink, G. R. Genet. Engng 1,
- Hinnen, A., Farabaugh, P. J., Ilgen, C., Fink, G. R. & Friesen, J. in Eukaryotic Gene Regulation, ICN-UCLA Symp. (eds Axel, R., Maniatis, T. & Fox, C. F.) 43-50 (Academic, New York, 1979).
- Mortimer, R. K. & Schild, D. Microbiol. Rev. 44, 519-571 (1980).
- Fink, G. R. & Styles, C. A. Genetics 77, 231-244 (1974).
 Fogel, S. & Mortimer, R. K. A. Rev. Genet. 5, 219-236 (1971)
- Esposito, M. S. Proc. nain. Acad. Sci. U.S.A. 75, 4436-4440 (1978).
 Fabre, F. Nature 272, 795-798 (1978).
- 23. Wildenberg, J. Genetics 66, 291-304 (1970).

yeast^{24,25}. Szostak and Wu²⁵ propose that unequal sister-strand exchange occurs at a high enough frequency in mitotic cells to account for the homogeneity. Meiotic analysis of a duplication of the leu2 gene in yeast has led Klein and Petes 42 to suggest that gene conversion is a more likely mechanism for rDNA rectification. Our data indicate that mitotic gene conversion is far more frequent than unequal sister-strand exchange and therefore more likely to be responsible for sequence homogeneity. These conversion events could be responsible for the sequence homogeneity found in other types of repeated genes in eukaryotes, for example, the histone⁴³ and globin genes^{44,45}

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Petes, T. D. Cell 19, 765-774 (1980).

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- 25. Szostak, J. W. & Wu, R. Nature 284, 426-430 (1980)
- Haber, J. E., Rogers, D. T. & McCusker, J. H. Cell 22, 277-289 (1980). Klar, A. J., McIndoo, J., Strathern, J. N. & Hicks, J. B. Cell 22, 291-298 (1980).
- Hicks, J. & Herskowitz, I. Genetics 85, 373-393 (1977) Strathern, J. & Herskowitz, I. Cell 17, 371-381 (1979).
- Harishima, S. & Oshima, Y. Genetics 84, 437-451 (1976). Naumov, G. I. & Tostruokov, I. I. Genetica 9, 82-91 (1973)
- Harishima, S., Nogi, Y. & Oshima, Y. Genetics 77, 639-650 (1974). Kushner, P., Blair, L. & Herskowitz, I. Proc. natn. Acad. Sci. U.S.A. 76, 5264-5268 (1979).
- Klar, A. & Fogel, S. Proc. natn. Acad. Sci. U.S.A. 76, 4539-4543 (1979).
 Hicks, J., Strathern, J. N. & Klar, A. Nature 282, 478-483 (1979).

- Nasmyth, K. A. & Tatchell, K. Cell 19, 753-764 (1980). Schweizer, F., MacKechnie, C. & Halvorson, H. W. J. malec. Biol. 46, 261-277 (1969).
- Schwelzer, F., MacKetting, C. & Havolson, H. W. J. Matter Man. Model. 1986.
 Bell, G. K. et al. J. biol. Chem. 252, 8118–8125 (1977).
 Cramer, J. M., Farrelly, F. W., Barnitz, J. T. & Rownd, R. M. Molec. gen. Genet. 151, 229-244 (1977).
- 40. Petes, T. D., Hereford, L. M. & Skryabin, K. G. J. Bact. 134, 295-305 (1978).
- Petes, T. D. & Bostein, D. Proc. natn. Acad. Sci. U.S.A. 74, 5091-5095 (1977).
 Klein, H. L. & Petes, T. D. Nature 289, 144-148 (1981).
- 43. Kedes, L. H. A. Rev. Biochem. 48, 837-870 (1979) 44. Efstradiatis, A. et al. Cell 21, 653-668 (1980).
- Lauer, J., Shen, C. J. & Maniatis, T. Cell 20, 119-130 (1980).
- 46. Cryer, D. R., Eccleshall, R. & Marmur, J. Meth. Cell Biol. 12, 39-44 (1975).
- Hirt, B. J. molec. Biol. 26, 365-369 (1967). Southern, E. M. J. molec. Biol. 98, 503-517 (1975).
- Maniatis, T., Jeffrey, A. & Kleid, D. G. Proc. nam. Acad. Sci. U.S.A. 72, 1184-1188 (1975).
- Bolivar, F. et al. Gene 2, 95-113 (1977)
- Sherman, F., Fink, G. R. & Lawrence, C. W. Methods in Yeast Genetics (Cold Spring Harbor Laboratory, New York, 1978).

Chromatin structure of endogenous retroviral genes and activation by an inhibitor of DNA methylation

Mark Groudine**, Robert Eisenman* & Harold Weintraub*

*Hutchinson Cancer Center, 1124 Columbia Street, Seattle, Washington 98104, USA †Department of Radiation Oncology, University of Washington Hospital, Seattle, Washington 98105, USA

The transcriptionally active ev-3 and inactive ev-1 endogenous retrovirus loci in chick cells differ in that ev-3 is undermethylated, preferentially sensitive to DNase I digestion, and contains nuclease hypersensitive sites in each of its two long terminal repeats. Transient exposure of cells to 5-azacytidine, a cytosine analogue which cannot be methylated at the 5 position, results in the hypomethylation and transcriptional activation of ev-1, as well as the acquisition of at least one nuclease-hypersensitive site within the chromosomal domain of ev-1.

ANALYSES from several laboratories have shown that expressed genes are packaged into an altered chromatin structure, as revealed by preferential DNase I digestion¹⁻⁴. These active regions are usually undermethylated at CpG dinucleotides 5-12. In many cases, gene activity is also correlated with a DNase I-hypersensitive site, which represents a very local alteration of chromatin that is associated with certain specific DNA sequences. These 'hypersensitive' regions are detected by the ability of DNase I to introduce double-stranded cuts at such

specific DNA sequences¹³. Thus, when nuclei are mildly digested with DNase I and the DNA isolated and redigested with restriction endonucleases, a sharp 'sub-band' appears on 'Southern' blotting. One end of this fragment is defined by the DNase I-generated double-stranded cuts in chromosomal DNA, and the other end by a neighbouring restriction site. Although many of the hypersensitive sites described thus far are very near the 5' border of transcriptionally active genes, others have been located in adjacent non-coding regions 14,15. The notion that hypersensitive sites may reflect important events in gene regulation is further supported by the finding that a change in location of these sites accompanies haemoglobin switching in the developing chicken embryo 14,16. Recently, we have shown that in precursor erythroblasts obtained from 22-h chick blastoderms, the globin genes are not transcribed; they are methylated, DNase I resistant and contain no hypersensitive sites. However, because all these parameters of gene activity become expressed coordinately (within the temporal resolution of our experiments) in progeny erythroblasts, it is not clear which, if any, of these structural features of active chromatin are primary and which secondary. We thus sought a system where we could experimentally manipulate such parameters.

In attempting to study the relationships between transcription, DNase I sensitivity, CpG methylation and hypersensitive sites, we have investigated these aspects of the endogenous avian retroviral genomes. Uninfected chicken cells contain one or more complete or nearly complete copies of genetically transmitted endogenous viruses, the prototype of which is called RAV-O. As described by Astrin¹⁷, most chickens contain at least one such genetic locus, ev-1, identifiable on 'Southern' blots as a 10.5-kilobase (kb) SsfI restriction fragment 18,19 Although ev-1 contains structural sequences nearly identical to RAV-O, it is basically silent in terms of expression²⁰. Astrin and co-workers21 have described at least 10 other avian genetic loci that contain structural genes for endogenous retroviruses, all of which are identifiable as specific Sst fragments. Of particular interest is ev-3, a deleted RAV-O locus which serves as the template for 31S and 22S viral RNA transcripts in uninfected chicken cells20,22. Although ev-3-containing cells do not produce either infectious virus or virus particles, they do contain a 120,000-molecular weight ev-3-coded polyprotein, P120Gs which represents an apparently non-functional, internally deleted form of the precursor to reverse transcriptase found in retrovirus infected cells 23,24 . The specific region of the ev-3deletion and detailed restriction maps of the various ev loci have been described recently 18,19,21,23,24

The present experiments demonstrate that, in contrast to ev-1, the conformation of ev-3 in chromatin results in sensitivity of this locus to digestion by DNase I and site-specific double-stranded cleavage at both the 5' and 3' ends of ev-3 (in long terminal repeat (LTR) sequence) by both DNase I and staphy-lococcal nuclease. In addition, and again in contrast to ev-1, several regions of ev-3 are undermethylated at cytosine residues at CpG dinucleotides. That undermethylation of at least some of these sites is important in permitting expression of the endogenous retroviral genome is strongly suggested by the transcriptional activation of an ev-1 locus by 5-azacytidine, a cytosine analogue which, due to a nitrogen substitution in the 5 position, cannot be methylated at this site²⁵. The activation of ev-1 by 5-azacytidine is also correlated with the generation of a

hypersensitive site within the chromosomal domain of this previously inactive retroviral genome. These results suggest that, at least for the ev-1 locus, undermethylation of at least part of the ev-1 locus results in the subsequent chromosomal activation of this genome as revealed by DNase sensitivity, transcription and synthesis of viral proteins.

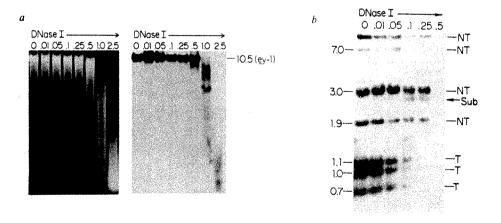
Preferential nuclease sensitivity of ev-3

Nuclei were isolated from red blood cells of 14-day chicken embryos characterized genotypically as either ev-1 or ev-1/ev-3. As previously reported 4.11.15, although such erythrocytes are no longer transcriptionally active, the globin genes previously expressed in these cells are preferentially sensitive to DNase I. The respective nuclear preparations were digested with very low levels of DNase I or staphylococcal nuclease 4.13.15 and the DNA was purified, restricted with Ssl, run on neutral 1% agarose gels, 'Southern' blotted and hybridized to 32P-RAV-O cDNA. (In all cases, identical results were obtained using cloned RAV-O sequence containing DNA as probe; however, we prefer to use the cDNA because it is usually higher in specific activity and gives much less background on blots.) Before restriction, most of this DNA had an average size of 15-20-kilobase pairs (kbp).

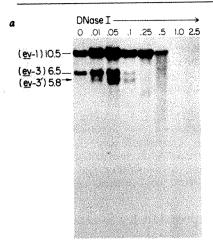
Figure 1 shows the results of DNase I digestion of nuclei from ev-1 red blood cells. As predicted from known restriction maps of ev-1 red blood cells. As predicted from known restriction maps of ev-1 red blood cells. As predicted from known restriction maps of ev-1 compared with bulk DNA, nor sub-bands indicative of site-specific double-stranded cuts within this locus are observed (Fig. 1a). Digestions with several other enzymes also fail to demonstrate sub-bands, indicating the lack of specific cuts in either the 5'- or 3'-flanking regions of this gene (not shown). As controls for the preferential digestion of active genes and generation of specific sub-bands in these DNA samples, restriction bands containing the α -globin genes once transcribed in these cells are no longer detectable in the sample digested with $0.25 \, \mu \mathrm{g} \, \mathrm{ml}^{-1}$ DNase I, and several samples show a specific sub-band containing globin flanking sequences (Fig. 1b).

Figure 2a reveals the results of a similar analysis performed with nuclei from cells containing both ev-1 and ev-3. In addition to confirming the relative 'insensitivity' of ev-1 to DNase I digestion, this figure demonstrates that ev-3 is much more sensitive to DNase I than is ev-1. Thus, the chromosomal conformation of ev-3 is similar to many transcriptionally active genes assayed by this technique. The kinetic analysis of the preferential digestion of the 6.5-kbp SsiI restriction fragment containing ev-3 also reveals a 5.8-kbp sub-band, indicating a site-specific double-stranded cut introduced into the chromosomal DNA in or around this locus. A similar sub-band is detected when nuclei from ev-1/ev-3 red cells are digested with increasing concentrations of staphylococcal nuclease (Fig. 2b), further indicating that a specific region of this endogenous viral locus is uniquely cut in the intact chromosome. Note,

Fig. 1 a, Resistance of ev-1 to low level DNase I digestion. Red blood cell nuclei from 14-day chicken embryos of ev-1 genotype were incubated with increasing amounts of DNase I at a DNA concentration of 1 mg ml-1 for 10 min at 37 °C. DNA was isolated, restricted with Ssd, electrophoresed on a neutral 1% agarose horizontal slab gel, stained with ethidium bromide. blotted on to a nitrocellulose filter and hybridized to ³²P-RAV-O cDNA. The dominant RAV-O related band in the Sst digest is 10.5 kbp, corresponding to ev-1. Numbers at the top of each lane refer to concentration (µg ml-1) of DNase I. b. Sensitivity of globin DNA to DNase I. The same DNA as shown in a was digested with Bam and EcoRI and hybridized to a genomic clone (α_s) containing transcribed (T) and nontranscribed (NT) regions of the chicken



 α -globin domain (the sub-band is generated from a hypersensitive site in a non-transcribed region of the α locus; see ref. 11 for details). The NT regions show a marked DNase sensitivity that has previously been shown to depend on HMG proteins, while the T regions show an 'intermediate' level of sensitivity that is not related to HMG binding. The ev-1 locus in a is classified as being DNase 'resistant'.



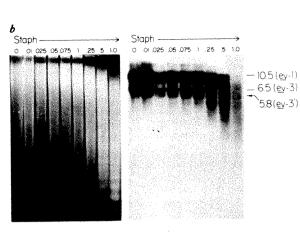


Fig. 2 Preferential sensitivity of ev-3 to DNase I and the generation of ev-3specific sub-bands by DNase I and staphyloccal nuclease. a, ev-1/ev-3 DNA was prepared and analysed after restriction with Sall as described for ev-1 DNA in Fig. 1. Two dominant RAV-O related bands in the Sst digest are the 10.5-kbp ev-1 and the 6.5-kbp ev-3. In addition to the preferential sensitivity of ev-3 to DNase I, a 5.8-kbp sub-band is revealed, b. DNA from staphylococcal nuclease-treased red blood cell nuclei from 14-day chicken embryos of ev-1/ev-3 genotype was prepared and analysed as above.

however, that the dramatic disappearance of ev-3 vis- \acute{a} -vis ev-1 seen in the DNase I digest (Fig. 2a) is not observed with staphycoccal nuclease digestion (Fig. 2b).

Previously, we reported the presence of RAV-O-related RNA and proteins in cells isolated from embryos of a particular flock of P120^{Gs}-negative, chick helper factor (chf) negative (gs chf), uninfected chickens²⁶. Using DNase I and solution hybridization we observed that these cells contained one 'active' and one 'inactive' retroviral genome. However, restriction mapping was not performed and the ev loci were not identified. Given the recent description by Astrin and co-workers²¹ of multiple ev loci associated with the gs chf phenotype, we reanalysed cells from this flock and observed that they contain both ev-1 and another 'phenotypically negative' ev locus, ev-8. Whereas ev-8 is DNase I sensitive in these cells, the ev-1 locus is resistant to DNase I, consistent with the above results. (A detailed analysis of these cells will be presented elsewhere.)

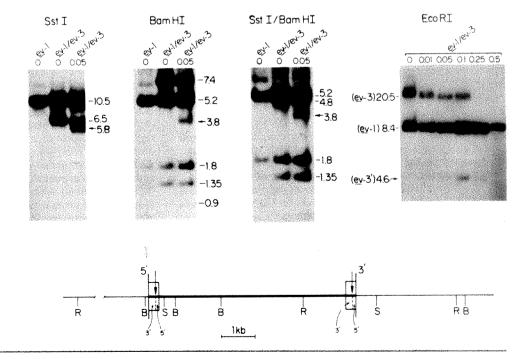
Mapping the hypersensitive site of ev-3

In an attempt to understand better the relationship between hypersensitive sites and gene activity, we mapped the region of ev-3 uniquely cut by DNase I and staphylococcal nuclease. Although all the mapping data presented here were generated from experiments with DNA from DNase I-digested nuclei, the same results are obtained with DNA samples from staphyloccal

nuclease-treated nuclei (not shown). Figure 3 shows the results of this analysis and a restriction map of ev-3. As indicated above (and in Fig. 3), a sub-band of 5.8 kbp is generated when the DNase I-treated samples are digested with Ssfl. Given the restriction map of ev-3, the hypersensitive site observed with Ssfl must be located either 700 bases 3' to the Sst site in the 5' end of the locus or within the LTR located on the 3' side of ev-3. When the same sample was digested with BamHI, a sub-band of 3.8 kbp was observed, indicating that the sub-band must be generated from a site within the 3' LTR. Ssfl/BamHI double digestion (Fig. 3) also shows a 3.8-kbp sub-band, providing further support for this conclusion. Given the location of Bam sites in the 5' end of the locus, this sub-band could only be generated by a DNase I-generated double-stranded cut in the 3' LTR.

As the LTR is repeated at both ends of ev-3, we wondered if a similar hypersensitive site was located in the LTR on the 5' side of the locus. Neither Sst nor Bam digestion would reveal such a site, because both cleave within the locus, 3' to the 5' LTR. We therefore hybridized an EcoRI-digested DNase series from ev-1/ev-3 cells with a ³²P-nick-translated DNA fragment containing sequences specific to the 'gag' gene located to the right of the 5' LTR. (The fragment was isolated from a pBR subclone provided by Drs W. DeLorbe and J.M. Bishop and their colleagues.) This probe detected the two expected EcoRIgenerated restriction fragments in the undigested sample

Fig. 3 Mapping the specific DNase Ihypersensitive Sites in ev-3. Th e restriction map of ev-3 is derived primarily from our own results, and the work of Hughes et al. 19. Undigested DNA from ev-1 and ev-1/ev-3 erythrocytes, and DNA from ev-1/ev-3 nuclei digested with 0.05 µg ml-1 DNase I were restricted with either Sstl or BamHI or doubly digested with Sstl and BamHI, and analysed as described in the previous figure legends. In addition, the DNase I-digested ev-1/ev-3 DNA 'series' presented in Fig. 2 was digested with EcoRI and analysed as above except that rather than using RAV-O cDNA, we used a ³²P-nick-translated DNA fragment containing sequences specific to the gag gene located to the right of the 5' LTR. In the EcoRI blot, the 20.5-kbp fragment is ev-3-specific, whereas the 8.4-kbp fragment is ev-1 specific. (In mapping the location of the nuclease-generated sub-bands with EcoRI, we have presented a kinetic analysis for optimal visualization of the ev-3 sub-bands.)



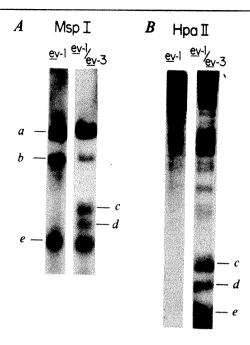


Fig. 4 Undermethylation of ev-3 relative to ev-1. DNA from ev-1 and ev-1/ev-3 erythrocytes was purified from nuclear preparations, digested with various restriction enzymes, electrophoresed on neutral, 1.4% agarose horizontal slab gels, blotted on to nitrocellulose and hybridized to 32 P-RAV-0 cDNA. A, MspI digestion of ev-1 and ev-3 DNA. B, HpaII digestion of the same samples. Note the generation by HpaII of fragments c, d and e in the ev-1/ev-3 cells, but not in ev-1 cells.

(20.5 kbp containing the 5' 5.0 kbp of ev-3 and 15.5 kbp of 5' flanking sequences, and an 8.4-kbp ev-1 specific fragment), but revealed a 4.6-kbp sub-band in the DNase I-digested samples (Fig. 3). As we have been unable to detect any retrovirus-related sub-bands in DNA from DNase-treated ev-1 nuclei using a variety of restriction enzymes and specific probes (Fig. 1 and other data not shown), the 4.6-kbp sub-band is probably generated from a hypersensitive site that maps in the 5' LTR of ev-3. This has been confirmed using several other probes and restriction enzymes, and we are now attempting to determine the exact sequence within the LTR that is cut by DNase I.

Thus, although no hypersensitive sites are observed in the ev-1 chromosomal domain, at least two such sites are apparent in ev-3, both located within regions of sequence homology (LTRs) on both the 5' and 3' side of the proviral coding regions. These LTR regions are of particular interest in terms of control of gene expression, given the sequence homology in this region to a number of putative control elements²⁷ (see discussion) and the ability of homologous regions in Rous sarcoma virus (RSV) to act as promoters in vitro²⁸.

Undermethylation of ev-3

Figure 4A and B show the results of restricting DNA from red cells of either ev-1 or ev-1/ev-3 genotype with Msp1 and HpaII, which recognize the sequence CCGG²⁹; however, Msp1 cuts at this site independent of the methylated state of the second C, whereas HpaII will not cut this sequence if the second C is methylated. As evident in Fig. 4A, ev-1/ev-3 DNA contains at least two more Msp1 restriction fragments (labelled c and d) than ev-1 DNA. Figure 4B illustrates that although ev-1 is highly methylated at CCGG sequences, the two ev-3-specific Msp1 fragments (c and d) are not methylated at such residues. In addition, several other regions are relatively undermethylated, given the generation of a broad band (e) of fragments with an average size of 300 base pairs (bp). We presume that these fragments come from ev-3 as they are not found in HpaII-digested DNA from ev-1 birds; however, we cannot formally rule out the possibility that under the influence of ev-3, these

fragments now become undermethylated at the ev-1 locus. We have also observed that whereas the 10.5-kbp SstI fragment characteristic of ev-1 is relatively stable on double digestion of ev-1 or ev-1/ev-3 DNA with SstI and HpaII, the 6.5-kbp SstI fragment characteristic of ev-3 disappears on Sst/HpaII double digestion of ev-1/ev-3 DNA (not shown). Thus, our results demonstrate qualitatively a clear correlation between the expressed ev-3 locus and undermethylation and an analogous correlation between the unexpressed ev-1 locus and hypermethylation. Although other investigators have also observed differences in the methylated state of retroviral genomes in various cells $^{30-32}$, correlation of expression and degree of methylation of a particular viral locus in these cells was not possible.

Induction of ev-1 expression by 5-azacytidine

In an attempt to understand the role of undermethylation in the control of gene activity, we analysed the effect of 5-azacytidine²⁵ on the expression of the inactive ev-1 locus. A continuous line of chicken lymphocytes containing only ev-1, MSB cells^{33,34}, was exposed to the cytosine analogue for 24 h (two or three generations), the medium containing the drug removed, fresh medium added and the cells assayed at various intervals for changes in ev-1 DNA methylation, RNA accumulation and virus-specific protein synthesis. DNA containing this analogue cannot be methylated at the substituted site²⁵. Moreover, during the 'chase' period most of the analogue is diluted out by replication. Thus, its effect on methylation occurs during the initial treatment, but the undermethylated state is nevertheless propagated to daughter cells independent of the continual presence of the analogue.

Figure 5A shows MspI and HpaII digestions of DNA from control and 5-azacytidine (AZA)-treated MSB cells. Although ev-1 MspI fragments are generally similar in red blood cells (Fig. 4) and MSB cells (Fig. 5), a small amount of what may be partial and limit restriction fragments is present in HpaII digests of MSB [for example, bands c and d in Fig. 5A (left)], but not erythrocyte DNA when blots containing restricted DNA from both cell types are exposed equivalently. As the MSB cells are routinely subcloned, these limit HpaII fragments may reflect the fidelity with which a particular methylation pattern is transmitted to daughter cells during DNA replication.

In contrast to the very small amount of signal observed in the limit restriction fragments from control MSB cells, exposure of these cells to azacytidine results in the genration by HpaII of the major MspI e fragments observed in control DNA. Based on the relative intensities of the e band observed in MspI and HpaII digests (Fig. 5A), within the limits of our assay, $\sim 50\%$ of the ev-1 Msp fragments in these cells are now undermethylated. We presume that this reflects the degree of substitution of 5-azacytidine during the initial treatment period.

To assay for gene expression, we isolated cytoplasmic RNA from control and azacytidine-treated MSB cells, 'dotted' the RNA on to nitocellulose35 and assayed for the appearance of RAV-O-related sequences by hybridization with ³²P-RAV-O cDNA (Fig. 5B). In these conditions we cannot detect significant signal from control cells, but signal from the AZA-MSB cells is observed as early as 24 h after removal of the analogue. Although the total amount of viral RNA increases between 24 and 48 h post-exposure, virus-specific RNA sequences change little between 48 and 72 h. To quantify ev-1-coded RNA sequences, we compared the intensities of the AZA-MSB RNA 'dots' with that from RNA isolated from chick embryo fibroblasts (CEF) infected with RSV, which by solution hybridization analysis contain ~5,000 copies of virus-specific RNA per cell (not shown). As the intensity of the 10 µg AZA-MSB 'dots' is comparable with that of the 0.1-µg RSV-CEF 'dots', we estimate that AZA-MSB cells contain ~50 copies of RAV-Orelated RNA per cell. This has also been confirmed by conventional hybridization analysis in solution. Obviously, this assay gives no information regarding the number of cells actually transcribing ev-1 (see below); however, here we are interested

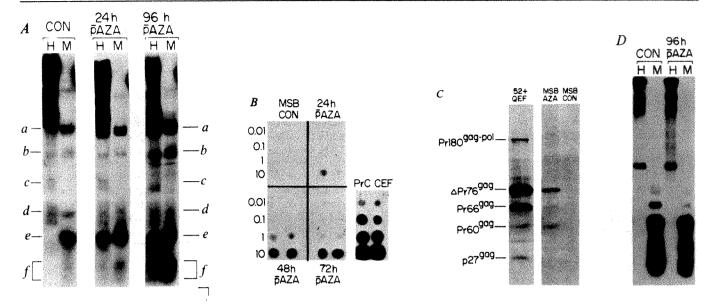


Fig. 5 Induction of ev-1 expression by 5-azacytidine. MSB cells were grown in media containing 3 μM 5-azacytidine (AZA) for 24 h, the analogue-containing medium was removed and the cells analysed 24, 48, 72 or 96 h after removal of the drug. A, Restriction of DNA from control (CON) and 5-azacytidine-treated (AZA) MSB cells with HpaII (H) and MspI (M) 24 and 96 h after removal of the analogue, and hybridization to ³²P-RAV-O cDNA. B, Dot blots' of cytoplasmic RNA isolated from control MSB cells, MSB cells at 24, 48 or 72 h after exposure to 5-azacytidine, and chick embryo fibroblasts (CEF) infected with RSV. 10, 1, 0.1 and 0.01 μg of each RNA sample were denatured at 65 °C, spotted in duplicate on to dry nitrocellulose filter paper (previously wetted with H₂O and soaked in 20 × SSC for 30 min), dried under a heat-lamp, and baked at 80 °C under vacuum for 2 h. The RNA-containing filters were prehybridized and then hybridized to ³²P-RAV-O cDNA. C, Analysis of retrovirus proteins in untreated or azacytidine-treated MSB cells. MSB cells were treated with 3 μM azacytidine for 24 h and allowed to grow for 3 additional days after removal of the drug. Untreated cells from the same culture were grown in parallel. The cells werewashed and incubated for 1 h at 37 °C with 200 μCi ³⁵S-methionine in methionine-deficient medium. Quail embryo fibroblasts (QEF) productively infected with the wild-type virus 52+ were similarly labelled. Cells were lysed as previously described^{23,24} and treated with 4 μl of a rabbit antiserum raised against purified retrovirus gag proteins and 80 μl of fixed Staphylococcus aureus. The immune complexes were washed extensively, dissolved in SDS (5%) electrophoresis sample buffer and subjected to electrophoresis on a 12.5% polyacrylamide slab gel; an autoradiograph of the dried gel is shown. The various gag-related polypeptides immunoprecipitated with the antiserum represent the different cleavage proteins generated from the primary precursor, Pr76²⁸C. D, Hybridization of a ³²P-nick tra

in any transcription of the retroviral locus in the AZA-cells compared with control MSB cells. Interestingly, analysis of viral RNA content in cells containing the active endogenous ev-3 genome has revealed that such cells also contain ~ 50 copies of RAV-O-related RNA per cell²².

We also investigated the synthesis of viral proteins in the 5-azacytidine-treated cells. MSB cells have been characterized as negative for endogenous retroviral gag, pol and env gene expression³⁶ and, as shown in Fig. 5C, we could not detect any of the viral internal structural proteins (gag) in the control MSB cells. However, Fig. 5C also shows that the gag precursor polyprotein Pr76gag and two of its cleavage intermediates, Pr66 and Pr60, are detectable in MSB cells assayed 72 h after exposure to azacytidine. Of significance, viral particles are also detectable in the supernatant medium from these cells, but they do not contain reverse transcriptase (R. Eisenman and K. Conklin, unpublished observations). The absence of reverse transcriptase actually facilitates our assay, for the particles are thus uninfectious, and our results cannot be attributed to reinfection of the AZA-MSB cells by RAV-O. (A detailed characterization of the viral RNA, protein and particles is in preparation.)

As expected from the results of Jones and Taylor²⁵, our results depend on incorporation of the analogue into cellular DNA; concurrent exposure of MSB cells to azacytidine and inhibition of DNA synthesis by cytosine arabinoside or hydroxyurea results in none of the observed changes in DNA methylation or viral RNA accumulation (not shown). We have also observed that 24 h exposure of MSB cells to concentrations of azacytidine $>7 \,\mu$ M results in severe toxicity, with only 10–20% of the cells remaining viable 48 h after its removal. At the concentration used in these experiments (3 μ M), a minimum of 50% of the cells are viable when assayed at 48 h post 'wash-out'. We have also observed ev-1 activation on 8 h exposure of MSB cells to 1 μ M azacytidine and, in these conditions, >90% of the cells remain viable. Finally, we have observed similar changes in ev-1

DNA methylation, RNA accumulation and protein synthesis on exposure of cultures of ev-1 containing chick embryo fibroblasts to the analogue (unpublished observations). Thus, azacytidine is effective both in activating whole programmes of differentiation in mouse cells²⁵ (see discussion) and in activating a particular gene in chicken cells, in a continuous line and in primary fibroblasts.

In contrast to the 5-azacytidine-induced undermethylation of regions of ev-1, we have observed no significant changes in the methylated state of five different α - and β -globin genes in these same 5-azacytidine-treated MSB cells. For example, using an embryonic β -globin probe and the same MSB AZA-DNA described above, none of the globin-specific MspI-generated fragments is observed with HpaII digestion (Fig. 5D). The methylated state of these various globin genes has been maintained in every MSB AZA-DNA sample tested. Thus, the undermethylated state resulting from 5-azacytidine treatment is apparently non-random (see discussion).

An ev-1 hypersensitive site induced by 5-azacytidine

As mentioned above, regions of DNase I hypersensitivity have been described for several transcriptionally active genes $^{7,13-15,37}$. Although HMG 14 and 17 are responsible, at least in part, for the preferential sensitivity of each nucleosome within a particular transcription unit 38 , the basis of the hypersensitivity of the particular point site is unclear (see ref. 39 for extensive discussion). Using β -globin genes, we demonstrated previously that the appearance of these sites coincides with the developmental activation of any one particular β -globin gene. Thus, although no β -globin-hypersensitive sites are observed in precursor haematocytoblasts, such sites are detectable near the embryonic β -globin genes in daughter, first generation erythroblasts 16 . The generation of hypersensitive sites during development suggests that these regions are not 'stable' areas in

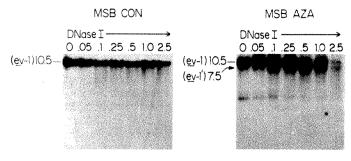


Fig. 6 Appearance of a DNase I-hypersensitive site after treatment of MSB cells with 5-azacytidine. Nuclei from control MSB cells and MSB cells 72 h after exposure to 5-azacytidine were incubated with increasing amounts of DNase I and analysed as described for red blood cell nuclei in Fig. 1. Although no sub-band is detectable in the control cells, a prominent 7.5-kbp sub-band (in addition to the expected 10.5-kbp ev-1 SsI fragment) is observed in these cells after exposure to the cytosine analogue.

terms of chromosomal conformation, but are the results of processes involved in the developmental activation of particular regions of the chromosome. We wondered, therefore, whether the transcriptional activation of ev-1 by 5-azacytidine was also correlated with the generation of a hypersensitive site within the chromosomal domain of this retroviral genome. Figure 6 shows the results of low-level DNase I digestions of control and azacytidine-treated MSB cells. Whereas no ev-1-related subbands are detectable in the SstI-restricted DNA from control cells, a prominent 7.5-kbp sub-band is observed in the DNA isolated from DNase I-digested nuclei of MSB cells, analysed 72 h after exposure to the analogue. In addition, the intensity of the sub-band and the kinetics of its disappearance compared with those of the 10.5-kbp ev-1 SstI fragment, indicate that the hypersensitive site is present in $\sim 50\%$ of the cells in the azacytidine-treated population. Thus, the activation of ev-1 by 5-azacytidine is associated with a change in the chromosomal conformation of this gene, paralleling the developmental activation of the chicken globin genes.

Extensive mapping of the azacytidine-induced ev-1 sub-band (similar to that described in mapping the ev-3 sub-band) has revealed site-specific double-stranded cuts in both the 3' and 5' LTRs of the endogenous retrovirus (unpublished observations), suggesting that transcriptional activity of two distinct endogenous retroviral loci is associated with a conformational change at a specific sequence located at both ends of these genomes.

Discussion

The experiments described above reveal that the expression of retroviral genomes, like many genes, is correlated with the conformation of these sequences in chromatin. Thus, the unexpressed ev-1 locus is no more sensitive to DNase I than bulk DNA, whereas the transcriptionally active ev-3 locus is preferentially sensitive to DNase digestion. In addition, and again as in several other systems, the unexpressed ev-1 is highly methylated at CCGG residues, whereas the 'active' ev-3 is relatively undermethylated at these sites. Similarly, although no hypersensitive site is observed on low level DNase digestion of ev-1, two hypersensitive sites are detectable (with both DNase I and staphylococcal nuclease) in ev-3. These sites are located within the LTR on either side of this retroviral genome. As described by Ju and Skalka²⁷, the LTRs of many retroviruses contain regions of homology with several other genetic elements. Particularly intriguing is a 15/20-bp sequence homology between the retroviral LTRs (CGTCATTCG'ACGATAG-TAG) and a tandemly repeated region in SV40 (CGT**TTCGTACG*TAG*AG), in which this sequence is present in a location recently described as essential for the initiation in vivo of early transcription⁴⁰. In addition, this same region of SV40 contains a DNase I-hypersensitive site associated with early transcription (C. Cremisi, unpublished observation) and is part of a larger region that is preferentially exposed to staphylococcal nuclease and various restriction endonucleases late in infection^{41,42}. The ev-3 sub-band mapping data presented above tend to localize the ev-3-hypersensitive sites to the border of the 5' and 3' sequences within the LTR (see Fig. 3); however, the error in this analysis is such $(\pm 100 \text{ bp})$ that the hypersensitive site could be located in the region of the LTR containing sequence homology with SV40. Several other sequences important in transcriptional control—a TATAA box and a cap site—are also located within this 200-bp region of the LTR. Given the location of such sites in both LTRs, we believe that a particular DNA sequence or sequences dictates the chromosomal conformation revealed by the DNase I-hypersensitive sites. Clearly, other factors (such as the pattern of DNA methylation) are also important because in normal chicken cells the LTRs present in ev-1 are not hypersensitive even though they contain sequences with the potential for generating hypersensitive regions, as evidenced by the appearance of such sites after treatment with azacytidine. Recently, Blair et al.43 have shown that ligation of LTRs to either the 5' or the 3' side of a virus-transforming gene increased the frequency of morphologically transformed colonies after DNA transfection. In the context of the present experiments, these results suggest that LTRs increase transcription not only by providing a highefficiency 'promoter'44, but also by 'opening-up' large tracts or domains of chromatin structure, possibly by phasing adjacent nucleosomes or segregating genes into a specific nuclear compartment required for transcription. If this is so, and if the LTR signal for this proposed change in chromatin structure is bidirectional (as its many inverted repeats suggest), it would not be surprising if these sequences have an effect when placed either 5' or 3' to a given gene. Although the biochemical basis for the hypersensitivity is unknown, these sites may represent regions of specific interactions with DNA sequence recognizing proteins. DNA 'footprinting' analysis of several DNA binding proteins has shown that sites within the binding domain of the protein can be 10-100-fold more sensitive to DNase I cutting when the protein is bound⁴⁵

We have also demonstrated that 5-azacytidine-induced undermethylation of the unexpressed ev-1 results in the transcriptional activation of this retroviral genome. In contrast to the induction of murine proviruses by such agents as BUdR and IUdR, induction of ev-1 in chickens by halogenated pyrimidines or other agents has not been demonstrated. However, several experiments 46,47, and our preliminary results, indicate that ev-1 cannot be efficiently induced by such agents. Although our azacytidine results strongly suggest that ev-1 activation is effected through a change in the methylated state of DNA, this has not been proved (for example, by DNA-mediated transfer). However, it is supported by the evidence presented above (change in cutting at HpaII sites; requirement for DNA synthesis; and generation of hypersensitive sites) and by the findings that ethionine and 3-deazaadenosine, two very different types of analogues which affect DNA methylation, also induce gene activity 48,49. Nevertheless, at present there is no direct experiment to exclude the possiblity that azacytidine leads to the activation of some other cellular locus which, in turn, activates the ev-1 locus. In this regard, results of transfection experiments in murine cells indicate that halogenated pyrimidines can affect viral gene transcription by acting at a site other than the viral genome⁵⁰. Our inability to observe 5-azacytidine-induced changes in the methylated state of several globin genes in the same cells in which regions of ev-1 are clearly undermethylated could be interpreted to support such a trans effect, assuming that the 'target size' for the putative regulating locus of ev-1 is much larger than that for the entire globin domain. Alternatively, it seems more reasonable that initially all genes are equivalently undermethylated, but that some type of selective correction mechanism leads to the re-methylation of the globin genes. Finally, the observed selective undermethylation of ev-1 in the AZA-MSB cells could be due to a non-random incorporation of the analogue. All these possibilities are being investigated.

The role of DNA methylation and gene function has been studied by several laboratories and recently reviewed by Razin and Riggs⁵¹. Our own work focuses on a particular gene and also suggests that undermethylation is important in generating a chromosomal structure permissive for transcription, for the transcriptional activation of ev-1 accompanies the appearance of a hypersensitive site, similar in location to that point site associated with the transcriptionally active ev-3. Our experiments seem to suggest that undermethylaton of all or a specific subset of CpG dinucleotide sites within the chromosomal domain of a given gene precedes the acquisition in chromatin by that gene of a conformational change permissive for transcription. It seems useful to compare our results on the activation of ev-1 with those of Jones and Taylor, who monitored cytodifferentiation, the presumed activation of entire programmes for gene activity. Using roughly comparable degrees of exposure to azacytidine, we estimate that at least 50% of the ev-1 loci are activated (see above), whereas Jones and Taylor observed about 1% of the cells in their cultures differentiating, for example, into muscle cells. We presume that the differences in frequency reflect the relative sizes of the respective targets needed to be substituted for the two assays. This could

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- Weintraub, H. & Groudine, M. Science 93, 848-858 (1976).
- Garel, A., Zolan, M. & Axel, R. Proc. natn. Acad. Sci. U.S.A. 74, 4867-4871 (1977)
- 3. Palmiter, R., McKnight, S., Mulvihill, E. & Senear, A. Cold Spring Harb. Symp. quant. Biol. 42, 639-647 (1977)
- Stalder, J., Groudine, M., Dodgson, J., Engel, D. & Weintraub, H. Cell 19, 973-980 (1980).

- McGhee, J. & Ginder, G. D. Nature 280, 419-420 (1979).
 Machee, J. & Cinder, G. D. Nature 280, 419-420 (1979).
 Mandel, J. & Chambon, P. Nucleic Acids Res. 7, 2081-2103 (1979).
 Kuo, T., Mandel, J. & Chambon, P. Nucleic Acids Res. 7, 2105-2113 (1979).
 Bird, A. P., Taggart, M. H. & Smith, B. A. Cell 17, 889-901 (1979).
 Van der Ploag, J. & Flavell, R. Cell 19, 947-958 (1980).

- Van der Ploag, J. & Flaveli, R. Cell 19, 947-936 (1980).
 Shen, S. & Maniatis, T. Proc. natn. Acad. Sci. U.S.A. 77, 6634-6638 (1980).
 Weintraub, H., Larsen, A. & Groudine, M. Cell 24, 333-344 (1981).
 Naveh, T. & Cedar, H. Proc. natn. Acad. Sci. U.S.A. (in the press).
 Wu, C., Bingham, P. M., Livak, K., Holmgren, R. & Elgin, S. C. R. Cell 16, 797-806 (1979).
 Wu, C. Nature 284, 856-860 (1980).
 Stalder, J. et al. Cell 20, 451-460 (1980).

- Groudine, M. & Weintraub, H. Cell 24, 393-401 (1981).
 Astrin, S. Proc. natn. Acad. Sci. U.S.A. 75, 5941-5945 (1978)
- Skalka, A., DeBona, P. Hishinuma, F. & McClements, W. Cold Spring Harb. Symp. quant. Biol. 44, 1097-1104 (1980).
- Hughes, S., Toyoshima, K., Bishop, J. & Varmous, H. Virology 108, 189-207 (1981).
 Hayward, W., Brauerman, S. & Astrin, S. Cold Spring Harb. Symp. quant. Biol. 44, 1111-1121 (1980).
- Astrin, S. et al. Cold Spring Harb. Symp. quant. Biol. 44, 1105-1109 (1980).
 Wang, S., Hayward, W. & Hanafusa, H. J. Virol. 24, 64-73 (1977).
 Eisenman, R., Shaikh, R. & Mason, W. S. Cell 14, 889-904 (1978).
 Eisenman, R., Mason, W. & Linial, M. J. Virol. 36, 62-78 (1980).

represent a requirement for greater substitution in a single target for myogenesis or, more likely, a requirement to activate more genes before muscle cytodifferentiation can be

Although the examples of chicken α - and β -globin genes^{5,11} and the active retroviral genomes described above, show a striking correlation between gene expression and hypomethylation of almost all the HpaII sites in a transcription unit, most studies in other systems do not support such a simple correlation (for example, ref. 10). Thus, we feel that undermethylation of specific regions of a locus^{6,9,10} may be the signal important in generating an active chromatin structure. This hypothesis predicts that exposure of MSB cells to very low levels of 5azacytidine should result in the transcriptional activation of ev-1in a subpopulation of the cells undermethylated only at one site or a few sites, possibly within the LTR. Analysis of subclones of such cells would then be expected also to reveal a specific hypersensitive site.

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- 25. Jones, P. & Taylor, S. Cell 20, 85-93 (1980).
- Groudine, M., Das, S., Neiman, P. & Weintraub, H. Call 14, 865-878 (1978).
 Ju, G. & Shalka, A. Cell 22, 379-386 (1980).

- Ju, U. & Shaika, A. Cell 24, 3/9-380 (1980).
 Yamamoto, T., de Crombrugghe, B. & Paston, I. Cell 22, 787-797 (1980).
 Wasilwyck, C. & Flavell, R. A. Nucleic Acids Res. 5, 4531-4641 (1978).
 Humphries, E., Glover, C., Weiss, R. & Arrand, J. Cell 18, 803-815 (1979).
 Guntaka, R., Rao, P., Mitsalis, S. & Katz, R. J. Virol. 34, 569-572 (1980).
- Cohen, J. C. Cell 19, 653-662 (1980).
 Akiyama, Y. & Kato, S. Biken J. 17, 105-116 (1974).
- Nazerian, K. & Lee, L. J. gen. Virol. 25, 317-321 (1974). Thomas, P. Proc. natn. Acad. Sci. U.S.A. 77, 5201-5205 (1980).
- Nazerian, K., Neiman, P., Okazaki, A., Smith, E. & Crittenden, L. Avian Dis. 22, 732-741
- ., Sundin, O. & Bohn, M. Cell 16, 453-466 (1979)
- Varsnavsky, A., Sundin, V. & Bolin, M. Cell 10, 433-450 (1977).
 Weisbrod, S., Groudine, M. & Weintraub, H. Cell 19, 289-301 (1980).
 Weintraub, H. Nucleic Acids Res. 8, 4745-4754 (1988).
 Benoist, C. & Chambon, P. Nature 290, 304-309 (1981).

- Sundin, O. & Chainfowl, P. Nather 230, 355-546 (1979).
 Sundin, O. & Varshavsky, A. J. molec. Biol. 132, 535-546 (1979).
 Varshavsky, A., Sundin, O. & Bahn, M. Nucleic Acid: Res. 5, 3469-3477 (1978).
 Blair, D., Mclements, W., Oskarsson, M., Fischarger, P. & Vande Wande, G. Proc. nam. Acad. Sci. U.S.A. 77, 3504-3508 (1980).
- Neel, B., Hayward, W., Robinson, H., Fang, J. & Astrin, S. Cell 23, 323-334 (1981).
 Schmitz, A. & Galas, D. Nucleic Acids Res. 6, 111-137 (1979).
- Schmitz, A. & Galas, D. Nutcie Actas Res. 9, 11-13 (1977).
 Weiss, R., Friis, R., Katz, E. & Vogt, P. Virology 46, 920-938 (1971).
 Robinson, H., Swanson, C., Hruska, J. & Crittendon, L. Virology 69, 63-74 (1976).
- Christman, J., Price, P., Pedriman, L. & Acs, G. Eur. J. Biochem. 81, 53-61 (1977).
 Chiang, P. Science 211, 1164-1166 (1981).
- Lowy, D. Proc. natn. Acad. Sci. U.S.A. 75, 5539-5543 (1978).
- 51. Razin, A. & Riggs, A. Science 210, 604-610 (1980).

Peculiar optical spectrum of the Red Rectangle

R. F. Warren-Smith*, S. M. Scarrott* & Paul Murdin†

- *Department of Physics, University of Durham, South Road, Durham DH1 3LE, UK
- †Royal Greenwich Observatory, Herstmonceux Castle, Hailsham BN27 1RP, UK

The Red Rectangle¹ is a nebula centred on the star HD44179. Within an amorphous blue nebula is embedded a hollow biconical red nebula with apex at the star. The generators of the hollow bicone show in projection as four spikes radiating from HD44179. The bicone has a hitherto totally unidentified spectrum. In high resolution spectra of the nebula we have identified a narrow-line component (Na D, Ca H and K, Hα) from a low excitation plasma and we have resolved broader features presumably from molecular bands. Here we give constraints on the molecules responsible, and propose as a candidate carbyne (linear chains of carbon with alternate single and triple bonds). The Red Rectangle may thus be a factory for the production of the raw material from which some interstellar molecules are made.

Low resolution (50 Å) spectra² of the Red Rectangle show a blue continuum spectrum identical to that of the central star, plus a broad red emission feature which extends over 2,100 Å and is centred near 6,400 Å. From polarization measurements3.4 it is clear that, while the blue spectrum is scattered star light from HD44179, the red spectrum is emission originating on the bicone. Polarization studies indicate4 that the bicone is formed of a concentration of dust grains as well as the unknown ingredient X_{RR} responsible for the red emission feature. Moderate resolution spectra (10 Å) break³ the feature into considerable structure, including resolved components grouped like molecular band-heads, presumably from a molecule X_{RR} in association with the dust grains which give the polarization behaviour. IR spectra^{1,5} of HD44179 itself show emission bands also usually seen in association with dust, but not the 9.7-µm feature which is a signature of silicates.

We have taken high resolution (1 Å) spectra of the Red Rectangle on the 3.9-m Anglo-Australian Telescope in several positions in the nebula, and spectra of the central star, in the wavelength range $\lambda 3,400-6,600$. Figure 1 illustrates the spatially summed spectrum of the nebula in the wavelength range λ 5,700-6,660, which includes most of the red emission feature. Figure 2 illustrates the blue spectrum 3,400-4,400 Å which shows prominently the Balmer absorption lines in the reflection spectrum.

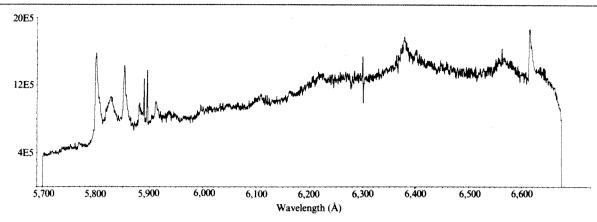


Fig. 1 Spectrum of section 1.2 arc s wide across the nebula, centred 7 arc s north of HD44179 at position angle 120°, as observed for 10,000 s with 0.5 Å spectral elements. Ordinate represents photons detectable per element in a perfectly efficient 3.9-m telescope. The D lines and H α are superimposed on a banded spectrum. (The feature at 6,300 Å is from night-sky emission.)

In these spectra of the nebulosity there are four distinct types of emission features superimposed on the scattered stellar continuum:

(1) The nebula contains narrow emission lines at the wavelengths Na D (strong), Hα (weak), and Ca II H and K (strong). The central star has strong narrow emission lines of Na D and strong H α emission, but no detectable H and K emission; on the contrary, there is weak K-line absorption, perhaps interstellar. Thus these nebular emission lines are not scattered starlight but are intrinsic emission from the nebula. Indeed, the ratio of the Na D lines varies across the nebula, being $\sim 2:1$ (optically thin) just outside and within the bicone but $\sim 1:1$ (optically thick) on the spikes, thus confirming the hollow structure of the bicone. There is no detectable broadening or splitting or velocity shift of the sodium lines across the nebula, and we estimate that projected bulk motions in this plasma are less than 15 km s⁻¹, and turbulent motion has a FWHM less than 35 km s⁻¹. The narrow emission lines are from a very low excitation plasma. The ionization potentials of Na I and Ca II are 5.1 and 11.9 eV; the weak H α indicates that ionization energies just up to 13.6 eV are available. The mean surface brightness of $H\alpha$ over the part of the nebula which we measured is 6.9×10^5 photons s⁻¹ sr⁻¹ cm⁻² which is consistent with ionization by the AO-B8 II-III star HD44179 (Strömgren sphere of 1-2 pc diameter) of $3-8 \times 10^{-6}$ solar masses of hydrogen (distance¹ of 330 pc, angular size = 0.5 arc min FWHM) with r.m.s. density in the range $40-100 \text{ cm}^{-3}$. The H α detection is consistent with upper limits to radio detection¹ at 8.4 and 15 GHz of ~ 0.05 Jy. The low excitation spectrum of the nebula indicates that its temperature is lower than the canonical 104K of a typical H II region. Other low temperature H II regions⁶, which may be cooled by abundance anomalies⁷ (metal rich), have temperatures in the region of 4,000 K, and judging by its low ionization, the Red Rectangle may be cooler still.

The other three types of feature in the spectrum of the Red Rectangle are difficult to identify.

- (2) There is the very broad feature, which ranges³ from $\lambda 5,400$ to 7,500.
- (3) There are R-type band-like features characterized by a sharp rise from the shorter wavelength and a gradual degradation to the red⁸; these occur at λ 5,799, 5,855, 5,880 and 6,615, the first two and the last one being most prominent. All these features have very similar structure and their relative intensities remain reasonably constant with position in the nebula. However, the degree of degradation to the red seems to decrease on the spikes at the edge of the biconical nebula. No velocity structure, as measured by the peak of the R-type features, can be seen over the nebula, to an accuracy of 50 km s⁻¹.
- (4) The emission from the Red Rectangle also contains M-type diffuse features that are broad (10-20 Å FWHM), peaked in the middle and centred at $\lambda 5,828$, 5,913, 5,938, 6,109, 6,208, 6,378, 6,420, 6,563. There may be weak features in the blue spectrum but because of low signal-to-noise and confusion with the Balmer series we cannot identify them well; we note the possible feature at 4,044-4,053.

The ingredient X_{RR} which gives rise to all these features may be simple molecules trapped on the grains abundant within the nebula, in which case the mode of attachment may modify the emission wavelengths and a comparison with laboratory spectra of 'free' molecules would not yield an identification. Alternatively the emission may be from the grains themselves undergoing a form of fluorescence as a result of some illumination from the central star, although no outstanding stellar spec-

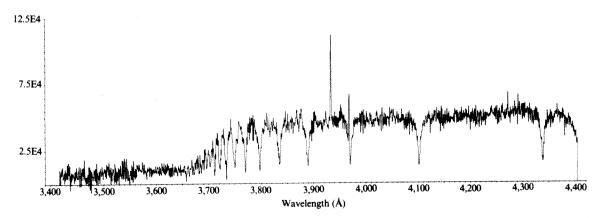


Fig. 2 Spectrum of the blue region of the nebular spectrum showing H and K emission superimposéd on the Balmer line continuum of scattered star light. Ordinate as Fig. 1, for 2,000 s integration.

tral component for this illumination can be identified. It is more likely³ that ingredient X_{RR} consists of free molecules in the nebular medium.

If the features are molecular emission lines³ then the very broad emission feature (2) could correspond to molecular dissociation, and features (3) and (4) could be transitions between vibrational states of different electronic states of the molecule. The diffuse or degraded structure could correspond to rotational broadening, and changes in the degradation from place to place correspond to a difference in excitation temperature. The question arises as to which molecule X_{RR} might be.

There are some coincidences³ between the spectrum of the Red Rectangle and that of H₂O⁺ (as found in comets⁹) and of C₃ but in each case there are additional spectral lines of these molecules that are absent from the spectra. The C₃ series is the better fit because in the laboratory it produces phosphorescent lines at 5,856, 5,874, 5,911, 5,969 and 6,308 Å in a neon trapping gas, with a 50-Å shift to the red in argon¹⁰. A 57-Å shift to the blue (in an arbitrary attempt to simulate the environment in the Red Rectangle) produces 5,799, 5,817, 5,854, 5,912 and 6,251 Å which may be identified with the X_{RR} transitions at 5,799, 5,828?, 5,855, 5,912 and 6,208?? Å but does not identify the third strongest band at 6,615. It is tempting to identify the weak blue feature at 4,050 Å with the strongest of the Swings' band of C₃, and this would indicate that there may be limited regions in the Red Rectangle with temperature ≤3,000 K.

There is, however, a general similarity between the spectrum of the Red Rectangle and those of some open-shell organic cations studied by Maier¹¹. They have a broad (~2,000 Å) dissociation spectrum in the red (5,000-8,000 Å region for the examples given) and red degraded or middle-peaked bands which are strongest on the blue end of the broad feature. Common building blocks of many of these linear organic molecules are the C≡C and C-C bonds. Maier¹¹ gives the vibrational frequencies of the stretching fundamentals of the C≡C and C-C bonds as 2,119 and 629 cm⁻¹ for the ground molecular state of dicyanoacetylene. These frequencies are not significantly different (±20%) for the same bonds in other linear cations quoted by him or for the neutral molecules which we anticipate will be present in the Red Rectangle. It so happens that in the Red Rectangle spectrum the stronger bands at λ 5,799 and λ 6,615 have an energy difference of 2,127 cm⁻¹ and the bands at $\lambda 5,880$ and $\lambda 6,109$ have a difference of 638 cm⁻¹. As both these energy differences in X_{RR} correspond well with those of the C≡C and C-C bond stretching frequencies, it is tempting to identify X_{RR} with an unknown linear organic molecule that is constituted in part by C≡C and C-C configurations. Linear carbon chain molecules have low transverse vibrational frequencies and these may also contribute in addition to rotational effects to the detailed structure of the observed band-like features.

Many of the organic molecules found in the interstellar medium contain some such configurations, for example cyanoacetylene^{12,13} $(H-C\equiv C-C\equiv N)$. Carbyne, which has been proposed14 as a constituent of interstellar material, is a linear structure of carbon with strings of C−C≡C chains. Webster 14 attributes some of the unidentified IR bands between 3.27 and 11.3 µm to carbyne; they are carried by something that is carbon-rich^{15,16} and are present⁵ in HD44179. Carbyne is stable 17 between 2,600 K and 3,800 K, consistent with the temperature of the narrow-line plasma in which the bicone is bathed. Carbyne might be produced¹⁷ above ~3,000K from C₃ molecules (C=C=C) by transfer of one bond and/or from graphite grains by fracturing the hexagonal planes of which graphite is composed. We have pointed already to the marginal evidence for the 4,050 Swings' C₃ band in the Red Rectangle and to the absence of the 9.7-µm feature in the grains in HD44179 so that in the absence of silicate grains 15,16 we therefore suppose they may be graphite in an oxygen-deficient environment. It is thus conceivable that carbon phases associated with carbyne are present in the Red Rectangle, and it is a likely candidate for X_{RR} although in the absence of a reference

spectrum the case is circumstantial. We cannot exclude other molecules as we have no explanations for several features in the spectra.

The formation of a linear molecule such as carbyne in the nebular environment around stars like the Red Rectangle may be an important initial step in the production of the many organic molecules found in the interstellar medium.

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- Cohen, M. et al. Astrophys. J. 196, 179 (1975).
 Greenstein, J. L. & Oke, J. B. Publ. astr. Soc. Pacif. 89, 131 (1977).
 Schmidt, G. D., Cohen, M. & Margon, B. Astrophys. J. Lett. 239, L133 (1980).
 Perkins, H. G., Scarrott, S. M., Murdin, P. & Bingham, R. Mon. Not. R. astr. Soc. 196, 635
- Russell, R. W., Soifer, B. T. & Willner, S. P. Astrophys. J. 220, 568 (1978).
- Shaver, P. A., McGee, R. X. & Pottasch, S. Nature 28, 476 (1979).
 Shaver, P. A., McGee, R. X., Murdin, P. & Goss, W. M. Mon. Not. R. astr. Soc. 190, 527
- 8. Pearse, R. W. B. & Gaydon, A. G. Identification of Molecular Spectra 4th edn (Chapman & Hall, London, 1976)
- Wehinger, P. A., Wycoff, S., Herbig, G. H., Herzberg, G. & Low, H. Astrophys. J. Lett. 190, L43 (1974).

- Weltner, W. & Mcleod, J. J. chem. Phys. 40, 1305 (1964).
 Maler, J. P. Chimia 34, 219 (1980).
 Morris, M., Turner, B. E., Palmer, P. & Zucherman, B. Astrophys. J. 205, 82 (1976).
- Avery, L. W. Interstellar Molecules (ed. Andrew, B. H.) (Reidel, Dordrecht, 1980). Webster, A. Mon. Not. R. astr. Soc. 192, 7P (1980).
- Aitken, D. K. Infrared Astronomy (eds Wynn-Williams, C. G. & Cruikshank, D. P. (Reidel, Dordrecht, 1981).
- Aitken, D. K. et al. Astrophys. J. 233, 925 (1979). Whittaker, A. G. Science 200, 763 (1978).

Inverse comptonization and the nature of the March 1979 γ -ray burst event

E. P. T. Liang

Institute for Plasma Research, Stanford University, Stanford, California 94305, USA, and Lawrence Livermore National Laboratory, University of California, Livermore, California 94550, USA

The central issue concerning the nature of the 5 March 1979 γ -ray burst is that of whether its source is the supernova remnant N49 in the Large Magellanic Cloud (LMC), whose extragalactic distance implies unusually high (super-Eddington) luminosity. Although the optically thin synchrotron mechanism proposed by Ramaty et al.1 has met many of the earlier theoretical objections, other evidence is needed to support or disprove such an extragalactic origin. I point out here that the observed burst spectrum seems to be most naturally and consistently interpreted as that of a synchrotron spectrum modified by inverse from ~MeV e*-pairs. scattering Comptonization describes the gain of energy by photons as a result of scattering with electrons of much higher energy.) This model then allows us to derive, from first principles, the intrinsic synchrotron luminosity of the burst source, which agrees basically with that expected from N49 (distance ~55 kpc).

The model spectrum of Ramaty et al. suggests that the spectral density above ~400 keV is greater than that from a simple redshifted pair annihilation line (Fig. 1). On the other hand, a power-law tail with a high energy cutoff (at 1.5-2 MeV in the present case, Fig. 1) is produced when a copious supply of soft photons (the synchrotron X rays below ~300 keV in the present case) is made more energetic by Compton scattering from hot electrons, provided that the Comptonization is unsaturated (the average fractional energy gain of the emerging photons = $y \le 1$)³⁻⁵. This has become the normal interpretation of the X-ray spectra of Cyg X-1 and of active galaxies. It is evident that the same relativistic pairs that are emitting the synchrotron X rays in the model of Ramaty et al. would automatically also Compton upscatter some of them, despite the low scattering depth. Hence, the dominant contribution to the y intensity above the break at ~300 keV (Fig. 1) is taken here to be comptonized photons, with only a small admixture of redshifted 511-keV photons producing the bump at 430 keV

(ref. 2). Uncertainty in the annihilation photon contribution does not matter in our estimates as long as it is smaller than the continuum. But, to be specific, the Compton continuum is taken as that lying below the dashed line in Fig. 1, with the excess due to the line contribution. The dashed line is the best fit to the data based on the general form of the unsaturated inverse Compton spectrum^{3.6} (see below).

In the limit when comptonization is highly unsaturated ($y = \text{Kompaneet parameter} \ll 1$)³⁻⁵, which is evidently the case here, the ratio of the hard comptonized energy flux F_{C} to the uncomptonized soft flux F_{S} is simply $F_{\text{C}}/F_{\text{S}} \approx y$ (refs 3, 5). Breaking the spectrum at $\sim 300 \text{ keV}$ (Fig. 1: S denotes synchrotron component; C, Compton component) we find

$$y \cong \frac{F_{\rm C}}{F_{\rm s}} \simeq 0.077 \tag{1}$$

Also, in the optically thin limit $(\tau_{es} \ll 1)$, the ratio of comptonized photons N_C to uncomptonized photons N_S is the mean scattering probability:

$$\tau_{\rm es} \cong \frac{N_{\rm C}}{N_{\rm c}} \cong 6.5 \times 10^{-3} \tag{2}$$

(from Fig. 1). In the relativistic regime^{7.8},

$$y = \frac{4}{3}\tau_{\rm es}\Gamma^2$$

where $\Gamma \equiv \bar{E}/m_e c^2$ is the average relativistic factor of the pairs. Hence,

$$\Gamma^2 \cong 8.9 \quad \text{or} \quad \Gamma \cong 3$$
 (3)

This is consistent with the apparent steepening of the tail above ~ 1 MeV. Pozdnyakov *et al.*⁶ have derived the spectral index of the power law tail for relativistic comptonization. The effective spectral index in the present case is⁶

$$\alpha = -\frac{\ln \tau_{\rm es}}{\ln (4\Gamma^2/3)} \approx 2.04 \tag{4}$$

using equations (2) and (3). Hence, the predicted photon number spectrum is in approximate agreement with the dashed curve (ref. 6, Fig. 4).

From equation (2), we find the pair column density

$$n_e h = (n_+ + n_-)h = \frac{\tau_{es}}{\sigma_{KN}} = 3.42 \times 10^{22} \text{ cm}^{-2}$$
 (5)

where the Klein-Nishina cross-section $\sigma_{\rm KN} \approx 1.9 \times 10^{25} \, {\rm cm}^2$ at $\Gamma \approx 3$. The soft component is essentially an exponential of decay constant $\sim \! 20$ to $30 \, {\rm keV}$. Interpreting this as the (redshifted) critical synchrotron frequency.^{7,8}, we have

$$h\nu_c \equiv 1.78 \times 10^{-11} B_{Gauss} \Gamma^2 \text{ keV}$$

 $\approx 30 \text{ keV}$ (6)

Hence,

$$B \simeq 1.9 \times 10^{11} \,\mathrm{G} \tag{7}$$

consistent with the field expected at a typical neutron star surface. From equation (5) and (7), we derive the intrinsic synchrotron luminosity

$$L_{\text{syn}} = 2.3 \times 10^{-2} B^2 \Gamma^2 n_{\text{e}} h f_{*}$$

= $f_{*} 2.5 \times 10^{44} \text{ erg s}^{-1}$ (8)

where f_* is the fraction of the surface area of a 15-km neutron star that is emitting. This (optically thin) synchrotron luminosity, which is derived without any reference to the assumed distance to the source, is so close to the quoted burst luminosity of $\sim 3 \times 10^{44} \, {\rm erg \, s^{-1}}$ at the distance of N49 that it serves as a consistency check on the LMC identification (f_* is expected to be of the order unity from the width of the subsequent 8-s period pulses²). The mean synchrotron self-absorption depth is $\bar{\tau}_{\rm syn} \sim 10^{-4}$, completely justifying the optically thin assumption (used in equation (8)). In fact, the frequency at which the self-absorption depth approaches 1 is $\sim 20 \, {\rm keV}$, consistent with the obser-

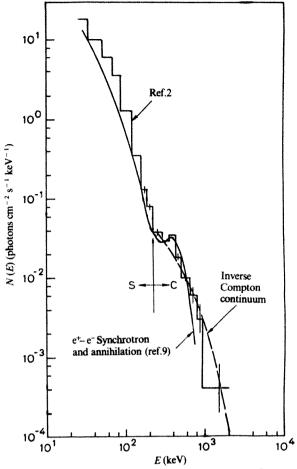


Fig. 1 Comparison of the models of the 5 March 1979 γ -ray transient, according to Mazets² and Ramaty *et al.*⁹ with the present inverse Compton continuum.

ved low-energy turnover. The synchrotron cooling time of the relativistic pairs is

$$t_{\text{syn cool}} \simeq \frac{6 \times 10^8 \,\text{s}}{R^2 \Gamma} \simeq 2 \times 10^{-15} \,\text{s}$$
 (9)

much shorter than bremsstralung cooling time or pair annihilation time. Hence, the pairs cool before annihilation, as expected. However, their probability of Compton-upscattering soft photons before cooling down is still high because of the large amount of soft photons present:

$$t_{\rm es} = \frac{1}{n_{\gamma}\sigma_{\rm KN}c} \sim 3 \times 10^{-15} \quad \rm s$$

Equation (9) also indicates that the long duration of the burst (\sim 120 ms) must be supported by a continuous supply of relativistic pairs which may be produced from slow dissipation of large energy reserves (for example, neutron star vibrations of ref. 9).

If we identify all the excess above the dashed line continuum in Fig. 1 as coming from pair annihilation, we find (now assuming a true distance of 55 kpc) for the pair annihilation tuminosity

$$L \approx 1.8 \times 10^{41} \text{ erg s}^{-1}$$
 (10)

or

$$n_+ n_- h f_* \approx 1.4 \times 10^{48}$$
 (11)

Assuming $n_{+} \approx n_{-}$, we have, combining equations (11) and (5),

$$n_{+} \simeq 10^{26} f_{*}^{-1} \text{ cm}^{-3}$$

and

$$h \approx 2 \times 10^{-4} f_* \text{ cm}$$
 (12)

These numbers are generally similar to those of Ramaty et al.¹ One clue as to why h is so small may be the extremely short

cooling time of the relativistic pairs which limits the distance over which a newly created pair can traverse before cooling down ($ct_{\rm syn\,cool} \simeq 6 \times 10^{-5}$ cm). Of course, we still need to explain why all pairs must be created in such a thin layer. As with all models dealing with an LMC origin, this model still faces problems related to the huge energy source, fast coupling, and the slow decay of the subsequent pulsations. Of course, interesting ideas have already been proposed (see ref. 9), but detailed analysis remains to be done.

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- Ramaty, R., Lingenfelter, R. E. & Bussard, R. W. Astrophys. Space Sci. (in the press). Mazets, E. P. et al. Nature 282, 587 (1979); 290, 378 (1981). Shapiro, S., Lightman, A. P. & Eardley, D. M. Astrophys. J. 204, 187 (1976).

- Katz, J. Astrophys. J. 206, 910 (1976).
- Sunyaev, R. A. & Titarchuk, L. G. Preprint (Space Res. Inst. USSR, 1979)
- Pozdnyakov, L. A., Sobol, I. M. & Sunyaev, R. A. Soviet Astr. 21, 708 (1977). Tucker, W. Radiation Processes in Astrophysics (MIT Press, 1975).
- Ginzburg, V. L. Elementary Processes for Cosmic Ray Physics (Gordon and Breach, New York, 1969).
- Ramaty, R. et al. Nature (in the press).
- 10. Cline, T. L. et al. Astrophys. J. Lett. (submitted).

Microanalysis by Raman spectroscopy of carbon in the Tieschitz chondrite

Mireille Christophe Michel-Levy* & Alain Lautie†

* Laboratoire de Minéralogie-Cristallographie associé au CNRS, Université Pierre et Marie Curie, 75230 Paris, France † Laboratoire de Spectrochimie IR et Raman, CNRS, 94320 Thiais, France

The Tieschitz H3 chondrite contains 0.25% bulk carbon but concentrations of as much as 2-6% (ref. 2) occur in black, 10-20 µm-wide rims around chondrules that are thought to have accreted before the agglomeration of the stone²⁻⁵. These rims are extremely fine-grained and compact, the main constituent being an iron-rich olivine (Fa 60-65) as shown by a Debye-Sherrer X-ray diffractogram, in good agreement with the FeO/FeO+MgO matrix value2.6. To further our understanding of the rim composition and of the thermal history of the stone, we used the MOLE Raman laser microprobe7 to characterize the carbon constituent. We report here that it is a highly disordered graphite with a progressive ordering as shown by samples heated to temperatures between 300 and 600 °C. Tieschitz could not have been heated to more than 300-350 °C.

Polished sections were prepared from Tieschitz samples previously heated in a nitrogen flux to 300 and 350 °C for 2 h, and to 400 and 600 °C for 1 h. These four samples were then compared with a section of Tieschitz that had not been heated. Areas of interest were precisely located under the microscope and recorded on photomicrographs. The specimens were then transferred to the MOLE microprobe (Jobin-Yvon). The power of the 5,145-A line of a Spectraphysics 160 argon ion laser is 10 mW at the sample surface, thus the laser beam was slightly defocused to avoid degradation of the sample during analysis and the resulting excited area was ~10 µm². The nominal magnification used was ×1,000 and width of the spectral slit was 6 cm⁻¹. As the width of the observed bands was large, the precision of the reported frequencies is ± 5 cm⁻¹. The reported relative intensities were not corrected for the spectrometer yield, thus the 1,605 cm⁻¹ line is underestimated by ~3% compared with the 1,355 cm⁻¹ line.

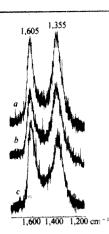


Fig. 1 Raman spectra between 1,100 and 1,800 cm⁻¹ of chondrule rims in the Tieschitz H3 chondrite; a, unheated sample; b, sample heated to 300 °C; c sample heated to 600 °C.

In the 2,500-1,000 cm⁻¹ region, the monocrystalline graphite Raman spectrum exhibits a single band at 1,575 cm⁻¹, whereas polycrystalline graphite and more or less disordered carbon materials show two main bands at 1,605 and 1,355 cm⁻¹, the relative intensities of which depend on the degree of 'graphitization'8-10. The 1,605 cm⁻¹ band, deriving from crystallized graphite, is related to vibrations in the layers. The 1,355 cm band may be assigned to distortion of the hexagonal lattice. The relative intensity ratio of the two bands $\rho = I(1,605)/I(1,355)$ can thus be used as a measure of the imperfection of the graphite layer planes. This was done for carbons heat-treated temperatures between 1,000 and 2,500 °C 9,10 . A relation between ρ and the size of the carbon particles was also found, although it remains to be established whether this is a causal relationship.

About 10 carbon-rich rim areas were studied for each of the Tieschitz sections between 900 and 2,300 cm⁻¹. No detectable degradation of the sample was observed during recording of spectra. To monitor possible effects of degradation, each spectrum was registered in a symmetric mode, sweeping the relevant frequencies both ways. In all cases studied, only the two bands of more or less graphitized carbon were found (Fig. 1). Carbynes, the occurrence of which is questioned in carbonaceous chondrites 11-13 were not detected here.

The relative intensity ratio ρ , determined for each specimen over 10 spectra, shows a deviation of <4% with respect to the mean. This should be ascribed to experimental errors (because of the rather low signal-to-noise ratio) rather than to the existence of real variations. For unheated Tieschitz $\rho = 0.92 \pm 0.03$. This ratio increases with increasing temperature for the heattreated specimens, and thus constitutes a convenient index for the degree of crystallinity of the sample. For t = 300, 350, 400 and 600°C, $\rho = 0.93$, 0.97, 1.05 and 1.23, respectively. In the relatively narrow temperature range investigated, ρ increased linearly with t (Fig. 2). The ρ for unheated Tieschitz is almost indistinguishable from that of the sample heated to 300 °C. We can therefore conclude that Tieschitz, after accretion, was not heated above 300-350 °C. Moreover, the ρ value of 0.92 for the untreated specimen allows us to give an approximate 40 Å limit to the size of the graphite layers occurring in Tieschitz chondrule rims; this corresponds either to crystallite size, or to the size of undisturbed domains. These small particles are dispersed in the entire rim, as the characteristic Raman spectra are identical at any point on the rims.

Independent confirmation of the 300-350 °C heating is given by the nickel-iron alloys that occur in Tieschitz: (1) according to Bevan and Axon¹⁴, interface Ni compositions of contiguous kamacite and taenite grains indicate equilibration to ~350 °C; (2) tetrataenite—the ordered 50% Ni-Fe alloy¹⁵—occurs either as rare isolated grains (their anisotropy is then observable), or in zoned taenite as a clear rim and in association with nickel-poor

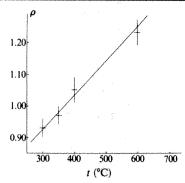


Fig. 2 Intensity ratio of Raman bands at 1,605 and 1,355 cm⁻¹ $\rho = I_{1,605}/I_{1,355}$ obtained on chondrule rims, plotted against temperature.

alloys, giving a cloudy appearance to etched zones. The ordering of this alloy may occur below 320°C and is only observed in slowly-cooled meteorites.

Further microanalyses by Raman spectroscopy of carbon in meteorites will hopefully help us to understand the complex and controversial thermal history of these extraterrestrial rocks.

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- 1. Otting, W. & Zahringer, J. Geochim, cosmochim, Acta 31, 1949-1960 (1967).
- Kurat, G. Earth planet. Sci. Lett. 7, 317-324 (1970)
- Christophe, M. Earth Planet Sci. Lett. 30, 143-150 (1976).
- Hutchison, R., Bevan, A. W., Agrell, S. O. & Ashworth, J. R. Nature 280, 116-119, (1979)
 Ashworth, J. R. Proc. R. Soc. A374, 179-194. (1981).
- Huss, G. R., Keil, K. & Taylor, G. J. Meteoritics 13, 495-497 (1978). Dhamelincourt, P. thesis, Univ. Lille (1979).
- Tuinst, F. & Koenig, J. L. J. chem. Phys. 53, 1126-1130 (1970).
 Sato, Y., Kamo, M. & Setaha, N. Carbon 16, 279-280 (1978).
- Vidano, R. & Fischbach, D. B. J. Am. ceram. Soc. 61, 13-17 (1978).
- 11. Hayatsu, R., Scott, R. G., Studier, H., Lewis, R. S. & Anders, E. Science 209, 1515-1517.
- Whittaker, A. G., Watts, E. J., Lewis, R. S. & Anders, E. Science 209, 1512-1514 (1980)
- 13. Smith, P. P. K. & Buseck, P. R. 12th Lumar planet. Sci. Conf. 1017-1019 (1981).
- Bevan, A. W. R. & Axon, H. J. Earth planet. Sci. Lett. 47, 353-60 (1980).
- 15. Clarke, R. S. Jr & Scott, E. R. D. Am. Miner. 65, 624-630 (1980)

Early Archaean gneisses from the Yilgarn Block, Western Australia

J. R. De Laeter, I. R. Fletcher & K. J. R. Rosman

School of Physics and Geosciences, Western Australian Institute of Technology, South Bentley, Western Australia 6102, Australia

I. R. Williams, R. D. Gee & W. G. Libby

Geological Survey of Western Australia, Perth. Western Australia 6000, Australia

The Yilgarn Block is an ancient crustal block which extends over ~650,000 km² in the south-west of Western Australia and consists of high-grade gneiss and granite-greenstone terrains1. Being one of the largest segments of Archaean crust in the world, it is important to studies of early crustal evolution. We report here a geochronological study of banded gneisses near Mount Narryer, in the north-west of the Yilgarn Block, which has given a Rb-Sr whole-rock isochron age of 3,348 ± 43 Myr with an initial 87Sr/86Sr ratio of 0.7037±0.0005. The data indicate that the rocks had a prior crustal residence time of ~200 Myr. The validity of this early Archaean age is supported by model Sm-Nd ages of 3,510 Myr and 3,630 Myr for two of the samples. Taken together, these ages represent the oldest evolutionary sequence so far identified in the Yilgarn Block, and are comparable with old ages from other Archaean cratons elsewhere in the world.

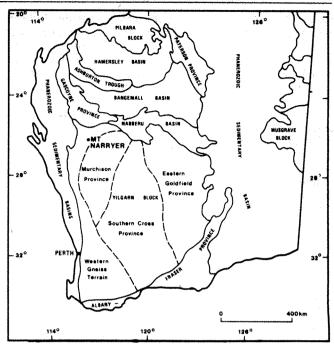


Fig. 1 Distribution of Precambrian tectonic units in relation to Mount Narryer, Western Australia.

Contrasting evolutionary models have been applied to the regional patterns of the Yilgarn Block. One model interprets the gneiss terrains as the metamorphosed and migmatized equivalents of the adjacent greenstones, infolded to deep crustal levels²; the other interprets the gneiss terrains as basement complexes on which greenstones were deposited3.4. However, this debate⁵ is by no means settled, either in the Yilgarn Block, or in other well studied Archaean cratons in the world.

It has been established that the gneisses of the Yilgarn Block occur mainly in an arc >1,000 km long along the western margin of the block, termed the Western Gneiss Terrain (Fig. 1). It was concluded on geological and equivocal geochronological evidence that the gneisses were old basement, and that the greenstone-granite terrains evolved in response to a widespread but relatively short-lived tectonothermal event ~2,700 Myr ago.

The importance of the Yilgarn Block as a reference for early crustal evolution further lies in the nature of the Western Gneiss Terrain. In addition to the extensive areas of banded orthogneiss, there are occurrences of paragneiss in which quartzite, BIF, metacarbonates, metapelite and metaconglomerate occur. The paragneiss is intruded by mafic and ultramafic rocks, now also highly metamorphosed, but with no identifiable volcanics. The paragneiss is clearly 'non-greenstone' in character, and points to sedimentary regimes in the earliest geological record, more uniformitarian than the volcanic regimes of the later greenstones, widely considered to be unique to the Archaean.

The extensive geochronological data for the Yilgarn Block have recently been reviewed⁶. The isotopic dates for most Yilgarn granitoids vary little from the range 2,600-2,700 Myr, and all Rb-Sr ages to date indicate ages for the greenstones in this range, although it has not been unequivocally established that these are volcanic ages as opposed to alteration ages. Early Rb-Sr studies' had indicated that the gneisses near the western margin of the Yilgarn Block may be older than 3,000 Myr. This is confirmed by the present work, and also by zircon U-Pb dates of 3,340 and 3,250 Myr from quartzite and orthogneiss in the Toodyay area, ~90 km east of Perth⁸.

The Rb-Sr data for the samples collected from the Mount Narryer area (26°27' S, 116°24'30" E), in the northern part of the Western Gneiss Terrain, is listed in Table 1 and plotted on an isochron diagram in Fig. 2. A least-squares regression of the data points, using the method of McIntyre et al., yields a slope and intercept corresponding to an age and initial 87Sr/86Sr ratio of 3.348 ± 12 Myr and 0.70383 ± 0.00008 (2 σ errors), with a

 0.85482 ± 14

MSWD = 10.8. The relatively large value for the MSWD indicates that the data do not fit within experimental error, and that some isotopic remobilization may have occurred. Therefore a more realistic evaluation of the age and initial ratio of the banded gneiss is that provided by model 4 (ref. 9), which gives 3.348 ± 43 Myr and 0.7037 ± 0.0005 (2 σ errors).

The samples were collected from a site ~13 km due west of Manfred Homestead and 9 km north-northeast of Mount Narryer 'trig' point. The Mount Narryer rocks are a high-grade amphibolite-granulite transition facies metasedimentary sequence. All samples are banded quartz-feldspar-biotite gneiss of granodiorite to adamellite composition. Microcline is present in all samples and plagioclase exists as oligoclase. Samples rich in quartz and microcline contain metamict allanite. All samples have been redeformed and recrystallized beyond the primary gneissose structure and in part are protomylonitic. Plagioclase is lightly saussuritized and biotite has been recrystallized and in part chloritized in the course of redeformation of the gneiss. Sample 60739 is largely epidote and has been excluded from the isochron.

Strontium evolution analysis of the data from Mount Narryer suggests a mantle evolution age of 3,550 Myr assuming singlestage evolution. This value was calculated from the determined age (3,348 Myr), the initial 87Sr/86Sr ratio (0.7037) and the arithmetic mean of measured ⁸⁷Rb/⁸⁶Sr ratios (1.32). Mantle Sr evolution was assumed to be linear from 0.6990 at 4,600 Myr to 0.7037 at present¹⁰. The projected path of strontium isotope evolution in the sample intersects the modelled mantle development curve at 3,550 Myr at a 87 Sr/ 86 Sr ratio of 0.7000. An independent determination of 0.700 for the initial 87Sr/86Sr ratio of a 3,350 Myr gneiss from the South Indian Craton¹¹ suggests that the mantle model which was used is realistic. On these assumptions, prior crustal residence of $\sim 200 \,\mathrm{Myr}$ is

As a test of the hypothesis that the gneisses at Mount Narryer had a substantial crustal residence time before the effects of dynamic metamorphism reset the Rb-Sr isochron at 3,348 Myr, Sm-Nd model ages were determined for two of the samples in the suite, as this geochronological technique has some advantages over the Rb-Sr method in determining ages of differentiation of crustal rocks from the mantle. The analytical data are given in Table 2.

The model $T_{\text{Chur}}^{\text{Nd}}$ ages of 3,510 Myr and 3,630 Myr are consistent with a crustal residence time of ~200 Myr for the gneisses that yield the Rb-Sr whole-rock isochron age of 3,348 Myr. The earlier ages are interpreted as the time of extraction of quartzo-feldspathic material, probably as granitic melts from the mantle; and the later age is taken to represent the age of the gneiss that was derived by metamorphic reworking of the primeval quartzo-feldspathic material.

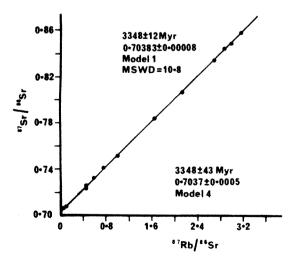


Fig. 2 Mount Narryer, Rb-Sr whole-rock isochron. Errors are quoted at 2σ level9.

Table 1 Rubidium strontium analytical data, Mount Narryer 87Rb/86Sr 87 Sr / 86 SrRb Rb/Sr Sample 0.013 ± 0.001 670 60739* 33 0.0045 0.71948 ± 9 60734 0.022 0.064 ± 0.001 0.70703 ± 12 6.7 310 0.70720 ± 7 60736 17 680 0.024 0.069 ± 0.001 0.433 ± 0.005 0.72439 ± 12 60737 60 387 0.150 0.72503 ± 11 69634 71 485 0.154 0.445 ± 0.005 69626 61 313 0.195 0.564 ± 0.005 0.73186 ± 15 0.254 0.736 ± 0.007 0.74007 ± 8 60735 67 265 69625 97 284 0.342 0.991 ± 0.009 0.75086 ± 14 69638 108 195 0.554 0.78263 ± 5 1.61 ± 0.01 60738 112 155 0.720 2.10 ± 0.02 0.80390 ± 11 153 2.64 ± 0.02 0.83225 ± 9 69637 138 0.905 0.959 69636 134 139 2.81 ± 0.02 0.84261 ± 8 69627 123 1.00 2.93 ± 0.03 0.84602 ± 12 123

The value of 87 Sr/ 86 Sr for the SRM 987 standard was 0.71021 ± 0.00008 normalized to a 88 Sr/ 86 Sr value of 8.3752. The value of yr 1 was used for the decay constant of 87 Rb. All errors are quoted at the 2σ level. The Rb and Sr concentrations (in p.p.m.) and the Rb/Sr ratios were determined by X-ray fluorescence spectrometry. The concentrations are accurate to ±5%.

 3.08 ± 0.03

1.05

134

69642

139

Table 2 Samarium-neodymium analytical data, Mount Narryer

Sample	¹⁴⁷ Sm/ ¹⁴⁴ Nd	¹⁴³ Nd/ ¹⁴⁴ Nd	fsm/Nd End	T CHUR (Myr)
60734	0.07892 ± 23	0.509884 ± 30	-0.5988 -53.4	3510 ± 50
60735	0.08751 ± 26	0.509996 ± 20	-0.5551 -51.2	3630 ± 40
BCR-1		0.51263 ± 3	and the second second	maintable

The samples were digested in HF/HClO₄/HCl acids in a Teflon bomb. A portion of each sample was spiked with a ¹⁴⁷Sm/¹⁵⁰Nd dual tracer, and the spiked and unspiked samples were separated using ion exchange procedures. Both Sm and Nd were analysed on triple rhenium filament assemblies as Nd^+ and Sm^+ ions. Nd isotopic ratios were measured with respect to ^{142}Nd and corrected for fractionation using $^{146}Nd/^{142}Nd=0.632265$ (ref. 12). Evidence for Ce contamination was checked at mass 140. The uncertainties are 95% confidence limits. The analytical blank contributed <1 part in 10^4 of the total Nd. $f_{\rm Sm/Nd}$, $\varepsilon_{\rm Nd}^{\rm Nd}$ and $T_{\rm CHUR}^{\rm Nd}$ are calculated using ($^{147}{\rm Sm}/^{124}{\rm Nd})_{\rm CHUR}^0$ = 0.1967 and ($^{143}{\rm Nd}/^{144}{\rm Nd})_{\rm CHUR}^0$ = 0.51262 (ref. 13).

These data strongly support the emerging picture in the Yilgarn Block of an ancient gneiss basement, now exposed in the west, overlain by greenstones, now exposed in the central part. Additional comparative Sm-Nd and Rb-Sr studies are planned on regional transects up to 800 km in length, across the greenstone terrains to clarify the chronological relations between the gneiss and greenstones, and to detect any regional variation in greenstone ages. Such studies may help distinguish between a model of lateral accretion of successively younger greenstones away from an embryonic craton, or a single widespread event of greenstone accumulation on a continuous basement. This may also clarify the curious situation observed in the Yilgarn Block of younger rocks occurring on the concave side of an arcuate belt of older rocks, a feature that does not fit with an island-arc accretionary model.

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- 1. Gee, R. D., Baxter, J. L., Wilde, S. A. & Williams, I. R. Geol. Soc. Australia Spec. Publ. No. 7 (in the press)
- Glikson, A. Y. & Lambert, I. B. *Tectonophysics* 30, 55–89 (1976). Archibald, N. J., Bettenay, L. F., Binns, R. A., Groves, D. I. & Gunthorpe, R. J. *Precambr.* Res. 6, 103-131 (1978). Gee, R. D. Tectonophysics 58, 327-369 (1979)
- Glikson, A. Y. Earth Sci. Rev. 15, 1-73 (1979).

 De Laeter, J. R., Libby, W. G. & Trendall, A. F. Geol. Soc. Australia Spec. Publ. No. 7 (in the

Omitted from isochron, severely epidotized.

- Arriens, P. A. Geol. Soc. Australia Spec. Publ. No. 3, 1-23 (1971).
 Nieuwland, D. A. & Compston, W. Geol. Soc. Australia Spec. Publ. No. 7 (in the press).
 McIntyre, G. A., Brooks, C., Compston, W. & Turek, A. J. geophys. Res. 71, 5459-5468
- 10. Faure, G. & Powell, J. L. Strontium Isotope Geology (Springer, Berlin, 1972).
- Beckinsale, R. D., Drury, S. A. & Holt, R. W. Nature 283, 469-470 (1980).
 Lugmair, G. W., Scheinin, N. B. & Marti, K. Earth planet. Sci. Lett. 27, 79-84 (1975).
- 13. Jacobsen, S. B. & Wasserburg, G. J. Earth planet. Sci. Lett. 50, 139-155 (1980).

Nd and Sr isotopic relationships in pelagic clays and ferromanganese deposits

S. L. Goldstein

Lamont-Doherty Geological Observatory and Department of Geological Sciences, Columbia University, Palisades, New York 10964, USA

R. K. O'Nions

Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, UK

The concentrations of heavy metals in seawater are exceedingly low and commonly less than 10^{-9} g per g. In marked contrast, their concentrations in pelagic clays and ferromanganese deposits occurring on the ocean floor are many orders of magnitude greater. This relationship is a reflection of both the low solubilities of heavy metals in seawater and their efficient sequestering from seawater by particulates. The behaviour of the heavy metal neodymium in the marine environment is further investigated in this study through the useful natural isotope tracer ¹⁴³Nd. ¹⁴³Nd/¹⁴⁴Nd and ⁸⁷Sr/⁸⁶Sr ratios are reported for hydrogenous ferromanganese sediments, the lithic substrate of a manganese nodule and pelagic clay samples occurring close to manganese nodules. Common sources for Nd in clays, ferromanganese deposits and pelagic clays are demonstrated, but significant differences exist between the Atlantic, Indian and Pacific Oceans.

Ferromanganese sediments are broadly divisible into hydrogenous and hydrothermal deposits¹. Hydrogenous deposits are considered to form by slow (0.1-1.0 mm Myr⁻¹) precipitation of Fe and Mn (refs 2-4) and include ferromanganese pavements on marine topographic highs, and concretions on abyssal plains. Hydrothermal deposits are considered to form by much more rapid (1-10 mm kyr⁻¹) precipitation of Fe and Mn (refs 3, 5) and include basement associated ferromanganese crusts and the so-called metalliferous sediments⁶⁻⁹.

The sources of elements incorporated into ferromanganese deposits vary according to the type of deposit and geographical location. Although the continents are usually considered to be the major source of the Fe and Mn in hydrogenous deposits, submarine volcanism may also contribute in some areas 10. Even within a single deposit, the provenances of the different major, minor, and trace elements may not be the same. Whilst Fe and Mn in metalliferous sediments occurring at the East Pacific Rise are thought to be derived predominantly from oceanic basalt^{8,9,11}, the isotopic compositions of O, S, Sr and U in these deposits differ from those found in oceanic basalts and are compatible with a seawater origin^{8,9}. Further, although Pbisotope compositions in the East Pacific Rise deposits are within the range found in mid-ocean ridge basalts^{9,12}, the range of Pb-isotope compositions in Pacific hydrogenous ferromanganese deposits encompasses many of the variations found in oceanic basalts and pelagic clays¹²⁻¹⁴.

In contrast with the large variability of Pb isotope compositions, the 143Nd/144Nd ratios of all measured Pacific hydrothermal and hydrogenous ferromanganese deposits vary within a narrow range, and are less than those measured for Recent mid-ocean ridge basalts and also less than the value for the model bulk Earth (Fig. 3a). These observations suggest that the

Nd in these deposits is predominantly continent-derived.

The median ¹⁴³Nd/¹⁴⁴Nd ratio of Pacific Ocean surface ferromanganese deposits is greater than for the Indian Ocean. which is in turn greater than the median ratio for the Atlantic Ocean (Fig. 3a). In each case, the ¹⁴³Nd/¹⁴⁴Nd ratios vary within a small range (less than $\pm 2 \times 10^{-4}$), indicating that each ocean is isotopically well-mixed with respect to Nd. ¹⁴³Nd/¹⁴⁴Nd ratios in seawater samples from the Pacific and Atlantic oceans are within the range of 143Nd/144Nd ratios for ferromanganese deposits within these oceans 15.16. 87 Sr/86 Sr ratios of all surface ferromanganese samples measured cluster around the present day seawater value (0.7091). These observations raise questions concerning the mechanisms by which different elements are transported to their sites of deposition. An understanding of such mechanisms must involve identification of the ultimate provenance of the elements, their fluxes between different reservoirs, and the degree of mixing within each reservoir. This study investigates the behaviour of Nd and Sr in the marine environment and focuses on the isotopic relationships of Nd in ferromanganese deposits and spatially related pelagic clays.

The locations of ferromanganese and oceanic clay samples analysed, together with locations of ferromanganese, pelagic clay and seawater samples previously analysed for Nd isotopes 12.15-17, are shown in Fig. 1. All of the Nd and Sr isotope analyses reported in Table 1 were made at the Lamont-Doherty Geological Observatory using techniques previously described 18,19. For those samples leached with acetic acid (HOAc), isotope data are reported for both leachates and

residues (Table 1).

The ¹⁴³Nd/¹⁴⁴Nd ratios for samples of surface ferromanganese deposits from the Pacific and Indian oceans (Fig. 3, Table 1) fall within the ranges reported previously for samples from these oceans 12,16. Sample D-9, described elsewhere5, from the Galapagos spreading axis consists of a hydrothermal ferromanganese crust layer in contact with a hydrogenous ferromanganese crust. The hydrothermal crust was deposited at, or near, the ridge axis, whereas the hydrogenous crust was apparently deposited at a locale where hydrothermal effects were absent. The ¹⁴³Nd/¹⁴⁴Nd ratios obtained for these distinct layers (Table 1) are indistinguishable and fall within the narrow range found for all other ferromanganese and seawater samples from the Pacific Ocean (Fig. 3a), which range from 0.5124 to 0.5126. 143 Nd/144 Nd ratios in ferromanganese samples from the Atlantic and Indian oceans are significantly lower than the Pacific Ocean samples and range from about 0.5119 to 0.5122 (Fig. 3a). The median of the Atlantic Ocean samples is at a lower 143 Nd/ 144 Nd ratio than the median of Indian Ocean samples, but the larger range of values for the Atlantic Ocean samples does indicate real variability of provenance. Piepgras and Wasserburg¹⁶ have shown that measurable variability in ¹⁴³Nd/¹⁴⁴Nd ratios extends to different depths within the same water column in the Atlantic Ocean. The total range of measured Atlantic seawater values is within the range found for Atlantic ferromanganese deposits. The relative differences in the 143Nd/144Nd ratios found in the Atlantic compared with the Pacific samples are compatible with an older overall crustal source of Nd in Atlantic ferromanganese deposits compared with Pacific

As part of a general survey of pelagic clays, 143Nd/144Nd ratios are presented in Table 1 for surface clay samples from the Atlantic, Indian and Pacific oceans (Fig. 1) that are spatially related to manganese nodules. The 143Nd/144Nd ratios of Indian and Atlantic samples are within the ranges established for the ferromanganese deposits from the same ocean (Fig. 3a, b), which suggests that the provenance of Nd in both clays and ferromanganese deposits within these oceans are closely related. The reported ¹⁴³Nd/¹⁴⁴Nd ratio of 0.512423 ± 21 for an eastern Pacific Ocean clay sample 17, is within the range established for Pacific Ocean ferromanganese deposits. Three clay samples

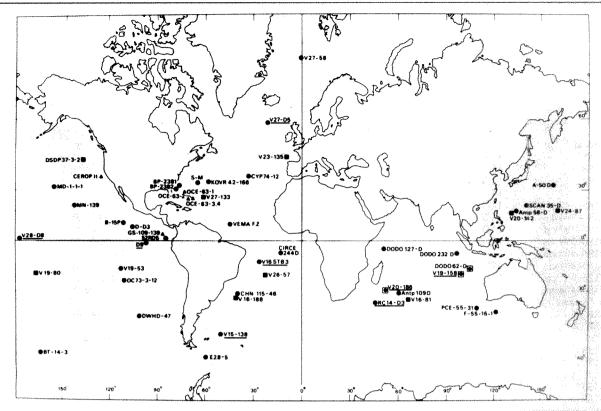


Fig. 1 Location of ferromanganese deposits (●) analysed as part of this study (underlined) and previously published ^{12,15}. Seawater sites (▲) from refs 15, 16. Locations of pelagic clay samples analysed as part of this study (□, ■) and red clays (DSDP-37-3-2 and DODO-62D) from refs 17, 15 respectively.

from the western Pacific (Table 1, Fig. 3b) have 143Nd/144Nd ratios close to 0.5122, lower than those of manganese nodules sampled from similar locations, indicating a decoupling between Nd in clays and nodules. In contrast, data for the Atlantic and Indian oceans suggest that Nd in both clays and nodules may be of the same derivation. If, for example, Nd in western Pacific manganese nodules is simply considered to be a mixture of a component isotopically similar to western Pacific pelagic clays 3Nd/14 4 Nd ≈ 0.5122) and a basaltic component $(^{143}\text{Nd}/^{144}\text{Nd} \simeq 0.5130)$, then the latter component would comprise ~25% of the Nd in these nodules. It remains to be shown whether the higher mean seafloor spreading rate in the Pacific compared with the Atlantic and Indian oceans is the cause of these observations.

The relationship between Nd and Sr isotopes in a manganese nodule (located at a depth of 400 cm in Vema core V20-186 from the Indian Ocean) and the enclosing lutite has been examined in addition to the general survey of pelagic clays. Six samples of sediment from above and below the manganese nodule, together with two samples of the nodule itself, were leached with HOAc. Measured ⁸⁷Sr/⁸⁶Sr ratios for leachates and residues, and ¹⁴³Nd/¹⁴⁴Nd ratios for the residues (Table 1, Fig. 2) demonstrate the existence of an HOAc leachable component in both the clays and the nodule with a 87Sr/86Sr ratio close to 0.709, the present day value of seawater. The 87Sr/86Sr ratios of clay residues are greater than 0.726. Those for the nodule residues are close to 0.710, significantly higher than present day seawater, and may reflect occlusion of some clay in the nodules. Although typical concentrations of Nd in manganese nodules are nearly an order of magnitude higher than pelagic clays²⁰, the ¹⁴³Nd/¹⁴⁴Nd ratios of the lutite and manganese nodule samples are very similar with a total range of 0.511940-0.512011 (Table 1, Fig. 2), and this cannot be attributed to the inclusion of clay into the nodules. This contrasts with the greater differences in Sr/86Sr ratios between the manganese nodule and lutite and suggests that, in contrast to Sr, the provenance of Nd in the lutite and the nodules may be the same. 143Nd/144Nd ratios for clay and nodule samples from the top of a second Indian Ocean core

(V19-158) are 0.512128 ± 24 and 0.512214 ± 28 respectively. These results are similar to the ratios obtained for an Indian Ocean clay-nodule pair $(0.512140\pm25$ and 0.512246 ± 26 respectively)¹⁵. In both cases the ¹⁴³Nd/¹³⁴Nd ratios of the clay and nodule samples are within the range of all Indian Ocean surface samples measured (Fig. 3a, b).

The relationships between the ¹⁴³Nd/¹⁴⁴Nd ratios in surface

ferromanganese deposits and pelagic clays within each ocean suggest that the Nd budget of ferromanganese deposits may be dominated by the same source that supplies pelagic clays. Ferromanganese deposits have a minor role in the overall Nd budget of the oceans. The largest repository of Nd in the ocean basins is pelagic clay (~40 p.p.m. by weight of Nd). Although manganese nodules possess a higher concentration of Nd than pelagic clay²⁰, the ratio of the rate of Nd accumulation in manganese nodules relative to pelagic clay is estimated (Table 2) to be about 0.05. The more restricted occurrence of manganese nodules limits the ratio of total accumulation in nodules to clay

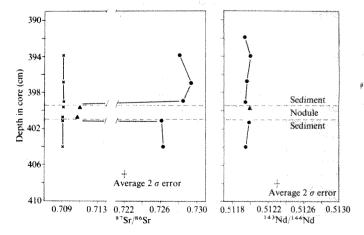


Fig. 2 87Sr/86Sr and 143Nd/144Nd in core V20-186 as a function of depth. △, Manganese nodule; ♠, sediment; ×, HOAc leachate.

Table 1	Sample lo	estione and	Nd and	Sr-isotope data
rabier	Sample loc	cations and	Na- and	Sr-isotone data

Sample	Type	Location	Depth (m)	¹⁴³ Nd/ ¹⁴⁴ Nd	⁸⁷ Sr/ ⁸⁶ Sr*	⁸⁷ Sr/ ⁸⁶ Sr†
Pacific Ocean	1,00	Location	Dopth (III)	144/ 144	31/ 31	31/ 311
D-9‡	HGC	3°02′ S, 95°09′ W	2,420	0.512486 ± 32	0.709184 ± 50	0.70923 ± 50
D-9	HTC	3°02′ S. 95°09′ W	2,420	0.512468 ± 30	0.709157 ± 50	0.,00,20,200
V28-D8	MN	0° N. 179° W	3,594-2,587	0.512465 ± 28	0.709159 ± 50	
V19-80	LUT(WR)	18°23′ S, 165°44′ W	4,839	0.512227 ± 30		
V20-142	LUT	17°11′ N, 133°16′ E	5,997	0.512251 ± 30		
V20-87	LUT	19°04′ N, 161°33′ E	4,819	0.512210 ± 24		
Indian Ocean						
RC14-D3	MN	34°45′ S, 44°46′ E	2,479-2,050	0.512142 ± 30		
V19-158	MN	18°11′ S, 99°24′ E	5,759	0.512214 ± 28		
V19-158	LUT(WR)	18°11′ S, 99°24′ E	5,759	0.512128 ± 24		
V16-81	LUT(WR)	30°38′ S, 70°00′ E	4,050	0.512210 ± 24		
V20-186-392 cm	LUT)		5,037	0.511935 ± 30		
V20-186-394 cm	LUT		•	0.512011 ± 26	0.728143 ± 50	0.709203 ± 50
V20-186-397 cm	LUT			0.511947 ± 30	0.729679 ± 59	0.709225 ± 50
V20-186-399 cm	LUT (0000 (I G . 5404 OI T)		0.511940 ± 26	0.728509 ± 52	0.709204 ± 50
V20-186-400 cm	MN	28°06′ S, 51°19′ E		0.511996 ± 32	0.709997 ± 58	0.709180 ± 48
V20-186-401 cm	MN				0.710866 ± 50	0.709245 ± 40
V20-186-401 cm	LUT			0.511989 ± 30	0.726174 ± 50	0.709178 ± 48
V20-186-404 cm	LUT			0.511943 ± 32	0.726241 ± 50	0.709263 ± 45
Atlantic Ocean						
V16-SBT3	MN	13°04′ S, 24°41′ W	4,415	0.512078 ± 28	0.709244 ± 48	
V16-SBT3	SUBST.	13°04′ S, 24°41′ W	4,415	0.512444 ± 30	0.707420 ± 56	
V27-D5	MN	56° N, 21° W	1,641-1,509	0.512109 ± 24	0.709150 ± 34	
V15-138	MN	49°35′ S, 48°05′ W	2,725	0.512206 ± 32		
V26-57	LUT	20°46′ S, 24°08′ W	5,297	0.512150 ± 20		
V27-133	LUT(WR)	25°58′ N, 60°19′ W	4,682	0.512056 ± 24	0.721152 ± 50	
V23-135	LUT(WR)	45°05′ N, 7°56′ W	5,868	0.5119825 ± 26	0.722680 ± 46	
V16-188	LUT	30°56′ S, 39°37′ W	4,665	0.512241 ± 24		

MN, manganese nodule; HGC, hydrogenous crust; HTC, hydrothermal crust; LUT, lutite; SUBST, lithic substrate of nodule V16-SBT3. 143 Nd/ 144 Nd data normalized to 146 Nd/ 144 Nd = 0.7219. 87 Sr/ 86 Sr ratios expressed relative to 87 Sr/ 86 Sr = 0.70800 for Eimer and Amend SrCO₃. Measured value for this standard is 0.70806.

Reported errors are 2σ on the mean.

to $\ll 0.05$. The total mass of Nd dissolved in seawater, $\sim 4 \times$ 109 kg using Hogdahl's²¹ estimate for the concentration of Nd in seawater (Table 2), is trivial compared with the mass of Nd in pelagic clay. For example, a mere 0.4 mm of abyssal carbonatefree sediment is estimated to contain an amount of Nd equal to the total dissolved Nd in a water column of average depth (Table 2). A much larger sediment depth maintains contact with seawater by bioturbation processes. In other words, a particulate clay concentration in seawater of 75 µg l⁻¹ would possess an amount of Nd equal to the estimated average dissolved load. Note that the pelagic clay sedimentation rate estimates suggest that seawater should contain an average of $\sim 50 \mu g l^{-1}$ of particulate material, or an amount of Nd in particulate material approximately equal to the dissolved load (Table 2). Particulate concentrations are much higher than average in the nepheloid zone, which contains pelagic clay swept back up into the water column, and may extend 1,000 m above the sediment-seawater interface.

An outstanding problem is the mechanism by which the related ¹⁴³Nd/¹⁴⁴Nd ratios in ferromanganese deposits, pelagic clays and seawater are established. For example, continual cation exchange between pelagic clay and seawater, particularly within the nepheloid zone, may control the Nd isotope composition of seawater. If cation exchange is the controlling factor, then the ¹⁴³Nd/¹⁴⁴Nd ratios of western Pacific pelagic clays require that some of the Nd in the clay be isolated from chemical contact with seawater, as occurs with Sr (ref. 22). Addy²³ has interpreted rare-earth element (REE) abundance patterns in Atlantic red clays in terms of a detrital Nd component (\approx 90%), and a hydrogenous component (\approx 10%). The hydrogenous component must be sequestered from seawater, and may preferentially exchange with it.

Degradation of particulate material in river systems provides the initial source of marine Nd; however, the 143 Nd/144 Nd ratios of water and particulates in different rivers are likely to be highly

variable. Even though some 98% of the annual river flux of Nd is in particulate material^{24,25}, the estimated average concentration of dissolved Nd in river water is over an order of magnitude higher than in seawater²⁴⁻²⁶. If the dissolved Nd concentration in seawater is at a steady state, the excess Nd in river water must be sequestered by particulate material in the oceans, and may constitute a portion of the Nd in the hydrogenous REE component of pelagic clays. The small but distinct range of ¹⁴³Nd/¹⁴⁴Nd ratios in ferromanganese deposits and seawater within each ocean is therefore a function of the residence time of Nd in seawater, the kinetics of any important exchange processes, and intra- and inter-ocean mixing times.

Estimates of the oceanic Nd-residence time 27,28 ($\approx 2-3 \times$ 10² yr) are comparable to estimates of the residence time of Pb

Table 2 Mass balances and residence time of Nd in ocean basins

Average concentration of Nd in seawater ²¹	$2.8 \times 10^{-9} \mathrm{g l^{-1}}$
Thickness of pelagic sediment (averaged, over oceans) containing mass of Nd equivalent to that in seawater	0.4 mm
Mass of clay particulate containing equivalent	$\sim 7.5 \times 10^{-5} \text{ g}$
amount of Nd as 1 litre seawater	7,57,10 8
Average amount of non-carbonate detrital particulate in seawater	$5 \times 10^{-5} \mathrm{g}\mathrm{l}^{-1}$
$\tau_{\rm Nd}$ (residence time of Nd) assuming particulate Nd isolated from dissolved Nd	3,100 yr

Calculations assume steady state and the following: Nd concentration in pelagic clay equal to average North American shale = 37 p.p.m.; density of dry abyssal sediment = 0.7 g cm^{-3} (ref. 3); abyssal non-carbonate sedimentation rate = $10^{-4} \text{ g cm}^{-2} \text{ yr}^{-1}$ (ref. 3); mean ocean depth = 3,729 m (ref. 3); pelagic sediment $\sim 90\%$ carbonate (ref. 3); manganese accumulation rate in nodules = 10^{-6} g cm⁻² yr⁻¹ (ref. 3); Nd concentration in river water = 3.79×10^{-7} g l⁻¹ (ref. 25); H₂O flux from rivers in oceans = 3.2×10^{16} 1 yr⁻¹ (ref. 38).

^{* 8&}lt;sup>7</sup>Sr/⁸⁶Sr of whole rocks (with the strength of the stren Sr of whole rocks (WR) or HOAc residue.

[‡] Sample D-9 described by Moore and Vogt⁵; all others from Lamont-Doherty Geological Observatory.

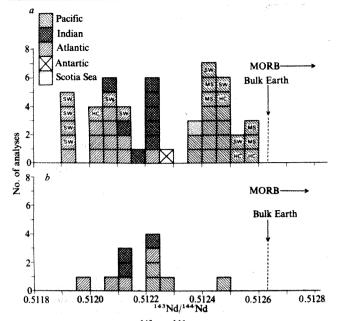


Fig. 3 a, Histogram of ¹⁴³Nd/¹⁴⁴Nd ratios in ferromanganese samples from this study plotted together with analyses from refs 12, 15. MS, metalliferous sediment; HC, hydrothermal crust; SW, seawater. Unlabelled boxes are manganese nodules. b, Histogram of pelagic clay samples from this study together with a sample from ref. 17 and one from ref. 15. All 143 Nd/ 144 Nd ratios are relative to 146 Nd/ 144 Nd = 0.7219.

(refs 29-33). In these estimates the abundance term is based on the concentration of dissolved Nd in seawater, and the input term is based on the total load of Nd entering the oceans per year. The actual residence times will be greater than these minimum values if portions of the REE which enter the oceans are isolated from chemical contact with seawater, as is indicated by the western Pacific clays. If the REE within particulates are completely isolated from seawater, for example, τ_{Nd} may be as long as 3,100 yr. The intra-ocean uniformity and inter-ocean differences in the 143Nd/144Nd ratios of surface ferromanganese deposits contrasts with the intra-ocean variability of Pb isotope ratios, and implies that τ_{Nd} is greater than τ_{Pb}

There have been various opinions on the direct source of the REE incorporated into ferromanganese deposits. Goldberg et al.27 noting the similarities between rare-earth patterns of manganese nodules and seawater, favour direct precipitation of the REE from seawater. Their suggestion has been supported for both manganese nodules 12,20,34,35 and East Pacific Rise metalliferous sediments^{8,9,12}. Ehrlich³⁶ and Bender³⁷, on the other hand, suggest that REE are incorporated into manganese nodules from proximal clay either by surface transfer mechanisms, or by clay occlusion into the nodule. The apparent conformability of Nd isotope ratios in Pacific seawater samples and ferromanganese deposits has led Piepgras et al. 15 to favour seawater as the source of Nd in ferromanganese deposits. A distinction between a seawater or pelagic clay source of Nd in manganese nodules cannot be determined from the results on Atlantic and Indian ocean samples (Fig. 3a, b). However, the demonstrated decoupling between Nd in clay and manganese nodules in the western Pacific suggests that Nd in the manganese nodules is not representative of the total Nd in the clay. Although pelagic clay may contain both labile and non-labile Nd components, the simplest explanation of the data on hand is to invoke seawater as the direct source of the REE in manganese nodules.

143Nd/144Nd ratios in surface pelagic clay samples from different oceans exhibit the same general relationship as those from ferromanganese deposits. That is, the median 143Nd/144Nd ratio of Pacific Ocean clays is higher than the median of Indian Ocean clays, which is in turn higher than the median of Atlantic Ocean clays. This result demonstrates the domination of continent derived detrital material in controlling the Nd-budget of clavs, ferromanganese deposits and seawater within each ocean basin, and highlights the differences between them.

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- 1. Bonatti, E., Kraemer, T. & Rydell, H. in Ferromanganese Deposits on the Ocean Floor (ed.
- Horn, D. R.) 149-166 (NSF, Washington DC, 1972). Ku, T.-L. & Broecker, W. S. Deep Sea Res. 16, 625-637 (1969).
- Bender, M. L., Ku, T.-L. & Broecker, W. S. Earth planet Sci. Lett. 8, 143-148 (1970).
- Turekian, K. K., Cochran, J. K., Krishnaswami, S., Langford, W. A. & Bauer, K. A. Geophys. Res. Lett. 6, 417-420 (1979).
- Moore, W. S. & Vogt, P. R. Earth planet. Sci. Lett. 29, 349 (1976). Boström, K. & Peterson, M. N. A. Mar. Geol. 7, 427-447 (1969).
- Corliss, J. B. J. geophys. Res. 76, 8128 (1971). Bender, M. L. et al. Earth planet, Sci. Lett. 12, 425-433 (1971)
- Dymond, J. R. et al. Bull. geol. Soc. Am. 84, 3355-3372 (1973)
- 10. Cronan, D. S. in Chemical Oceanography Vol. 5, 217-263 (eds Riley, J. P. & Chester, R.)
- Academic, (New York, 1976). Edmond, J. M. et al. Earth planet. Sci. Lett. 46, 19-30 (1979)
- 12. O'Nions, R. K., Carter, S. R., Cohen, R. S., Evensen, N. M. & Hamilton, P. J. Nature 273,
- Chow, T. J. & Patterson, C. C. Geochim. cosmochim. Acta 26, 263-308 (1962).
 Reynolds, P. H. & Dasch, E. J. J. geophys. Res. 76, 5124-5129 (1971).
- Piepgras, D. J., Wasserburg, G. J. & Dasch, E. J. Earth planet. Sci. Lett. 45, 223-236 (1979). 15
- Piepgras, D. J. & Wasserburg, G. J. Earth planet. Sci. Lett. 50, 128-138 (1980).
- McCulloch, M. T. & Wasserburg, G. J. Science 200, 1003 (1978).
 O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. Earth planet. Sci. Lett. 34, 13 (1977).
- 19. Zindler, A., Hart, S. R., Frey, F. A. & Jakobsson, S. P. Earth planet. Sci. Lett. 45, 249-262
- 20. Piper, D. Z. Geochim. cosmochim. Acta 38, 1007-1002 (1974)
- Høgdahl, O. T., Melsom, S. & Bowen, V. Adv. Chem. 73, 308–325 (1968).
 Dasch, E. J. Geochim. cosmochim. Acta 33, 1521–1552 (1969).
 Addy, S. K. Geochim. cosmochim. Acta 43, 1105–1115 (1979).

- Martin, J.-M., Høgdahl, O. & Philippot, J. C. J. geophys. Res. 81, 3119-3124 (1976). Martin, J.-M. & Maybeck, M. Mar. Chem. 7, 173-206 (1979).
- Turekian, K. K. in Impingement of Man on the Oceans (ed. Hood, D. W.) (Wiley, New York,
- 27. Goldberg, E. D., Koide, M., Schmitt, R. A. & Smith, N.H. J. geophys. Res. 68, 4209-4217
- Wildeman, T. R. & Haskin, L. J. geophys. Res. 70, 2905-2910 (1965).
 Rama, K. M. & Goldberg, E. G. Science 134, 98 (1961).

- Rama, K. M. & Goldberg, E. U. Science 134, 96 (1963).
 Craig, H., Krishnaswami, S. & Somayajulu, B. L. K. Earth planet. Sci. Lett. 17, 295 (1973).
 Nozaki, Y. & Tsunogai, S. Earth planet. Sci. Lett. 20, 83 (1973).
 Nozaki, Y., Thomson, J. & Turekian, K. K. Earth planet. Sci. Lett. 32, 304 (1976).
 Schell, W. R. Geochim. cosmochim. Acta 41, 1019 (1947).
- Piper, D. Z. in Ferromanganese Deposits on the Ocean Floor, 123-138 (ed. Horn, D. R.) (NSF, Washington DC, 1972).
- 35. Glasby, G. P. Mar. Chem. 1, 105-125 (1973). 36. Ehrlich, A. M. thesis, Mass. Inst. Technol. (1968).
- Bender, M. L. in Ferromanganese Deposits on the Ocean Floor, 73-80 (ed. Horn, D. R.) (NSF, Washington DC, 1972).
- 38. Garrels, R. M. & MacKenzie, F. T. Evolution of the Sedimentary Rocks (Norton, New York

15N abundance in Antarctica: origin of soil nitrogen and ecological implications

Eitaro Wada & Reiko Shibata

Mitsubishi-Kasei Institute of Life Sciences, Minamiooya 11, Machida, Tokyo 194, Japan

Tetsuva Torii

Chiba Institute of Technology, Tsudanuma 2-17-1, Narashino, Chiba 275 Japan

Antarctica offers a unique natural environment from the point of view of nitrogen cycling. Ice-free dry areas, 'Antarctic oases', and penguin rookeries are of particular interest because of the characteristic distribution of nitrate deposits 1-4 and biological materials such as algae and guano. As the distribution of 15 N/14N in a geochemical system reflects sources and metabolic pathways of nitrogen⁵, a study of the nitrogen isotopic composition of Antarctic samples would help us to understand the Antarctic nitrogen cycle. Here we report that nitrate in Antarctic soils is extremely depleted in 15N compared with biogenic nitrogen, and that algae collected from a nitrate-rich saline pond and from a penguin rookery exhibit, respectively, the lowest and the highest ¹⁵N/¹⁴N ratios among terrestrial biogenic nitrogen so far observed. We also discuss possible causes of these extreme nitrogen isotopic compositions.

Table 1 Distribution of chemical components and δ^{15} N of nitrate for soil materials from Wright Valley, Victoria Land, Antarctica

Sample	e Loc	ation	Mansell colour	Texture	pH(H ₂ O)	Cl ⁻ (mequiv. per g)		NO ₂ -N m N per g	NH‡-N dry soil)	δ ¹⁵ N _{NO} ³ (%)		Remarks	-
On nor	thern side of	Lake Vanda	175 m heig	ht									
V-1	77°31.1′ S	161°41.1′ E	5Y5/2	Silty clayey loam	6.28	2.06	63.6	0.00	0.08	-23.4	Slope of valley	f a U-sh	aped
V-2	77°30.9′ S	161°39.9' E	5Y6/4	Sandy loam	7.58	0.999	22.9	0.00	0.01	-22.2	In the d	elta	
V-3	77°30.9′ S	161°38.4E	7.5Y6/2	Loam	7.28	0.681	19.3	0.00	0.00		In the ing whit		
Near B	ull Pass												
P-1	77°31.3′ S	161°49.8′ E	7.5Y7/2	Silty loam	7.89	0.479	37.9	0.00	0.00	-18.3	Above layer	pecten	fossil
P-2	77°31.3′ S	161°49.8′ E	5Y6/4	Sand	8.10	0.191	58.6	0.00	0.04	-18.6	Below layer	pecten	fossil
On sout	thern side of	Lake Vanda											
2	77°32.7′ S	161°31.8′ E	5Y8/3	Loam	8.59	0.346	10.0	0.00	0.00	-11.5			
3	77°32.7′ S	161°31.8′ E	2.5Y6/4	Sandy loam	8.51	0.095	10.0	0.00	0.00	-16.1			
4	77°32.7′ S	161°31.8′ E	2.5Y6/4	Sandy loam	8.02	0.100	53.6	0.00	0.00	-14.8			
6	77°32.7′ S	161°31.8′ E	10YR4/4	Loam	7.46	0.994	179	0.00	0.00	-14.1			

Table 2 Distribution of $\delta^{15}N$ in various nitrogenous substances from Wright Valley, Victoria Land and Ross Island, Antarctica

Sample	Lo	cation	δ ¹⁵ N (%)	Remarks
Epibenthic algae				
V-5	77°31.7′ S	161°38.6' E	-9.0	Southern shore of Lake Vanda, a saline and meromictic lake
L-1	77°32.4′ S	160°45.2′ E	-47.8	Unnamed saline pond, Labyrinth, near the edge of the
			-49.0	Wright Glacier
C-2	77°32.8′ S	161°31.4′ E	+4.8	Eastern shore of Lake Canopus, a freshwater lake
B-1	77°31.5′ S	161°42.2′ E	-9.1	Lake Bull, a freshwater lake
			-9.3	,
Algal felt				
R-1	77°12.3′ S	166°28′ E	+30.7	Northern penguin rookery at Cape Bird, Ross Island
Organic nitrogen				, , , , , , , , , , , , , , , , , , , ,
Soil	77°12.3′ S	166°28′ E	-28.6	Northern penguin rookery at Cape Bird, Ross Island Mansell colour,
				7.5YR 1.7/1; texture, sand; $pH(H_2O)$, 7.6; Cl, 0.032 mequiv. g^{-1}
Sediment	77°32.1′ S	161°32.2′ E	-7.3	Bottom sediment of Lake Vanda depth, 67.5 m

'The Dry Valleys' in southern Victoria Land, McMurdo Sound region is a large polar desert extending over an area of $\sim 2,500 \text{ km}^2$ and containing several valley systems—Wright Valley being the most famous. Precipitation is $< 100 \text{ mm yr}^{-1}$ and mean air temperature is between -18 and -25 °C. Fallen snow is mostly dissipated by sublimation, but melting of glaciers occurs during the warm season and produces fresh and saline lakes and ponds where algal communities flourish. According to Webb⁶, the Wright Valley was below sea level in the Pliocene (3-4 Myr BP).

Soil materials and algal samples were collected from Wright Valley and Cape Bird in Ross Island in January 1979. Procedures for isotopic analysis were described elsewhere^{7.8}. Nitrogen isotopic composition is expressed as follows:

Nitrogen isotopic composition is expressed as follows:
$$\delta^{15}N(\%) = \left\{ \frac{\binom{1^5N/^{14}N)_{\text{sample}}}{\binom{1^5N/^{14}N}_{\text{air}}} - 1 \right\} \times 1,000$$

Water soluble salts were analysed by the following methods: for ammonium, phenol-hypochlorite method⁹; for nitrate, diazotization method¹⁰; for nitrate, cadmium reduction method¹¹; and for chloride, mercuric thiocyanate method¹². Organic nitrogen was determined by Kjeldahl digestion¹³.

Nitrate was the major component among nitrogenous compounds in Antarctic soils (Table 1). Concentrations of organic nitrogen (0.34-6.4 μ g atom N per g dry soil) were one to two orders of magnitude lower than those of nitrate except for samples collected from a penguin rookery, Ross Island. Our data of δ^{15} N for soil nitrate in Antarctica exhibited quite low values (-11 to -24%). The minimum value of -23.4% was found for soil materials collected from a slope of a U-shaped valley near Lake Vanda. The natural abundance of ¹⁵N in biogenic nitrogen in the literature generally ranges from -17 to +20%. The average δ^{15} N values for nitrate are: 3.2% for forest soils; 6.4% for cultivated soils; 7.0% for oceanic waters; and

-6.6% for rain waters¹³⁻¹⁵. The δ^{15} N values for Antarctic nitrate were, thus, quite different from that for oceanic nitrate, and gave the lowest ranges among terrestrial nitrate.

Various origins have been considered for the salts in the Dry Valleys: direct marine incursion16, marine aerosols or sea spray³, chemical weathering of minerals¹⁷, and NO_x produced by auroral activities¹⁸. According to Moore¹⁹, gaseous ammonia (-10.0%) and nitrogen dioxide (-9.3%) in clean air are depleted in 15N as compared with those in aerosols (NH₄, +5.6% and NO₃, 5.0%). He interpreted the low ¹⁵N content in gaseous compounds as being due to the nitrogen isotope fractionation associated with isotopic equilibria between gaseous and condensed phases with subsequent removal of the condensed phase. The successive removal of the condensed phase during long distance transportation from the tropical and temperate region to the polar region may further enhance depletion of 15N in nitrogenous compounds in the atmosphere of the polar region. The $\delta^{15}N$ data suggest that nitrate in Antarctica soils is mainly derived from atmospheric precipitation which carries NO_x depleted in ¹⁵N. NO_x depleted in ¹⁵N might be produced by photochemical reactions³ as well as by auroral activities¹⁸. No other processes can produce nitrogen oxides of such low δ^{15} N.

In the case of algae, $\delta^{15}N$ values ranged between -49 and +30% (Table 2). The lowest value (-49%) was found for the algae collected from a small saline pond located near the edge of Wright Upper Glacier. This is the lowest $\delta^{15}N$ value ever reported for terrestrial samples. It has been demonstrated that the isotope fractionation during nitrate assimilation by a marine diatom, *Phaeodactylum tricornutum*, is inversely related to its growth rate at low light intensities and a high nitrate concentration (10 mg atom NO_3^-N l⁻¹). A fractionation factor as high as 1.03 has been obtained as an extreme. A characteristic feature of Antarctic saline ponds is the high concentration of nitrate

in this pond was 8.7 mg atom NO₃-N l⁻¹. Growth of the epibenthic algae at high nitrate concentration and low light intensity may thus be accompanied by a large nitrogen isotope fractionation. The extremely low $\delta^{15}N$ values for the epibenthic algae can thus be explained by the combination of low $\delta^{15}N$ for source nitrate and large isotope fractionation associated with nitrate assimilation.

High ¹⁵N enrichments of +30.7 and +28.6% were found in algal felt and organic nitrogen collected from the Adelie penguin rookery at Ross Island, respectively (Table 2). The enrichment of ¹⁵N along a food chain has been reported elsewhere²². The 815N value for marine plankton ranges from 2 to 5% and for fishes from 15 to 20% (ref. 22). Adelie penguins at Cape Bird feed on krill and fish, and deposit faecal materials including uric acid in the soils of the rookery. Soil microorganisms convert uric

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- Jennen, H. I. Rep. Sci. Innext Br. Anteres. Expds. Geol. 2, 1907–1909 (1916).
 Johannesson, J. K.-& Grisson, G. W. Nenser 194, 567–568 (1962).
 Chridge, G. G. C. & Campbell, L. B. Nenser 219, 428–430 (1968).
 Morlkawa, H., Minsto, I., Ossaka, J. & Haysaki, T. Mess. nets. Inst. polar Res., Spec. Int. 4,
- Wade, E. in (eds Goldberg, E. D. & Horibe, Y.) 375–398 Leosope Marine Chamming (Ucinda-Rokalmino, Tokyo, 1980).
 Webb, P. N. Zasteret, J. U.S. 7, 226–234 (1972).
- 7. Kreitler, C. W. Rep. Denest. No. 83 (Bureau of Beconomic Geology, University of Texas at Aneth. 1975).
- Weids, E. & Hattori, A. Geochin. connection. Acts 48, 249-251 (1976).
 Solorzmo, L. Linuel. Oceanogr. 14, 799-801 (1969).
 Bendschneider, K. & Robmson, R. J. J. mar. Res. 11, 87-96 (1952).

acid and other organic nitrogen to ammonia which evaporates with a high nitrogen isotope fractionarion. This may leave nitrogenous materials rich in ¹⁵N in soils. Kreitler⁷ obtained an apparent fractionation factor of 1.038 for NH₃ volatilization from barnyard soils. In the cold climate of Antarctica, the biogeochemical processes in question might proceed at an extremely slow rate. If so, a much greater fractionation of the nitrogen isotope is expected, and the high 15N enrichments in organic nitrogen and algae in the penguin rookery can be explained.

Torii et al.²¹ observed a high pH of 9.4 in the surface water of Lake Canopus where intensive photosynthesis occurred. The isotope fractionation associated with ammonia volatilization thus also explains 15N enrichment of epibenthic algae in this lake (C-2 in Table 2).

- Wood, E. D., Armstrong, F. A. & Ruchards, F. A. J. mer. Biol. Am. U.E. 47, 23-31 (1967)
 Iwasaki, L., Utsum, S., Hagano, K. & Ozawa, T. Biol. chem. Soc. Jap. 29, 860-864 (1956).
 Wada, H., Kadonaga, T. & Matsuo, S. Grechen, J. 9, 139-148 (1975).
 Swensey, R. E., Liu, K. K. & Keplan, I. R. in Stable Distings in the Berth Science (ed. Robbason, R. W.) (DSIR Bull. No. 220, 9-26 (1978)).
- 15 Weds, E. & Nekamura, K. Off-puskagath 14, 7–15 (1980)
 16, Hendy, C., Wilson, A. T., Popplowall, K. B. & House, D. A. N.Z. J. Gool. Geophys. 28, 1103–1122 (1977).
- Behling, R. E. & Calkin, P. E. Arssert, J. U.S. 4, 128–129 (1969). Parker, B. C., Hesskell, L. E. & Thompson, W. J., Nature 271, 651–652 (1978). Moors, H. Aimer Braw. 11, 1239–1243 (1977).

- Wada, E. & Hattori, A. Gesencrobology, J. I., 85–101 (1978).
 Torii, T. et al. Mean. nam. Inst. polar Res., Spec. Izz. 4, 5–29 (1975).
 Milyako, Y. & Wada, E. Rec. Oceanogr. Works Jup. 9, 176–192 (1967).

Chemical flux in an acid-stressed stream

Ronald J. Hall* & Gene E. Likens

Section of Ecology and Systematics, Division of Biological Sciences, Cornell University, Ithaca, New York 14850, USA

The acidity of rain and snow falling on widely separated areas of the world has been increasing during the past 30 yr (refs 1-3). Acid rainfall consists of a dilute solution of sulphuric and nitric acids due to the oxidation and hydrolysis of airborne sulphur and nitrogen⁴ and frequently has a pH of <4.0. Recent studies have shown that acid rain alters the chemistry and biology of streams and lakes in large regions of the world. Results from reconnaissance studies in the field. and physiological studies in the laboratory show that diversity and numbers of aquatic organisms of all major trophic levels are affected by low pH (high acidity). The quantitative effects of such acidification on biogeochemistry and biological function in natural streams have received little attention. In contrast, much is known about aquatic ecosystems affected by acid mine drainage^{10–13} However, waters receiving acid mine drainage show effects caused by metal contamination and deposition of iron oxide particulates, as well as acidification. In our study, we experimentally acidified a third-order section of a small mountain stream in the Hubbard Brook Experimental Forest, West Thornton, New Hampshire, USA. Our aim was to measure the effects of increased acidity on chemical and biological export in the stream. It was found that experimental stream acidification to pH 4 did alter the chemical and biological flux. The most significant inorganic component affected by the experiment was alaminium. A significant net flux of carbon and nitrogen occurred in the biologically bound forms but not in dissolved substances. Net flux of phosphorus was significant in biologically bound forms. The increased loss of nutrients in the particulate organic fraction was also important, particularly if scaled to the total stream ecosystem. ..

Areas affected by acid precipitation are often located in northern latitudes where snow accumulates during long, cold winters. Snowmelt in spring then releases a large quantity of water and ions to streams and lakes 14,15. For example, on average, 54% of the annual streamwater discharge within the Hubbard Brook Experimental Forest occurs between March and May, with as much as 30% occurring in April¹⁵. It has been reported that when snow thaws, the first portions of the melt water may be very acidio¹⁶. Thus in such areas, late winter or early spring thaws may cause sudden decreases in pH in aquatic ecosystems. During such snowmelt periods, pH values as low as 4.0 have been recorded in streams in Norway¹⁷, in the Adirondack Mountains of New York State 18, and in the Hubbard Brook Experimental Forest, New Hampshire (N. Johnson, personal communication). Trout deaths have been observed in Norwegian streams during flushes of acidic melt water¹⁷.

Our study was done at Norris Brook, a tributary of Hubbard Brook 19-21. This stream flows through deciduous forest underlain by granitic bedrock and is subject to inputs of sulphuric and nitric acids in rain and snow at an annual volume-weighted pH near 4 (range 2.85 (ref. 22)-5.95 (ref. 15) for individual storms). The annual pH of Norris Brook in the study area normally ranges from 5.4 to 6.4. We experimentally decreased the pH of a third-order section of the stream to 4 (range 3.9-4.2) by continuously adding sulphuric acid (0.05-0.5M) from 18 April to 22 September 1977. The experimental design and biological effects for the entire study are detailed elsewhere 19-21. Sampling stations for the reference area (A) and the treatment area (B) were located 1 m above and 15 m below the acid addition point, respectively. The treatment reach of the stream between points A and B was $26.9 \text{ m}^2 \pm 0.6 \text{ (f} \pm \text{s.e.}, n = 3)$ in area. The effects of hydrogen ion stress on the biogeochemistry of the stream are described here for the first 30 days (18 April to 17 May 1977). We separated our results into the first 5 days ('acute response') and the remaining 25 days ('chronic response') of hydrogen ion stress. A mass-balance approach was used in which the inputs to the treatment area minus the outputs yielded a net flux of chemicals from the area during the experimental period.

Methods for collection of water samples and chemical analysis are detailed elsewhere 20. For fine particulate organic matter (FPOM) measurements, aliquots of stream water were filtered through ashed 0.45-µm glass fibre filters. Filters were dried for ~2 days at 60 °C. Carbon concentrations of the particulate

^{*} Present address: Ecosystems Research Center, Center for Environmental Research, Cornell University, Disco., New York 14853, USA.

Table 1 Mean daily biomass flux of drifting invertebrates and coarse particulate organic matter (CPOM), and flux of fine particulate organic matter (FPOM) and selected dissolved substances (Ca, Al, N, organic carbon) during 5-day (acute response) and 25-day (chronic response) periods of increased hydrogen ion stress

	\bar{x} (g per day) \pm s.e. $(n = 5)$						
	Acute response			Chronic response			
	Site A	Site B	% Loss or gain	Site A	Site B	% Loss or gain	
Invertebrates	0.065 ± 0.011	0.025 ± 0.065	-285	0.057 ± 0.011	0.09 ± 0.017	-58	
CPOM	5.5 ± 0.53	11.4 ± 1.6	-107	11.56 ± 5.89	9.49 ± 4.3	+18	
FPOM	715 ± 45.9	700 ± 66.4	+2	1.320 ± 575	1400 ± 590	-6	
DOC	$1,400 \pm 198$	1.400 ± 198	0	2.000 ± 759	2.000 ± 749	ő	
Al	10.4 ± 0.89	27 ± 2.9	-160	70 ± 29	130 ± 51.4	-86	
Ca	$1,700 \pm 22.4$	1.700 ± 161	0	2.000 ± 1.300	2.000 ± 1.400	0	
N	$< 8.2 \pm 0.72$	$< 8.2 \pm 0.72$	Õ	$<15\pm7.33$	$<15\pm7.33$	Ŏ	

Site A was in the reference area and just upstream of the treatment area; site B was at the downstream end of the treatment section. The durations of the acute and chronic responses were determined by a dose-response curve of drift density (biomass per volume of water). For the first 5 days of depressed pH, the density of invertebrate drift leaving the acidified zone exceeded that entering by a factor of 1.7-3.3, with peak drift appearing only after 2 days of acid addition. For the remaining 25 days, drift density at the treatment site was 1.6-fold greater than at the reference site. The mean daily (\pm s.e., n = 5) streamwater concentrations at site A for Ca, Al, N and DOC during the 5-day period were 1.65 ± 0.012 , 0.01 ± 0 , $0.01 \pm$

matter were determined by conversion to CO₂ and IR gas analysis²³. A conversion factor of 2.22 (ref. 24) was used to change fine particulate organic carbon to FPOM.

Drift nets were positioned upstream and downstream of the acid addition point²⁰. Invertebrates and coarse particulate organic matter (CPOM) collected in drift nets were dried to a constant weight at 60 °C. CPOM is defined as all materials (conifer and deciduous leaves, very small branches, and other organic particulates) retained in the 253-um mesh drift nets. Chemical analyses of the invertebrates and organic matter were done using a conductive Jarrell-Ash (model 975) Multi-Channel Plasma Atom-COMP/Flame Emission spectrophotometer and a Perkin-Elmer (model 303) spectrophotometer, respectively. The coefficient of variation for chemical concentrations in invertebrates and organic matter ranged from 5 to 28% for all samples. We determined the net flux (A-B) of invertebrate, particulate (CPOM, FPOM), and dissolved (Al, Ca, N and organic carbon) transport modes operating in our experimental area (site B) relative to the reference section (site A). Biomass flux of invertebrates and particulate (CPOM and FPOM) matter is the product of chemical concentration (mg per g) and mass of biota collected per unit time (g day⁻¹), whereas streamwater flux (dissolved ions) is the product of chemical concentration (mg l^{-1}) and discharge of water (1 day⁻¹).

A comparison of some of the major biotic and abiotic components between the upstream and downstream sites yields information on acute and chronic effects of hydrogen ion stress (Table 1). The biomass of exported invertebrates and concentration of dissolved Al were higher in the acidified area (site B), with the per cent net loss $[100(input-export)(input)^{-1} = net$ ecosystem flux] being greater during the first 5 days than during the remaining 25-day period (negative values equal loss and positive values equal gain between A and B, Table 1). For example, a 285% (Students' t, one-tailed, P < 0.025) and 58% (P < 0.025) net loss of invertebrate biomass from the acidified zone (26.9 m² study area) occurred during the 5-day (acute) and 25-day (chronic) periods, respectively; a 160% (P < 0.005) and 86% (P < 0.05) net loss of dissolved Al occurred during these same two periods. Although the net export of CPOM increased significantly (107%, P < 0.01) after just 5 days of acidification, the net flux of FPOM (P > 0.25) and dissolved Ca, N and organic carbon did not change (Table 1). Thus, increased acidity augmented the export of biomass and some dissolved chemicals to downstream reaches with a higher rate of loss occurring immediately after acidification.

Concentrations of Al, Ca, C, N, P and S in invertebrate biomass and CPOM were determined (Table 2), and the net flux (concentration \times mass per unit time) of each element between site A and B is shown in Fig. 1a (acute period) and b (chronic

period). The net flux (A-B) of these elements in the dissolved fraction (from Table 1) is also included in Fig. 1. A significant increase in net flux of Al, Ca, C, N, P and S in invertebrate biomass (P < 0.01) occurred for the 30-day period (Fig. 1a, b). A significant net flux of these six elements in the CPOM fraction occurred during the 5-day study period (P < 0.01, Fig. 1a) but not during the subsequent 25-day period (P > 0.1, Fig. 1b).

The increased drift of invertebrates may have been due to the increased hydrogen ion concentrations, or to increased aluminum concentrations, or to a combination of both. Schofield has shown that deaths of brook trout in laboratory experiments occur in dilute solutions with a pH of 4.5-5.2 and containing $0.2-1.0~{\rm mg\,I^{-1}}$ aluminum, whereas brook trout survive in pH 4.5-5.0 with $<0.1~{\rm mg\,I^{-1}}$ aluminum. The invertebrates in our study may have been affected in a similar manner $^{19-21}$.

Although loss of chemicals in invertebrate and CPOM fractions was significantly increased with elevated acidity, the total amounts of Al, Ca, C and N in those fractions were small compared with the amounts of these ions in the dissolved form (Table 1). However, if an assessment had been made of chemicals in the biomass of trout, sculpins, salamanders and frogs that left the experimental section during acidification, the estimates of Al, Ca, C, N, P and N lost in the biotic component would have been considerably higher. Thus the loss of biologically bound nutrients such as C, N and P is at a level that could be significant to the stream ecosystem as a whole during acute hydrogen ion stress, as these nutrients would no longer be available for use in the stressed area.

One surprising effect of higher stream acidity was the increased flux of CPOM during the first 5 days (Fig. 1a). The increased loss of CPOM may be due to: (1) a decrease in the number of collector organisms²⁵ (filter- and deposit-feeders)

Table 2 Chemical content of aquatic insects and coarse particulate organic matter collected from Hubbard Brook streams

	\bar{x} (mg per g dry mass \pm s.e.)		
Chemical	Aquatic insects $(n=7)^*$	$ \begin{array}{c} \text{CPOM} \\ (n=5)^{\dagger} \end{array} $	
C	473 ± 1.1	213 ± 35.2	
N	75 ± 3.0	10 ± 2.0	
P	9.1 ± 0.6	0.64 ± 0.13	
S	5.4 ± 0.49	0.66 ± 0.18	
Ca	2.0 ± 0.4	1.3 ± 0.30	
Al	0.84 ± 0.14	8.2 ± 0.70	
C:N:S:P	52:8.2:0.59:1	333:15.6:1.03:1	

^{*} Norris Brook, this study.

[†] Headwater streams in the Hubbard Brook Experimental Forest (R. Bilby, personal communication).

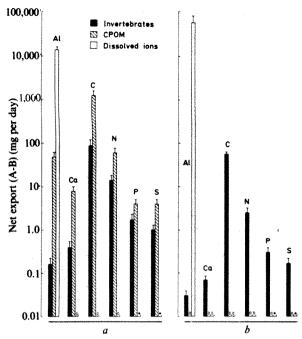


Fig. 1 Mean daily a, acute (5-day) and b, chronic (25-day) effects of elevated hydrogen ion concentration on flux of Al, Ca, C, N, P and S in invertebrates (), CPOM () and dissolved components in Norris Brook (

). Zero designates no change in net flux at site B relative to A. Vertical bars represent standard error. A greater mass was exported from B than A, thus A - B is a negative number. Asterisks indicate that flux of dissolved sulphur and phosphorus, due to elevated acidity, could not be evaluated for the 1977 study; that is, concentrated H2SO4 was added to the stream water to lower the pH and the quality of the dissolved phosphorus data was poor. However, based on preliminary results from an experiment in which HCl was used to lower the pH, no change in dissolved sulphate concentration was detected above and below the experimental addition, and total phosphorus was mobilized in the stream water in the experimental area relative to the reference section.

that use particulate organic matter as food and store it as biomass. In the acidified reach we observed an increase in export of collector and scraper organisms²⁰. Scrapers ingest relatively more periphyton than organic detritus in natural streams^{20,26,2} However, with a decrease in the number of collector organisms, one would also expect an increased loss of FPOM, but the expected loss of FPOM did not occur with the exception of a 25% increase in the net flux of FPOM during the first 3 h after acidification. In addition, we suspect that collectors filter such a small portion of the total water column that increases in FPOM net flux due to decreased filtering (fewer collectors present) may not be detectable. (2) Increased hydrogen ion stress may have caused organisms to move quickly off the bottom or out of accumulations of leaf debris, thus dislodging organic matter and allowing it to be transported downstream by water currents. Based on our quantitative data we believe that the latter factor is more important.

The acute (5-day) results of our acidification experiment may approximate what happens in streams during spring snowmelt in the northeastern US. Hydrogen ions are thought to migrate to the perimeter of snow crystals during the melt-refreeze cycle 16,28,29. It has been reported that as much as 80% of the acidic contaminants in the snowpack may be released in the first 30% of snowmelt²⁹. Depending on rate of melt, soil frost, and other environmental conditions, when an acid-stratified snowpack begins to thaw, these hydrogen ions enter streams and may depress the pH of the water substantially. As the snowmelt period is brief $^{16,17,28-30}$, and because neutralization of hydrogen ions takes place rapidly due to aluminum dissolution reactions in the soil^{31,32} and the streambed (our study) and cation exchange on clay minerals³³⁻³⁵, the period of high acidity may be relatively short and/or limited to headwater tributaries. Our results show, however, that a significant increase in net flux of chemical

components, incorporated in the biomass of organisms and organic matter, occurred within the first 5 days of stream acidification

The 25-day response of the biota to H+ stress may simulate results from chronic acidification from acid rain. An important repercussion of acid precipitation is the elimination of sensitive aquatic organisms²⁰. The reduction in numbers of biotic communities may have important consequences for the budget of organic matter in the headwaters of mountain streams. A greater than normal proportion of detrital material may be used by the decomposer food chain, or transported downstream, or both if detritivores had ingested and temporarily assimilated this organic matter. The altered storage, processing, and export of litter and nutrients may have either beneficial or detrimental effects on the downstream macrobenthos that otherwise would not have been affected directly by upstream acidification.

Because increased burning of coal and other fossil fuels may intensify acid precipitation and deposition in snowpacks, and because low pH values during initial snowmelt periods occur in first-, second- and third-order streams 17, the loss of nutrients, in particular phosphorus and nitrogen, and energy-abundant organic detritus may be of great ecological significance in headwater mountain streams.

This is a contribution to the Hubbard Brook Ecosystem Study. The NSF, the International Paper Company Foundation, and the Environmental Protection Agency, Duluth, provided financial support for the field study at Hubbard Brook. The Northeastern Forest Experiment Station, US Department of Agriculture, Forest Service, Broomall, Pennsylvania, furnished the facilities. We thank B. Bilby for chemical data on CPOM; J. Eaton, B. Wesley and B. Moore for technical assistance, and N. Johnson, C. Driscoll, J. Harte and M. Pratt for critical reviews.

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- 1. Braekke, F. W. Res. Rep. 6/76 (SNSF Project, Oslo, 1976).
- Likens, G. E., Wright, R. F., Galloway, J. & Butler, T. Scient. Am. 241, 3-11 (1979).

 Drabløs, D. & Tollan, A. Proc. int. Conf. on Ecological Impact of Acid Precipitation (SNSF

Project, Oslo, 1980).

- Galloway, J. N., Likens, G. E. & Edgerton, E. S. in Proc. 1st int. Symp. on Acid Precipitation and the Forest Ecosystem (USDA, Northeastern Forest Experiment Station, Broomall,
- Sutcliffe, D. W. & Carrick, T. F. Freshwat. Biol. 3, 437-462 (1973)
- Almer, B., Dickson, W., Ekstrom, C., Hornstrom, E. & Miller, U. Ambio 3, 30-36 (1974).
- Grahn, O., Hultberg, H. & Lander, L. Ambio 3, 93-94 (1974). Hendrey, G. R. & Wright, R. F. in J. Great Lakes Res. 2, suppl. 1, 192-207 (1976).
- Muniz, I. P. & Leivestad, H. in Proc. int. Conf. on Ecological Impact (eds Drabløs, D. & Tollan, A.) 84-92 (SNSF Project, Oslo, 1980).
- Parsons, J. D. Arch. Hydrobiol. 25, 25-50 (1968).
 Herricks, E. E. in Recovery and Restoration of Damaged Ecosystems (eds Cairns, J. Jr., Dickson, K. L. & Herricks, E. E.) 43-71 (University Press of Virginia, Charlottesville,
- 12. Whitton, B. A. & Say, P. J. in River Ecology Vol. 2 (ed. Whitton, B. A.) 286-311 (University
- of California Press, Berkeley, 1975).

 13. Tomkiewicz, S. M. Jr & Dunson, W. A. Wat. Res. 11, 397-402 (1977).

 14. Schofield, C. L. in Proc. Conf. on Emerging Environmental Problems: Acid Rain 76-86
- Schoneld, C. L. in Proc. Conf. on Emerging Environmental Protections: Acta Kain Insert (Publ. EPA-902/9-76-00, Environmental Protection Agency, Washington DC, 1975).
 Likens, G. E., Bormann, F. H., Pierce, R. S., Eaton, J. S. & Johnson, N. M. Biogeochemistry of a Forested Ecosystem (Springer, New York, 1977).
 Johannessen, M., Dale, T., Gjessing, E. T., Henriksen, A. & Wright, R. F. in Proc. Grenable Symp. on Isotopes and Imputities in Snow and Ice 115-120 (IAHS Publ. No. 118, 1977).
 Leivestad, H. & Muniz, I. P. Nature 259, 391-392 (1976).
- 18. Schofield, C. L. Research Program Technical Comprehensive Report No. A-072-NY, 1-26 (Office of Water Research Technology, US Department of Interior, Washington DC,
- Hall, R. J. & Likens, G. E. in Atmospheric Sulfur Deposition: Environmental Impact and Health Effects (eds Shriner, D. S., Richmond, C. R. & Lindberg, S. E.) 443-462 (Ann Arbor Science, Michigan, 1979).
- Hall, R. J., Likens, G. E., Fiance, S. B. & Hendrey, G. R. Ecology 61, 976-989 (1980).
 Hall, R. J. & Likens, G. E. in Proc. int. Conf. on Ecological Impact of Acid Precipitation (eds Drables, D. & Tollan, A.) 375-376 (SNSF Project, Oslo, 1980).
 Likens, G. E., Bormann, F. H. & Eaton, J. S. in Proc. NATO Advanced Research Institute on
- Effects of Acid Precipitation on Terrestrial Ecosystems (eds Hutchinson, T. S. & Havas, M.) 443-464 (Plenum, New York, 1980).
- 443-404 (Flenum, New York, 1989).
 Menzel, D. W. & Vaccaro, R. F. Limnol. Oceanogr. 9: 138-142 (1964).
 Likens, G. E. in Primary Production of Inland Aquatic Ecosystems (eds Lieth, H. & Whittaker, R. H.) 185-215 (Springer, New York, 1975).
 Wallace, J. B., Webster, J. R. & Woodhall, W. R. Areh. Hydrobiol. 79, 506-532 (1977).
 Merritt, R. W. & Cummins, K. W. An Introduction to the Aquatic Insects of North America (Kandall/Hunt. Publishers, 1978).
- (Kendall/Hunt, Dubuque, 1978).
- Cummins, K. W. Bioscience 24, 631-641 (1974).
 Hagen, A. & Langeland, A. Envir. Pollut. 5, 45-57 (1973)
- Johannessen, M. & Henriksen, A. Wat. Resour. Res. 14, 615-619 (1978).
 Schofield, C. L. & Trojnar, J. R. in Proc. Conf. on Polluted Rain (Plenum, New York, 1980).
- Mulder, J. thesis, The Agricultural Univ. Wageningen (1980)
- 32. Johnson, N. M., Driscoll, C. T., Eaton, J. S., Likens, G. E. & McDowell, W. H. Geochim. Johnson, N. M., Driscoli, C. T., Eaton, J. S., Likens, W. E. & W. cosmochim. Acta (in the press).
 Johnson, N. M. Science 204, 497–499 (1979).
 Cronan, C. S. & Schofield, C. R. Science 204, 304–305 (1979).
 Driscoll, C. T. thesis, Cornell Univ. (1980).

¹³C evidence for dietary habits of prehistoric man in Denmark

Henrik Tauber

Carbon-14 Dating Laboratory, National Museum, Ny Vestergade 10, Copenhagen K, Denmark

Carbon isotopes are fractionated by natural processes such as photosynthetic assimilation of CO₂ and its absorption in water. Carbon fractionation is affected by the type of metabolism used by the plant to fix CO₂ and differs in marine and terrestrial plants. This natural fractionation pattern is passed down the food chain and may therefore be used to elucidate questions on the origin of carbon compounds in nature. Here, the ¹³C fractionation pattern has been used to investigate the dietary habits of prehistoric man in northwestern Europe. The results show that whereas Danish Mesolithic man lived on a diet dominated by sea food, in the Neolithic the human diet consisted predominantly of terrestrial food.

Isotopic fractionation in natural carbonaceous materials relevant to prehistory has recently been analysed $^{1.2}$ and is shown in Fig. 1 as δ ^{13}C values relative to the PDB standard. Due to kinetic effects, terrestrial plants that follow the normal Calvin (C₃) photosynthesis are depleted in the heavy carbon isotopes, as shown by a change in δ ^{13}C values from -6 to -8% in atmospheric CO₂ to ~ -22 to -30% in terrestrial plants ^{13}C content, although with a slight shift towards higher δ ^{13}C values, partly because the respired CO₂ may be enriched in the light isotopes 4 . The magnitude of the fractionation effect in animals may therefore vary both with the nature of the foodstuff and with the position in the natural food chains.

Absorption of CO_2 in water and the subsequent formation of bicarbonate are governed by kinetic effects and by thermodynamic equilibrium processes which lead to an enrichment in the heavy carbon isotopes to $\delta^{13}C$ values close to 0% PDB (Fig. 1). When marine bicarbonate is assimilated during photosynthesis of submerged plants, reaction kinetics again result in a depletion in the heavy isotopes, in this case to $\delta^{13}C$ values of about -10 to -18% and this fractionation is also reflected in marine animals.

This simple isotopic separation between terrestrial and marine plants and animals is partly obscured if Hatch-Slack photosynthesis (C_4) has dominated in the terrestrial plants. Hatch-Slack photosynthesis occurs in several plants growing in arid and subtropical regions, especially in grasses of the family Panicoideae, and leads to δ ¹³C values of between -10 and -18% (ref. 3) (hatched boxes in Fig. 1). The most important cultivated C_4 plants are maize, sugar cane and millet. However, if as in the present study, which concerns temperate northwestern Europe, the influence of C_4 plants can be excluded, the ¹³C fractionation pattern may be used to distinguish between a predominant reliance by man on sea food and terrestrial food^{5,6}. Figure 2 shows the ¹³C content of organic remains of bone

Figure 2 shows the ¹³C content of organic remains of bone collagen or tissue from 28 humans living from 5200 BC to AD 1750, together with ¹⁴C dates of the samples, calibrated according to Clark⁷. Collagen from the bones was isolated as a gelatine by the method by Longin⁸, and the gelatine was converted to CO₂ which was used partly for the ¹⁴C determination in a proportional counter and partly for the ¹³C determination by a mass spectrometric analysis with a reproducibility better than 0.3% and an overall uncertainty of about 0.7%. Samples of human tissues were thoroughly extracted with acids and alkalis to remove extraneous carbon compounds before conversion to CO₂. It was checked that the chemical procedures applied to collagen and tissue samples had only a negligible effect on the isotopic compositions^{6,9}.

The series also included bones of three Eskimos from Greenland whose feeding habits are known or may be safely deduced. Two of these lived at Angmagssalik, East Greenland, before contacts with Europeans, and were therefore forced to subsist almost entirely on sea foods. This is substantiated by the difference between their apparent ¹⁴C age and their historical age, which suggests a 90-95% intake of sea food^{5,6}. The third Eskimo sample was from West Greenland and the difference in ¹⁴C content of human tissue and a reindeer fur, found together, suggests a dietary supply of sea food of about 70% (ref. 6).

The measurements on humans from Denmark proper show a clear distinction between the ¹³C content of humans from Mesolithic time and from the Neolithic and later periods. All six samples from the Danish Mesolithic had a ¹³C content similar to that of the Eskimos from historic times, and thus must represent people with a predominant reliance on sea food, comparable with that in Greenland.

This conclusion is at variance with common archaeological estimates for the period, based on bones and shells found in Mesolithic dwelling places in Denmark, However, fish bones are small and fragile and may easily have been overlooked or under-represented in the early excavations of Mesolithic sites. This is also suggested by a modern excavation of coastal Mesolithic dwelling places at Vedbæk, North Zealand, from where three of the Mesolithic skeletons originate. By a careful sieving of all the excavated materials, several thousand fragments of fish bones were recovered per square metre of the thin cultural layers (ref. 10 and K. Aaris-Sørensen, personal communication). In addition to sea food, high δ^{13} C values in humans may indicate a consumption of fish and birds from streams and lacustrine waters. When comparisons are made with archaeological estimates, it should be remembered that faunal remains from dwelling places often represent consumption during a short or seasonal stay only, whereas the ¹³C content of human bones gives an integrated measure of the composition of the mean food intake over an extended period, probably of the order of 5-10 yr.

All Mesolithic sites shown in Fig. 2 are coastal sites. A strong reliance on sea food therefore seems natural. However, all eight Neolithic skeletons represented in Fig. 2 also originate from sites or graves situated either directly on the coast or within 100–200 m of the sea. These Neolithic people were thus coast dwellers, as were the Mesolithic people, but they did not utilize the resources of the sea to the same extent as did the Mesolithic people. This suggests that a highly developed Neolithic cultural complex, with a different method of subsistence and therefore different food habits, rapidly succeeded a Mesolithic economy.

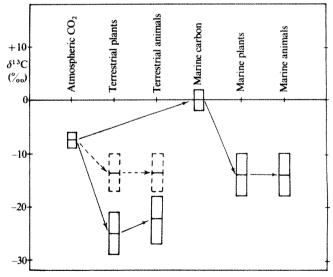


Fig. 1 Fractionation of carbon isotopes in nature. Variations in the ¹³C content are measured as

$$\delta^{13}$$
C = $\left(\frac{^{13}\text{C}/^{12}\text{C sample}}{^{13}\text{C}/^{12}\text{C standard}} - 1\right) \times 1,000\%$

with the Chicago sample of Cretaceous belemnite from the Peedee formation, South Carolina, as a standard.

& 13 C/% - 5 MARINE ANIMALS - 10 ~15 -20 TERR. ANIMALS -75 BRONZE AGE I RON AGE HIST, TIME MESOLITHIC NEOLITHIC 1000 2000 5000 A000 1000

Fig. 2 ¹³C content of human beings from Denmark and Greenland measured relative to the PDB standard versus calibrated 14C dates of the samples.

With a natural scatter in the δ ¹³C values as indicated in Fig. 1, estimates of the relative proportions of dietary components from δ ¹³C measurements are rather unreliable. It is also uncertain whether part of the isotopic fractionation is influenced by species-related or individual differences in the enzymatic processes during metabolism. It is therefore only concluded that the individuals analysed from the Danish Mesolithic subsisted on a diet dominated by sea food, while those analysed from the Neolithic and later periods lived predominantly on terrestrial food, although in some cases with a significant contribution of marine food.

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- Olsson, I. U. & Osadebe, F. A. N. Boreas 3, 139-146 (1974).
 Stuiver, M. & Polack, H. A. Radiocarbon 19, 355-363 (1977).
- 3. Troughton, J. H. Proc. 8th int. Conf. Radiocarbon Dating, E 39-57 (Royal Society of New

4. DeNiro, M. J. & Epstein, S. Geochim cosmochim. Acta 42, 495-506 (1978).

The number of individuals investigated is rather small, and one should therefore be cautious in drawing far-reaching conclusions about Mesolithic food habits, but the present investigation demonstrates the potential of such measurements for the elucidation of the dietary habits of prehistoric man.

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- 5. Tauber, H. Proc. 9th Int. Radiocarbon Conf., 447-452 (University of California Press,
- 6. Tauber, H. in Det Skabende Menneske (ed. Egevang, R.) 112-126 (National Museum, Copenhagen, 1981).
- Clark, R. M. Antiquity 49, 251-266 (1975). Longin, R. Nature 230, 241-242 (1971).
- Tauber, H. Kuml 1979, Aarhus, 73-78 (1980). 10. Aaris-Sørensen, K. Vidensk. Meddr. dansk naturh. Foren. 142, 139-149 (1980).

Carbon isotope analysis of separate chemical phases in modern and fossil bone

Charles H. Sullivan* & Harold W. Krueger†

- * Department of Anthropology, The University of Arizona, Tucson, Arizona 85721, USA
- † Geochron Laboratories Division, Krueger Enterprises, Inc., Cambridge, Massachusetts 02139, USA

Studies evaluating the distribution of stable carbon isotopes in bone and other tissues of both human and non-human animals have recently been reported1-6. Those investigations which examined the isotopic composition of bone have concentrated on the analysis of bone collagen and demonstrated that the ¹³C/¹²C ratios in bone collagen are directly related to the ¹³C/¹²C ratios of primary photosynthesizing plants in the diets of the animals concerned6-8. With regard to archaeological applications, such analyses have been limited to relatively young samples because of the instability of collagen in bone. We have extended the isotopic method of dietary analysis by using both the organic and inorganic phases of bone with equally good results. In the case of material over a few thousand years old, unless special conditions have preserved collagen, analysis of the organic phase of bone is no longer practical due to its deterioration9. The technique reported here allows dietary analysis of bone over 10,000 years old by using the inorganic phase, which is more stable in fossil material.

Dietary analysis using carbon isotopes is based on the fact that most plants photosynthesize by one of two pathways, the Calvin (C_3) cycle or the Hatch-Slack (C_4) pathway^{10,11}. These pathways produce very different carbon isotope ratios 12,13 which can be readily quantified by standard mass spectrometric techniques. The carbon isotope ratios of animal tissues reflect the proportions of C3 and C4 plants in the animal's diet. Fresh modern bone contains at least three distinct carbon-bearing phases14. Most carbon is in the form of collagen, a complex polypeptide which represents ~10-15% by weight of fresh bone. Hydroxyapatite, also called 'bone apatite', comprises 80-90% by weight of typical bone samples. It contains a small amount of carbon, probably as carbonate ion substitutions for hydroxyl or phosphate ions. A third phase, also inorganic in nature, behaves chemically like a normal carbonate.

The main phases of bone can be separated for isotopic study by appropriate techniques. The 'normal carbonate' phase can be removed and its carbon collected as CO2, by reaction with cold dilute acetic acid under vacuum, leaving the insoluble hydroxyapatite and collagen. After washing to remove acetic acid residues, this material can then be reacted with dilute HCl under vacuum to hydrolyse hydroxyapatite and release its carbon as CO2 for analysis. The collagen remains insoluble and can be filtered off and refined to pure bone gelatin15. Procedures developed for use in radiocarbon dating with kilogram-sized bone samples^{9,16} have been scaled down for isotopic studies of the geochemistry of bone using samples of ≤5 g.

Carbon isotope analyses were performed on CO2 directly evolved from hydroxyapatite, and also on CO2 prepared by a microcombustion procedure from purified bone gelatin-these

Table 1 Carbon isotopic compositions of bone fractions

Samp	le						Indicated %
no.	Species				δ ¹³ C (‰)	δ ¹³ C (‰)	of C ₄ plants
Mode	rn bone			Location	Gelatin	Apatite.	in diet
1	Masai giraffe			Kenya	-21.1	-13.7	0
2	Cape buffalo			Kenya	-10.3	-2.1	75
3	Domestic cow			Kenya	-13.5	-5.5	55
4	Hartebeest			Kenya	-7.8	+0.6	90
5	Grant's gazelle			Kenya	-18.7	-11.7	15
6	Waterbuck			Kenya	-10.0	-2.3	75
7	Zebra			Kenya	-10.5	-0.8	80
8	Thompson's gazelle			Kenya	-13.6	-4.8	55
9	Hippopotamus			Kenya	-11.2	-3.7	65
10	Domestic cow			Kenya	-9.3	-0.5	85
11	Warthog			Kenya	-10.6	-2.7	70
12	Domestic cow			Idaho	-19.6	-10.9	15
13	Domestic cow			Idaho	-22.6	-16.4	0
14	White-tailed deer			Connecticut	-18.9	-12.1	15
		n = 14	r = 0.991	m = 1.113	Apatite – Gelat	$\sin = 7.94\% \pm 0.93$	
Archa	neological bone (<2,0	00 years old)				
15	Musk ox	, , , , , , , , , , , , , , , , , , , ,	,	N. Canada	-22.1	-14.6	0
16	Arctic hare			N. Canada	-21.2	-13.8	ŏ
17	Bison			New Mexico	-8.7	-0.4	85
18	Bison			New Mexico	-9.4	-0.2	85
19	Unknown animal			Kenya	-6.8	+0.9	95
		n = 5	r = 0.997	m = 1.061	Apatite – Gelat	$in = 8.02\% \pm 0.75$	
Archa	eological bone (older	than 2 000	vearc)				
20	Domestic cow	than 2,000	years)	Kenva	-9.7	-0.3	85
21	Unknown animal			Kenya	-6.3	+0.8	95
	Human			New Hampshire	-17.0	-9.2	30
23	Human			Peru	-19.4	-11.2	15
24	Domestic cow			Kenya	-8.8	-0.6	85
		n = 5	r = 0.989	m = 0.988	Apatite - Gelat	$in = 8.14\% \pm 0.84$	
		١٥٥)					
	bone (older than 10,0	900 years)		No. Town	-20.2	-11.7	10
25	Mastodon			New Jersey	-20.2 -16.1	-11.7 -9.1	30
26	Fossil camelid			Idaho	-10.1	-9.1	30
		n=2			Apatite – Gelat	$in = 7.75\% \pm 1.06$	
All an	alyses						
		n = 26	r = 0.991	m = 1.067	Apatite - Gelat	$in = 7.98\% \pm 0.84$	

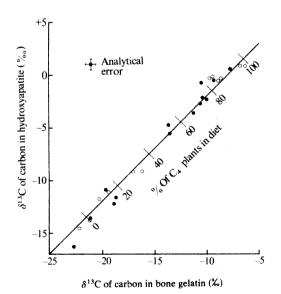


Fig. 1 Relationship between δ^{13} C of carbon in bone gelatin and hydroxyapatite. \bullet , Modern animals; \bigcirc , archaeological or fossil animals.

were done using either Micromass 602D or 903 mass spectrometers (at the Geochron Laboratories). Results are reported in standard δ notation relative to the PDB standard¹⁷.

To examine the relationship between δ^{13} C of bone gelatin and of hydroxyapatite, bone samples were chosen to represent a wide variety of conditions which might affect the isotopic relationships. Several samples were from animal species which are known to have preferences for either C₃ or C₄ plants^{18,19} including species that have dietary inputs of almost pure C₃ or C4. Samples of differing age were analysed to evaluate the isotopic relationship between gelatin and hydroxyapatite as a function of time and to determine what other geochemical interferences, if any, might cause problems. Samples were selected to provide a broad geographical base for the data, including samples from Africa, various sites in North America, and one site in South America. As far as possible, samples from environments with unusual preservation conditions, ranging from the Canadian Arctic to Kenya, New Mexico and the Peruvian desert, were selected. Among the fossil bone samples are several which have very different conditions of burial and preservation.

The analyses of 26 bone samples are given in Table 1 and the data points are shown in Fig. 1 as a plot of δ^{13} C of carbon in hydroxyapatite against δ^{13} C of carbon in gelatin. The analyses in Table 1 are derived from animals having almost the full range of plant diets, from 100% C_3 to 100% C_4 dietary inputs. Vogel et al. ²⁰ have estimated the average isotopic compositions of the basal photosynthetic food webs and assigned δ^{13} C values of -26.5% and -12.5% for C_3 and C_4 plants, respectively. These values represent the total carbon in the plants and may not

accurately represent the carbon that is actually used for metabolism or synthesis of animal tissues. We agree with Tieszen²¹ that there is a need for compound specificity when discussing the minute details of isotopic fractionation. Van der Merwe and Vogel⁶ have determined a δ^{13} C of -21.4% for collagen from North American humans with known C3 diets, and Vogel² reports a value of -21.2% for collagen from African ungulates with pure C₃ diets. Our data for those animals having amost pure C_3 diets indicate a δ^{13} C of ~-21.5%, which confirms the reported shift of $\sim +5\%$ from the basal food web.

Our analyses of gelatin from animals having essentially pure C_4 diets indicate a $\delta^{13}C$ of $\sim -6.5\%$, which is identical to the value given by Vogel² and close to the value of -7.5% calculated by Van der Merwe and Vogel⁶. The analyses of C₄-derived gelatin suggest an isotope enrichment of ~+6% relative to a C4 food source. The difference between the δ^{13} C of C₃-derived gelatin and that of C₄-derived gelatin is ~15% and closely approximates the isotopic difference in the original food materials.

Isotopic analyses of the hydroxyapatite phase of bone are quite different from those of gelatin for the same samples. In all samples the carbon in hydroxyapatite is ~8% heavier than that in the gelatin. Bone apatite derived from pure C_3 diets has a $\delta^{13}C$ of $\sim -13.5\%$ while that derived from pure C₄ diets has a value of $\sim +1\%$. The hydroxyapatite data thus show the same spread of isotopic values as the gelatin data, but exhibit consistently greater ¹³C enrichment relative to diet. While protein synthesis to produce collagen apparently induces an isotopic enrichment of $\sim +5\%$ from the basal food web, the carbon incorporated into hydroxyapatite is consistently enriched by ~+13% relative to the food web. It seems most likely that the carbon contained in hydroxyapatite is derived from dissolved bicarbonate in blood plasma. Analogous isotopic enrichments exist between marine shells and seawater bicarbonate.

Despite the greater isotopic fractionation between diet and hydroxyapatite, it is clear from Fig. 1 that dietary information is accurately reflected. The relationship between δ^{13} C of bone gelatin and δ^{13} C of hydroxyapatite is linear and is approximately represented by the equation $\delta^{13}C_{(apatite)} = \delta^{13}C_{(gelatin)} + 8$. Figure 1 defines a straight line for the data with a slope very close to unity (correlation coefficient = 0.99). This relationship can be used to estimate the percentages of C4 plants in the diet using bone gelatin data, bone apatite data, or both together. The particular value of this relationship derives from the fact that it can be used to gain dietary information, using only hydroxyapatite analyses, from older bone samples which no longer intact collagen. In this case $\delta^{13}C_{(apatite)} =$ contain $\delta^{13}C_{\text{(food web)}} + 13$, and the proportion of C_4 plants in the diet can be obtained directly from the hydroxyapatite axis of Fig. 1.

This model of dietary analysis using bone will have important applications in archaeology, ecology, climatology and other areas and will allow the extension of such studies well back into the Pleistocene.

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- Minson, D. J., Ludlow, M. M. & Troughton, J. H. Nature 256, 602 (1975).Vogel, J. C. S. Afr. J. Sci. 74, 298–301 (1978).
- Yogel, J. C. M. J. & Epstein, S. Geochim. cosmochim. Acta 42, 495–506 (1978).
 Teeri, J. A. & Schoeller, D. A. Oecologia 39, 197–200 (1979).
 Lyon, T. D. B. & Baxter, M. S. Nature 273, 750 (1978).
 van der Merwe, N. J. & Vogel, J. C. Nature 276, 815–816 (1978).

- Silberbauer, F. B. thesis, Univ. Cape Town (1979). Burleigh, R. & Brothwell, D. J. archaeol. Sci. 5, 355-362 (1978).
- Krueger, H. W. Proc. 6th Int. Conf. Radiocarbon and Tritium Dating, 332-337 (Washington
- Krueger, H. W. Proc. 6th Int. Conf. Radiocarbon and Finum Dating, 332–337 (No. 1)
 State University Press, Washington, 1965).
 Hatch, M. D. & Slack, C. R. Biochem. J. 101, 103–111 (1966).
 Hatch, M. D., Slack, C. R. & Johnson, H. S. Biochem. J. 102, 417–422 (1967).
 Smith, B. N. & Epstein, S. Pl. Physiol. 47, 380–384 (1971).
- Bender, M. M. Radiocarbon 10, 468-472 (1968).
- 14. McLean, F. C. & Urist, M. R. Bone: Fundamentals of the Physiology of Skeletal Tissue (University of Chicago Press, Chicago, 1968). 15. Longin, R. Nature 230, 241-242 (1971).
- Haynes, C. V. Science 161, 687-688 (1968) Craig. H. Geochim, cosmochim. Acta 3, 53-92 (1953)
- 18. Tieszen, L. L., Hein, D., Qvortrup, S. A., Troughton, J. H. & Imbamba, S. K. Oecologia 37, 351-359 (1979).
- Tieszen, L. L. & Imbamba, S. K. Afr. J. Ecol. 18, 237-242 (1980).
 Vogel, J. C., Fuls, A. & Ellis, R. P. S. Afr. J. Sci. 74, 209-215 (1978).
 Tieszen, L. L. Nature 276, 97 (1978).

Mycorrhizal infection and resistance to heavy metal toxicity in Calluna vulgaris

R. Bradley, A. J. Burt & D. J. Read

Department of Botany, University of Sheffield, Sheffield S10 2TN, UK

Although studies of the biology of mycorrhizas have repeatedly demonstrated their capacity to enhance nutrient capture in circumstances where essential elements are present in growth limiting quantities1-3, knowledge of their function in environments containing potentially toxic concentrations of metallic elements is lacking. It is known that mycorrhizal infection can increase the uptake of copper and zinc from soil solutions containing low concentrations of these metals4, but any such increase in circumstances of high metal concentration would clearly be disadvantageous. Because of the large number of situations, both natural and man-made, in which heavy metal toxicity can restrict plant growth, it is of great ecological and applied interest to increase our understanding of those factors which permit growth of plants on heavily contaminated soils. One of the most successful colonists of such soils in northern Europe is the strongly mycorrhizal ericaceous plant Calluna vulgaris5. Calluna has a characteristic 'ericoid' mycorrhizal infection which, in contrast to the situation found in vesiculararbuscular (VA) mycorrhizas, has been shown primarily to enhance nitrogen rather than phosphorus uptake^{6,7}. We have examined the possibility that mycorrhizal infection may influence the resistance of Calluna to high levels of heavy metals. The growth, survival and heavy metal content of two races of Calluna, one from a metal-polluted site and one from an unpolluted natural heathland, have been compared when plants were grown in the mycorrhizal (M) and non-mycorrhizal (NM) condition in sand cultures supplemented with different levels of copper and zinc. We report here that whereas NM plants show no tolerance of these metals at high concentrations, mycorrhizal infection provides a major degree of resistance to the toxicity and that infection leads to significant reduction of the heavy metal content of the shoot.

Calluna seeds were collected from plants growing on heavy metal-contaminated spoil at Parys Mountain, Anglesey, North Wales, and from natural heathland at Stanton Moor, Derbyshire, England. Seedlings of both races were grown aseptically on water agar and then transferred to sterile soil in Petri dishes. Half of the seedlings of each race were inoculated with an isolate of the mycorrhizal fungus Pezizella ericae, which had been obtained from seedlings growing in the original locality of that race. After incubation for 6 weeks in the presence of the appropriate endophyte, M seedlings of both races showed typical heavy endomycorrhizal infection of the ericoid type7. At this stage M and NM plants were transferred to pots of sterile acid-washed sand in which they were supplied with a dilute nutrient solution (1/10th Rorison's)8 containing either copper or zinc, which are the major metallic pollutants at Parys and at several other sites colonized by Calluna in the UK.

The nutrient concentration was selected after preliminary experiments had shown that, with twice weekly addition, macronutrient levels were adequate to sustain growth of both M and NM plants of Calluna, which is an intrinsically slow-growing species. P levels were not, however, high enough to cause inhibition of mycorrhizal infection, or precipitation of added metals. The recoverability of N, P and metals was shown in parallel experiments without plants to be greater than 95% at all treatment levels. In addition, levels of P in this medium were the same as those previously reported as 'extractable' from Parys mine spoil and heathland soil⁵, and were thus considered to be both ecologically and physiologically appropriate for this type of experiment. Copper treatments were at 0, 10, 25, 50 and

Table 1 Mean yield (mg) and standard error of plants of two races of Calluna vulgaris grown in the mycorrhizal (M) and non-mycorrhizal (NM) condition with different treatments of copper or zinc

Treatment	Pa	rys	Sta	nton	Tantanant	Pa	rys	Star	nton
Cu (mg l ⁻¹)	М	NM	M	NM	Treatment Zn (mg l ⁻¹)	М	NM	M	NM
0	47.9 ± 2.1	32.0 ± 1.4	48.4 ± 3.0	32.7 ± 2.0	0	47.2 ± 2.4	26.7 ± 3.4	51.9 ± 3.4	24.2 ± 1.6
10	17.6 ± 1.3	1.5 ± 0.4	15.5 ± 2.6	1.3 ± 0.1	25	$47.3 \pm 4.0*$	$18.9 \pm 2.2 \dagger$	16.6 ± 2.6	1.7 ± 0.7
25	10.0 ± 0.9	0.9 ± 0.1	13.9 ± 1.1	1.0 ± 0.1	50	$23.9 \pm 2.6*$	1.0 ± 0.1	14.3 ± 1.9	1.4 ± 0.3
50	8.6 ± 0.9	0.6 ± 0.1	8.9 ± 0.8	1.5 ± 0.1	100	$15.7 \pm 1.2*$	0.8 ± 0.2	9.9 ± 0.8	1.4 ± 0.1
75	9.7 ± 0.8	0.7 ± 0.2	6.1 ± 0.7	0.8 ± 0.2	150	15.0 ± 2.0 *	0.8 ± 0.1	6.1 ± 0.1	1.5 ± 0.1

All M values are significantly different from NM at P = < 0.05.

* Yield of Parys M plants significantly greater than those of the Stanton race at P < 0.05; † Parys NM value significantly different from Stanton NM at P < 0.05, n = 6.

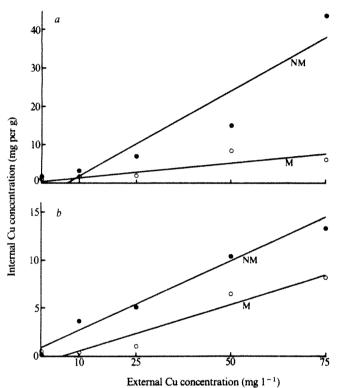


Fig. 1 Copper contents of mycorrhizal (\bigcirc) and non-mycorrhizal (\bigcirc) plants of Parys (a) and Stanton (b) races of Calluna vulgaris together with fitted regression lines showing the overall relationship between the external and shoot concentrations of the metal. In both cases the slopes of the M and NM regression lines differ significantly at P < 0.05.

75 mg l⁻¹ and zinc at 0, 25, 50, 100 and 150 mg l⁻¹. After 12 weeks all seedlings were harvested, their roots and shoots were separated, oven dried, weighed and further processed for analysis of Cu and Zn concentrations by atomic absorption spectrophotometry. The results of the shoot Cu and Zn measurements were subjected to a first order polynomial regression analysis to determine whether external metal concentration influenced tissue concentrations and to compare the magnitudes of any such influence in M and NM plants.

The major response of both races of Calluna to addition of heavy metals was that, with one exception (Calluna Parys, 25 mg l⁻¹ Zn), the NM plants failed to show any growth in the presence of the metals. Growth of both M and NM control plants was vigorous compared with that of NM plants exposed to metals, which indicates that major nutrient deficiencies were not experienced by the plants. M plants, in contrast, showed some growth even at the highest concentrations of metal and in all cases their yields were significantly greater than those of their NM counterparts (Table 1). The main difference between NM plants of the two races was that mortality was somewhat higher in the Stanton plants. Yield differences between M plants of the two races were small and insignificant in the copper treatments, but yields were significantly higher in Parys M plants in the zinc

treatments. This is the most distinctive difference between the races in the M condition. Because neither metals nor nutrients were precipitated at the concentrations used, it is likely that the main treatment effect in the NM plants is directly attributable to the added metal, rather than to interaction between metals and nutrient availability.

Regression analyses applied to the results of shoot copper (Fig. 1a, b) and zinc (Fig. 2a, b) determinations show that NM plants contain significantly higher contents of these metals in all cases except Stanton zinc. Premature death of Stanton NM plants in the Zn treatment probably contributed to the lower accumulation of Zn at the high treatment levels.

At the highest metal concentrations the root systems of NM plants of both races were so poorly developed that they were not suitable for metal analysis. Metal levels in roots of M plants were analysed and were found to be significantly higher than those of the shoots. This suggests that metals are being retained by the mycorrhizal fungus in the roots.

The severity of the growth inhibition in metal-treated NM plants compared with controls, and its association with a marked increase of metal concentration suggest that metal accumulation is primarily responsible for damage. High metal contents in roots relative to shoots of mycorrhizal plants also suggest that the capacity to maintain growth is associated with metal exclusion and hence avoidance of toxicity. It has been shown in

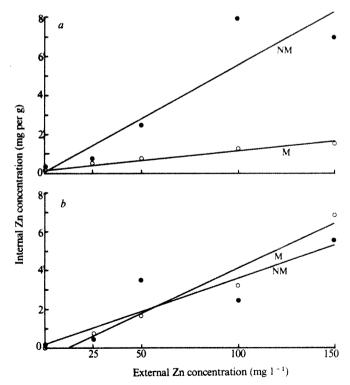


Fig. 2 Zinc contents of M and NM plants of Parys (a) and Stanton (b) races of Calluna vulgaris. Other details as in Fig. 1. The slopes of the M and NM regression lines are significantly different in the Parys race (P < 0.05) but not in the Stanton race.

the case of copper-tolerant races of the grass Agrostis tenuis that the metal is confined to the roots9, the main site of the complexing being the cell wall¹⁰. At high levels of metal, however, inhibition of root extension and hence of complexing ability occurs. In these circumstances the presence of an endomycorrhizal fungal symbiont could be of considerable importance. Fungal cell walls, particularly those of the class Ascomycotina to which Pezizella belongs, are known to have strong affinities for metallic cations11, and the ericoid infection, which provides a much greater concentration of fungal material in the root than that seen in VA mycorrhizas⁷, would be expected to provide a particularly efficient exclusion mechanism. Elaborate hyphal coils occupy most of the cortical cells of Calluna roots in those regions through which absorption occurs and these would provide a greatly increased surface for a retention of metal ions. The capacity, provided by mycorrhizal infection, to avoid metal accumulation may also help to explain the relative success of ericaceous plants on natural heathland soils in which the low pH increases the availability of metallic cations, in particular aluminium and manganese, to levels which are toxic to many non-ericaceous species¹². We are now assessing the ecological and physiological implications of the relationship between mycorrhizal infection and heavy metal resistance in a range of ericaceous species.

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1. Harley, J. L. in The Biology of Mycorrhiza, 1-334 (Hill, London, 1969).

- Tinker, P. B. in Symp. Soc. exp. Biol. 29, 325-349 (1975).
 Stribley, D. P. & Read, D. J. in Endomycorhizas (eds Sanders, F. E., Mosse, B. & Tinker, P. B.) 195-207 (Academic, London, 1975).
- Lambert, D. H., Baker, D. E. & Cole, H. J. Soil Sci. Soc. Am. 43, 976-980 (1979). Marrs, R. H. & Bannister, P. New Phytol. 81, 753-761 (1978). Read, D. J. & Stribley, D. P. Nature 244, 81-83 (1973).

- 7. Read, D. J. & Stribley, D. P. in Endomycorrhizas (eds Sanders, F. E., Mosse, B. & Tinker, P. B.) 105-117 (Academic, London, 1975).
- 8. Hewitt, E. J. in Commun. agric. Bur. tech. Comm. 22, 190-191 (1966).
 9. Wu, L., Thurman, D. A. & Bradshaw, A. D. New Physol. 75, 225-229 (1975).
 10. Turner, R. G. & Marshall, C. New Physol. 71, 671-676 (1972).
- Ashida, J., Higashi, N. & Kikuchi, T. Protoplasma 57, 27-32 (1963)
- Rorison, I. H. in Proc. Int. Symp. in Acid Sulphate Soils (ed. Dost, H.) 223-253 (Int. Inst. Land Reclamation & Improvement, Wageningen, 1973).

Increased growth of ryegrass exposed to ammonia

D. W. Cowling & D. R. Lockyer

The Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR, UK

Fluxes of ammonia (NH₃) between the soil/plant system and the atmosphere are components of the nitrogen cycle, but knowledge of them is inadequate 1,2. Grassland in the UK annually receives 8.4×105 tonnes of fertilizer nitrogen3 and 5×105 tonnes of nitrogen through the excreta of grazing ruminants⁴. Emissions of NH₃ from these sources may be 10⁵ tonnes annually and, apart from the loss of a plant nutrient, they may pollute the air. We report here that NH3 has beneficial effects on growth of perennial ryegrass (Lolium perenne L.), apparently through leaf sorption. In air containing NH3 at 16 µg m similar to the level above a grazed pasture⁶, sorption would be 48-224 ng m⁻² leaf area s⁻¹, depending on the density of leaves in the canopy. For grass with a leaf area index of 4, this process would add nitrogen at 6-28 g ha⁻¹ h⁻¹. These results support the suggestion7 that some of the flux of NH3 from soil may be sorbed by overlying plants.

The concentration of NH₃ in the air has been found to decrease with increasing height above the soil within the canopy of a pasture and it was suggested that some NH₃ volatilized from a soil may be sorbed by the overlying plants⁷. Recent studies of a

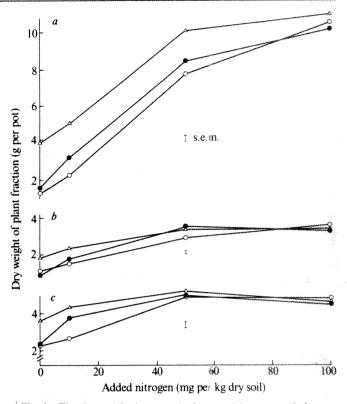


Fig. 1 The dry weight (g per pot) of perennial ryegrass (Lolium perenne L.) cv. S23 harvested in three fractions: a, 'shoots', cut 30 mm above the soil; b, 'stubble', bases of shoots cut to soil level; and c, 'roots' (ash-free basis) recovered by sieving and washing. The plants were exposed to filtered air with NH₃ added to give 11 (\bigcirc), 148 (\blacksquare), and 550 (\triangle) μ g m⁻³ throughout a 16-h photoperiod from day 45 after sowing, when they were first cut to 30 mm, until day 71. Fifteen plants were grown in each 15-cm diameter pot containing 4.2 kg of a sandy loam of pH 7.5, described else-, to which nitrogen (as NH₄NO₃) was added at either 0, 10, 50 or 100 mg kg⁻¹, together with uniform amounts of phosphorus, potassium, sulphur and magnesium (as CaH4 (PO4)2, KH2PO4, K₂SO₄ and MgCl₂). The soil surface was covered with a 10 mm layer of polyethylene granules to minimize sorption of NH3 and evaporation of water. Water was added frequently to the pots to bring the soil to an average tension of 7.4 kPa. Sets of four pots, representing the four rates of added nitrogen, were housed in each of nine chambers which provided three replicates of the three NH₃ concentrations. The chambers, which have been fully described elsewhere ¹⁸, were modified by the removal of the original perforated ceilings, and they received air at 121s⁻¹ to give two air changes per min. In these conditions, the boundary layer resistance to the transfer of water vapour measured just within the leaf canopy was 0.04 s mm⁻¹. The concentration of NH₃ was monitored continually in the outlet air and adjusted daily on the basis of determinations both by UV absorption spectrometry (Lear Siegler SM1000 Ambient Air Monitor, Colorado, USA) and by a colorimetric method using Nessler's reagent

number of species⁸⁻¹² have shown that NH₃ may be sorbed by leaves from air containing 4-14,000 µg m⁻³, sometimes with benefit to yield 8,10. In some studies 12,13, there was no evidence of sorption with NH₃ at $< 4 \mu g m^{-3}$, rather there was release from senescent leaves.

We examined the effects on plant growth of increments in NH₃ concentration and nitrogen supply to the roots (Fig. 1). The weight of shoots increased with NH3 concentration, and increased linearly with the addition of nitrogen to the soil. except at the highest rate. Although the stubble and roots (Fig. 1b, c) responded similarly to the shoots, the effects of the treatments were less clear. The concentration of nitrogen in shoots, stubble and roots (Fig. 2a-c) always increased with NH₃ concentration and with the addition of nitrogen to the soil, except at the lowest rate.

When nitrogen supply to the roots was inadequate, the growth of the plants was enhanced by exposure to NH₃. This

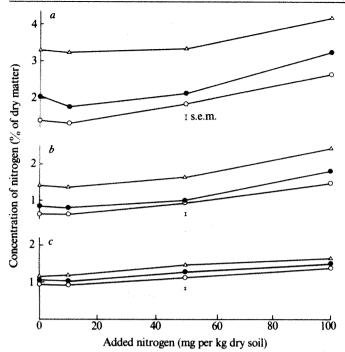


Fig. 2 The concentration of nitrogen (per cent of dry matter) in perennial ryegrass (Lolium perenne L.) cv. S23 harvested in three fractions: a, 'shoots'; b, 'stubble'; and c, 'roots', after exposure to filtered air containing NH₃ at 11 (○), 148 (●) and 550 (△) µg m as described in Fig. 1 legend. The concentration of nitrogen in duplicate oven-dried samples of the three replicate fractions of plant material was determined using an automated Dumas procedure (Coleman 29A Nitrogen Analyser, Illinois, USA).

enhancement was almost certainly through leaf absorption, which has been found for other crops with NH₃ (refs 9, 10) as well as with nitrogen dioxide^{14,15}. The term sorption, as used here, includes absorption into leaf tissue and adsorption on the leaf surface.

The high nitrogen concentration in the shoots exposed to NH₃ at 148 and $550 \,\mu\mathrm{g}\,\mathrm{m}^{-3}$ (Fig. 2a) probably results from leaf sorption of NH₃; sorption by soil and subsequent root uptake was unlikely to have been important 16. The concentration in the shoots of plants grown without added nitrogen and exposed to NH₃ at 11 µg m⁻³ was 1.4%, it increased to 2.6% with the highest rate of added nitrogen but, surprisingly, increased to 3.3% with exposure to NH₃ at 550 µg m⁻³. This comparison was made between the shoots of plants differing greatly in dry weight. A more appropriate comparison may be made by interpolating the shoot weight of plants grown without added nitrogen and exposed to 550 µg m⁻³ NH₃ (that is, 3.97 g per pot) onto the regression line produced for the shoot weight of plants grown with increased added nitrogen but with NH3 at 11 µg m (Fig. 1a). Thus, a similar shoot weight would have been obtained with nitrogen added at 22 mg kg⁻¹ soil; the shoots would have contained 1.5% nitrogen (Fig. 2a), that is, less than half the concentration resulting from exposure to 550 µg m⁻³ NH₃. This suggests that ammonia-nitrogen sorbed by leaves may be less available for transport and metabolism than nitrogen taken up through the roots. Ammonia may have reacted with the leaf surface, or its accumulation may have had both beneficial and injurious effects on growth.

The rate of sorption of NH₃, based on: (1) the leaf area, which was assumed, not unreasonably, to have developed linearly, and (2) the extra nitrogen in plants due to NH₃, may be expressed as a deposition velocity:

$$V_{\rm g}({\rm m~s^{-1}}) = \frac{{\rm rate~of~sorption~of~NH_3~(\mu g~m^{-2}~s^{-1})}}{{\rm concentration~of~NH_3~in~air~(\mu g~m^{-3})}}$$

where NH₃ concentration is reduced by 11 μg m⁻³, to allow for background in the filtered air. This expression is useful for comparing rates of sorption. Velocity ranged from 3 to and was lower for high-yielding plants; a greater 14 mm s

density of leaves presumably increased aerodynamic resistance to NH₃ sorption. In other studies, short-term exposures to NH₃ of isolated plants in chambers have given V_g of 10.4 and 15.4 mm s⁻¹ for two grasses¹¹ and 13.0 mm s⁻¹ for maize⁹.

The sorption of NH₃ by leaves may be important in reducing potential losses to the atmosphere, especially in heavily fertilized, intensively grazed grassland.

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- Terman, G. L. Adv. Agron. 31, 189-223 (1979)
 - Cooke, G. W. J. Soil Sci. 30, 187-213 (1979)
- Church, B. M. & Leech, P. K. Ministry of Agriculture, Fisheries & Food Rep. No.
- Cooke, G. W. in Agriculture and Water Quality, 5-57 (HMSO, London, 1976). Healey, T. V., McKay, H. A. C., Pilbeam, A. Scargill, D. J. geophys. Res. 75, 2317-2321
- Denmead, O. T., Simpson, J. R. & Freney, J. R. Science 185, 609-610 (1974).
- Denmead, O. T., Freney, J. R. & Simpson, J. R. Soil Biol. Biochem. 8, 161-164 (1976). Faller, N. Z. PflErnähr. Düng. Bodenk. 131, 120-130 (1972).

- Hutchinson, G. L., Millington, R. J. & Peters, D. B. Science 175, 771-772 (1972).

 Porter, L. K., Viets, F. G. & Hutchinson, G. L. Science 175, 759-761 (1972).

 Rogers, H. H. & Aneja, V. P. Envir. exp. Bot. 20, 251-257 (1980).

 Farquhar, G. D., Firth, P. M., Wetselaar, R. & Weir, B. Pl. Physiol. 66, 710-714 (1980).
- Farquhar, G. D., Wetselaar, R. & Firth, P. M. Science 203, 1257-1258 (1979).
 Matsumaru, T., Yoneyama, T., Totsuka, T. & Shiratori, K. Soil Sci. Pl. Nutr. 25, 255-265
- Rogers, H. H., Campbell, J. C. & Volk, R. J. Science 206, 333-335 (1979).
- Denmead, O. T., Nulsen, R. & Thurtell, G. W. Soil Sci. Soc. Am. J. 42, 840-842 (1978). Cowling, D. W. & Jones, L. H. P. Soil Sci. 110, 346-354 (1970).
- Lockyer, D. R., Cowling, D. W. & Jones, L. H. P. J. exp. Bot. 27, 397-406 (1976).
 Williams, P. C. Analyst 89, 276-281 (1964).

Sensing of $\Delta \bar{\mu} H^+$ in phototaxis of Halobacterium halobium

V. A. Baryshev, A. N. Glagolev & V. P. Skulachev

Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

Phototaxis in Halobacterium halobium is governed by two distinct photosystems1. Cells are attracted by green light, which is absorbed by bacteriorhodopsin, effectively a light-driven proton pump^{2,3}, and repelled by UV and blue light absorbed by a retinal-dependent pigment and by carotenoids^{1,4-10}. One possibility is that the bacterium detects changes in light intensity directly by the interaction of excited absorbent molecules with the tactile system, in which case the phototactic receptors would be analogous to bacterial chemoreceptors¹¹. However, H. halobium may sense light changes indirectly as changes in the chemical potential of hydrogen ions $(\Delta \tilde{\mu} H^+)^{12,13}$; the coincidence of the action spectra for positive phototaxis and photosynthesis 14,15 indeed support the suggestion that the cells may possess a specific $\Delta \bar{\mu} H^{\dagger}$ receptor, called a 'protometer'. We now describe experiments which indicate that positive phototaxis in H. halobium is governed by $\Delta \bar{\mu} H^+$ sensing, but that negative phototaxis is mediated by a specific photoreceptor system.

Shortly after an increase in blue light intensity, or a decrease in green light intensity, H. halobium cells reverse their direction of travel and, for a certain time period, the rate of reversals remains increased, gradually returning to the basal rate⁸. In peritrichous bacteria (for example, Escherichia coli), the adaptation time to a stimulus is directly proportional to the number of excited receptor molecules¹⁶. In the case of H. halobium the quantitative significance of the adaptation time is unclear and thus the only adequate method of determining taxis is by recording the fraction of cells that respond to a stimulus. Moreover, it seems reasonable to assume that the first reversal is an immediate response to the chemotactic signal, whereas adaptation reflects the process of inhibiting the chemotactic signal transmission. Therefore the first reversal will provide more accurate information about the reception process than the

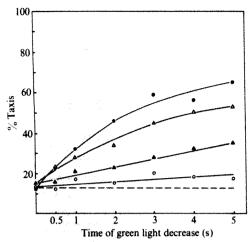


Fig. 1 The effect of inhibiting respiration and ATPase on the sensitivity of *H. halobium* phototaxis. *H. halobium* R₁M₁ lacking gas vacuoles and bacterioruberin (supplied by Dr N. Dencher) were grown in white light at 37 °C on a complex peptone medium (Oxoid) for 96 h. Motility of collected cells was greatly improved by incubation for 24 h in nutrient-free salts media. Cells were observed by phase contrast using a Univar microscope (Reichert). The initial intensity of the green light (500–650 nm) was 9 mW cm⁻². Cell reversal was recorded if the cell reversed within 5 s after insertion of a neutral filter that decreased the intensity by a factor of 1.4. The experimental points are means of 20–60 individual cell recordings. ○, Control; ♠, 0.1 mM DCCD; △, 5 mM KCN; ♠, 0.1 mM DCCD and 5 mM KCN. The baseline indicates the level of spontaneous reversals.

adaptation time. If, during phototaxis, bacteria actually sense changes in $\Delta\bar{\mu}\,H^+$ rather than changes in the concentration of excited bacteriorhodopsin, we would expect the inhibitors of oxidative phosphorylation to increase their sensitivity. Indeed, if the respiratory chain is blocked by CN⁻ and the H⁺-ATPase by DCCD, bacteriorhodopsin remains as the only operating proton pump, and minor changes in light intensity will effect significant changes in $\Delta\bar{\mu}\,H^+$. In the absence of inhibitors the same changes in light, due to the operation of the redox chain, will have little or no effect on the magnitude of $\Delta\bar{\mu}\,H^+$.

There are two ways in which the light stimulus can be varied: by changing the duration of the stimulus, or by altering its magnitude. The first approach was used in the experiment shown in Fig. 1. A given decrease in light was applied to the cell suspension for various periods of time. The sensitivity of phototaxis seemed to be much higher in the presence of CN⁻ and DCCD than in the absence of inhibitors. Oxygen consumption,

Table 1 Responses of H. halobium cells to monochromatic and mixed light

A STATE OF S	Per cent cells reversed	Motility rate
Illumination	in 3 s	$(\mu \text{ s}^{-1})$
Orange background: 0.12 mW	cm ⁻² ,	
$\lambda < 520 \text{ nm}$	<5	1.5
Increase in orange light to		
7.2 mW cm ⁻²	<5	2.7
Orange background + blue ligh	t	
$(\lambda < 400 \text{ nm}), 0.03 \text{ mW cm}^{-3}$	73	1.5
Increase in orange light to		
$7.2 \text{ mW cm}^{-2} + \text{blue light},$		
0.03 mW cm^{-2}	42	2.8
Orange background + intense		
blue ($\lambda < 490 \text{ nm}$)	100	2,7
Increase in orange light to		
$7.2 \text{ mW cm}^{-2} + \text{intense blue}$		
$(\lambda < 490 \text{ nm})$	100	2.7

Cells were incubated in a salt medium with NaCl mainly substituted by KCl^{23} . The slide was kept at 37 °C using a Reichert Biotherm microscopic thermostat. Blue light with $\lambda < 490$ nm (bottom two lines) was saturating for phototaxis.

monitored using a Clark electrode, was completely inhibited by 5 mM CN⁻, and light-driven ATP synthesis measured using firefly luciferase was found to be depressed by 90% in cells preincubated for 30 min with 0.1 mM DCCD (data not shown). In control cells lacking inhibitors a significant taxis response was observed only on a strong decrease in light. We found that the smaller the changes in light intensity, the greater the difference in phototaxis sensitivity between cells in the presence and absence of inhibitors, respectively (Fig. 2a). Control cells showed only a slight response to a twofold decrease in light intensity, while in the presence of inhibitors (closed circles) they responded with an 80% taxis effect.

These data indicate that phototaxis sensitivity strongly increases in conditions in which a sudden decrease in illumination produces a significant change in the energy level. However, the overall phototaxis could result from the mutual action of a protometer and a truly specific bacteriorhodopsin light receptor. A given decrease in light intensity would cause a given drop in the $\Delta \bar{\mu} H^+$ level in cells with inhibitors. A stronger decrease in illumination would result in the same decrease in $\Delta \bar{\mu} H^+$ in the control bacteria. As the decrease in $\Delta \bar{\mu} H^+$ is the same in both cases, although the changes in light intensity differ, an equal phototaxis response would rule out a specific photoreceptor mechanism. If the phototaxis activity were higher in the strongly illuminated sample, that is, in that lacking inhibitors, this would suggest a specific light-sensing process.

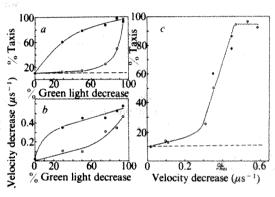


Fig. 2 a, The correlation between taxis activity and changes in motility rate with decreases in illumination. Conditions essentially as described for Fig. 1 legend. Initial intensity of the green light (500-650 nm) was 9 mW cm⁻². Reversals were recorded after the insertion of various neutral filters for 5 s: ○, control; ♠, 0.1 mM DCCD + 5 mM KCN. b, Motility rate was recorded by means of an ocular scale and a stopwatch before and after insertion of the same neutral filters as were used in a. The ordinate is the difference between control motility rate (2.4 μ s⁻¹) and motility rate after a decrease in light: ○, control; ♠, 0.1 mM DCCD + 5 mM KCN. c, Plot combining the data from experiments a and b.

As bacterial motility was found to be directly supported by $\Delta \bar{\mu} \, H^+$ (refs 17–20) and its rate to be positively correlated with the magnitude of $\Delta \bar{\mu} \, H^+$, we used motility rate as a probe for $\Delta \bar{\mu} \, H^+$. Figure 2 shows results of an experiment in which both motility rate and taxis were studied as a function of light intensity. When the phototactic response was plotted against the decrease in swimming velocity (Fig. 2c), the experimental points obtained both with control cells and those with inhibitors all lay on the same curve. This means that differences in light intensity changes per se are non-essential and that phototaxis is governed by changes in the energy level, presumably by $\Delta \bar{\mu} \, H^+$.

It seems to be important to correlate phototactic response with the absolute values of changes in $\Delta \bar{\mu} H^+$, although it is technically difficult to measure $\Delta \bar{\mu} H^+$ both rapidly and quantitatively in H. halobium in given conditions. Experiments on this are being done.

One may think that ATP, not $\Delta \bar{\mu} H^+$, is the principal effector in phototaxis. The possible existence of a specific ATP receptor in enterobacteria has been discussed elsewhere ¹³. However, this is not the case in *H. halobium*, as the addition of DCCD

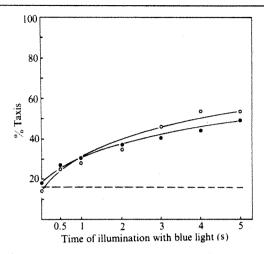


Fig. 3 The effect of inhibiting respiration and ATPase on the avoidance of blue light. Conditions were essentially as described in Fig. 1 legend. The intensity of the green background (500-580 nm) illumination was 36 mW cm^{-2} . Blue light stimuli ($\lambda < 400 \text{ nm}$, $1.4 \times 10^{-2} \,\mathrm{mW \, cm^{-2}})$ were applied through an incident light illuminator of the Univar microscope equipped with a 200-W mercury arc.

increased, rather than inhibited, the sensitivity of the phototaxis

The approach used in studying positive phototaxis was applied to test the negative phototaxis system. Blue light is known to affect the bacteriorhodopsin photocycle and thus to decrease $\Delta \bar{\mu} H^+$ (ref. 21). Wagner et al.²² found that UV light (360 nm) partially inhibited K⁺ uptake by H. halobium cells. They speculated that the behavioural response to blue light might be due to a decrease in membrane potential²². However, the inhibiting action of blue light, with a maximum at 412 nm, cannot be solely responsible for negative phototaxis, which has two main maxima, at 280 and 370 mm¹. Dencher³ and Hildebrand6 suggested that the blue light acts by decreasing the membrane potential, opening an ion channel associated with the pigment P₃₇₀. If the repelling action of blue light were indeed due to a decrease in $\Delta \bar{\mu} H^+$, one would expect the sensitivity of phototaxis to depend on the activity of the cellular proton pumps and it should thus be increased by CN and DCCD

Pulses of blue light with durations varying from 0.5 to 5 s were applied to cell suspensions with and without CN and DCCD (Fig. 3). Avoidance of blue light did not show a dependence on the ability of the stimulus to change the energy level. To test further the possible dependence of negative phototaxis on changes in $\Delta \bar{\mu} \, H^+$, a simultaneous stimulation by blue and green light was used; effects were shown to sum up algebraically⁸. Cells were partially de-energized by preincubation in dim light. As the K^+ gradient may support the energization²³ and motility²⁴ in H. halobium cells for a prolonged period of time, bacteria were incubated in a medium with a high KCl concentration. After incubation for 2 min the motility rate decreased from 2.7 to 1.5 μ s⁻¹ and could be brought to the initial level by illuminating the suspension with intense light for 10 s (Table 1). A mixture of blue and orange light elicited a reversal of 42% of H. halobium cells in 3 s. This reversal was accompanied by a significant increase in the motility rate, indicating that the repellent response of the cells to blue light is not due to a decrease in $\Delta \bar{\mu} H^{+}$. Increase in blue light alone produced 73% reversal of cells, which indicates that the effect of mixed light was a summation of an attractant (orange) and repellent (blue). Blue light alone had no effect on motility rate. The increase in motility rate was the same whether the intensity of orange light was increased alone or in combination with blue. If a high intensity blue light was used, the rate of reversals became independent of a simultaneous increase in orange light. The data shown in Table 1 clearly demonstrate that negative phototaxis does not result from changes in $\Delta \bar{\mu} H^{+}$.

The differences in the mechanisms of positive and negative phototaxis in H. halobium, that is, an indirect sensing of green light by means of a protometer and a direct sensing of blue light by a specified photoreceptor, would explain the striking differences in light sensitivity. Blue and UV light evoke a response at 1/10 and 1/500 the intensity of green light¹. This difference seems to be natural if we consider that the more sensitive system operates as a specialized photoreceptor.

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- Hildebrand, E. & Dencher, N. Nature 257, 46-48 (1975).
- Oesterhelt, D. & Stoeckenius, W. Proc. nam. Acad. Sci. U.S.A. 70, 2853-2857 (1973). Kayushin, L. P. & Skulachev, V. P. FEBS Lett. 39, 39-42 (1974).

- Hildebrand, E. Biophys. Struct. Mechanism 3, 69-77 (1977).

 Dencher, N. in Energ. Struct. Halophil. Microorg. (eds Caplan, S. R. & Ginzburg, M.) 335-340 (Elsevier, Amsterdam, 1978). Hildebrand, E. in Taxis and Behavior (ed. Harzebauer, G. L.) 35-73 (Chapman and Hall,
- London, 1978)
- Dencher N. A. & Hildebrand E. Z. Naturf. 34, 841-847 (1979)
- Spudich, J. L. & Stoeckenius, W. Photobiochem. Photobiophys 1, 43-53 (1979). Nultsch W. & Häder M. Ber. dt. bot. Ges. 91, 441-453 (1978).
- Sperling, W. & Schims, A. Biophys. Struct. Mechanism 6, 165-169 (1980).
 Koiwai O. & Hayashi H. J. Biochem. 86, 27-34 (1979).

- Glagolev, A. N. 12th FEBS Meet. Abstr. N 1867 (1978) Glagolev, A. N. J. theor. Biol. 82, 171-185 (1980).
- Clayton, R. K. Arch. Mikrobiol. 29, 189-212 (1958)
- Harayama, S. & Iino, T. J. Bact. 131, 34-41 (1977)
- Spudich, J. L. & Koshland, D. E. Jr Proc. natn. Acad. Sci. U.S.A. 72, 710-713 (1975). Belyakova, T. N., Glagolev, A. N. & Skulachev, V. P. Biokhimiya 41, 1478-1483 (1976). Manson, M. D., Tedesco, P., Berg, H. C., Harold, F. M. & van der Drift, C. A. Proc. natn.
- Acad. Sci. U.S.A. 74, 3060-3064 (1977)
- Matsuura, S., Shioim J.-I. & Imme, Y. FEBS Lett. 82, 187-190 (1977).
 Glagolev, A. N. & Skulachev, V. P. Nature 272, 280-282 (1978).
- Dancshazy, Zh., Drachev, L. A., Ormos, P., Nagy, K. & Skulachev, V. P. FEBS Lett. 96,
- Wagner, G., Geissler, G., Linhardt, R., Mollwo, A. & Vonhof, A. in Plant Membrane Transport (eds Spanswick, R. M. et al.) 641-644 (Elsevier, 1980).
- Wagner, G., Hartmann, R. & Oesterhelt, D. Eur. J. Biochem. 89, 169-179 (1978).
- Brown, I. I. thesis, Univ. Moscow (1981).

Control of mucus hydration as a Donnan equilibrium process

Patrick Y. Tam & Pedro Verdugo*

Center for Bioengineering and Department of Biological Structure, University of Washington WD-12, Seattle, Washington 98195, USA

The physical properties of the mucus secreted by the uterine cervix of primates and other animals undergo periodic changes which are important in the regulation of fertility. During the luteal phase of the sexual cycle, cervical mucus is thick, rubbery and impenetrable by spermatozoa; by the time of ovulation, it is a watery gel easily penetrated by spermatozoa. Although these changes in the rheological properties of mucus have been attributed to variations in the covalent cross-linking between long chains of glycoproteins, the main macromolecular constituent of cervical mucus^{1,2}, recent studies^{3,4} suggest that the macromolecular matrix of cervical mucus is probably not covalently crosslinked. Rather it is an entangled random network of long mucins3, in which case the observed changes in the physical properties of mucus would be explained by varying degrees of hydration, the mechanism of which is not understood5. We now describe experiments which show that the hydration of cervical mucus may be controlled by a Donnan equilibrium process.

Polyionic gels can behave as a semi-permeable membrane and become hydrated following a Donnan equilibrium. As mucins are polyionic species, mucus hydration could also follow a Donnan equilibrium in which the glycoproteins may function both as structural components of a stable continuous network, analogous to a semi-permeable membrane (which prevents the movement of polyions), and as the entrapped polyionic species that generate the osmotic drive. A typical feature of Donnan equilibria is the dependence of the movement of water on the ionic concentration and pH of the solvent. As the transport of ions in mucus secretory epithelia is known to be physiologically regulated5, it seems reasonable to propose that if mucus swelling follows a Donnan equilibrium, mucus hydration could be phy-

^{*} To whom correspondence should be addressed.

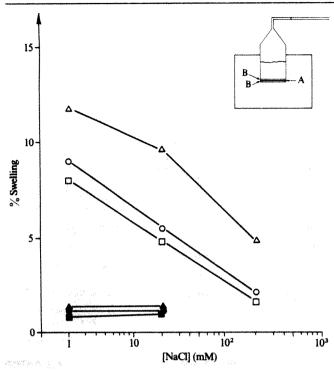


Fig. 1 Relationship between relative (%) swelling of cow cervical mucus at pH 7 and NaCl concentration in the swelling medium for three different samples of luteal (open symbols) and three samples of oestrous mucus (solid symbols). The inset is a diagram of the swelling chamber. Exchange of H_2O , ions and low molecular weight species is established across the Nucleopore filter membrane (A), which is sandwiched between two washers made of stainless steel fine wire mesh (B). See text for more detailed description.

siologically regulated by movement of water and electrolytes (including \mathbf{H}^+) across the epithelia.

Six samples each of luteal and oestrous fresh cow cervical mucus were obtained from the Animal Research Facility of Carnation Farms in Washington. The stages of the oestrous cycle were determined by rectal palpation. Mucus swelling was measured as the relative volume expansion of mucus, using an osmometer-like swelling chamber (Fig. 1 inset). In this chamber 0.1 ml of mucus sits on a diaphragm of Nucleopore filter (0.8 µm pore size) at the bottom of a small cylindrical container. The upper portion of the cylindrical container, above the mucus, is filled with silicone oil, forming a single fluid column that ends in a capillary tube (10 cm long and 16.7 µl volume). The water transfer between the mucus and the swelling solution is established across the filter and the volume expansion of the mucus is measured by the displacement of the oil meniscus in the capillary tube. In these experiments, the bottom of the mucus container was immersed in 50 ml of swelling medium which was continuously agitated at room temperature. Swelling medium was a water salt solution containing 2, 20 or 200 mM NaCl at neutral pH, or 150 mM NaCl at pH 8.0, 7.6, 6.99 or 6.46. The pH was adjusted with a phosphate buffer by changing the ratio of monobasic to dibasic sodium phosphate, but keeping the total phosphate concentration constant at 5 mM.

Figure 2b shows the typical time course of swelling of oestrous and luteal mucus in a swelling solution containing 150 mM NaCl at pH 6.46. Note that oestrous mucus demonstrates a very small amount of swelling whereas luteal mucus allows a 13% swelling at equilibrium in ~ 200 min. Figures 1 and 2 show the relationship between swelling at final equilibrium and salt concentration, and swelling and pH, respectively, in both oestrous and luteal mucus. Whereas oestrous mucus does not undergo swelling, luteal mucus demonstrates significant and irreversible swelling inversely proportional to the \log [NaCl] in the swelling medium, and at constant salt concentration (150 mM NaCl), linearly proportional to the pH of the swelling solution.

A unique feature of the Donnan equilibrium is the redistribution of water and ions in the presence of a barrier that limits the mobility of an electrostatically charged (polyionic) molecule. In the most common experimental demonstration of a Donnan equilibrium, this barrier is a semipermeable membrane? However, there are other ways of hindering the mobility of a polyionic molecule. For example, in polyionic gels the mobility of an electrostatically active polymer is limited by topological constraints (cross-links and/or entanglements) between long chains of the polymer itself. In the particular case of the mucus, the topological constraints of the molecular matrix prevent the glycoproteins from diffusing out of the gel. Therefore, in mucus, glycoproteins have a dual role as the structural elements of the entrapping molecular network, and as the entrapped polyions responsible for the osmotic drive.

The dependence of the movement of water on the concentration of ions and the pH of the solvent is a typical characteristic of a Donnan equilibrium and is due to the interaction of polyionic species. The phenomenon can be easily visualized by considering the glycoproteins as electrostatically charged chains. Although the role of ions in water movement depends on their colligative effects, pH affects water movement by changing the total electrostatic charge of the entire chain in a way that is

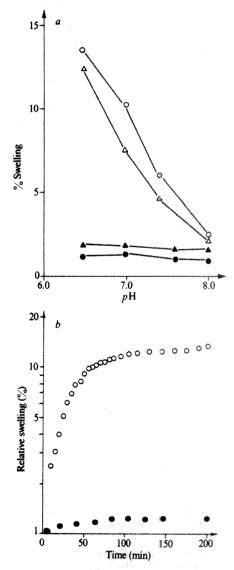


Fig. 2 a, Relationship between relative (%) swelling of cow cervical mucus and pH, in a swelling medium containing 150 mM NaCl. Samples ○, △ are luteal, and ▲, ● are oestrous mucus. b, Time course of swelling as relative (%) volume increase for oestrous (●) and luteal (○) cow cervical mucus in a medium containing NaCl 150 mM, pH 5.46, at 20 °C.

dependent on the ionization constant of dissociable groups in the glycoproteins. The dominant dissociable groups in mucus glycoproteins are axial sialic acidic groups in the oligosaccharide side chains of the molecule⁸. Therefore, the decrease in mucus hydration with increasing pH and/or ionic concentration found in these experiments is indeed predicted by the Donnan equilibrium.

The rheological properties of mucus are important in transcervical migration of sperm into the uterus as well as in mucociliary transport in the airways. Hydration of mucus is a critical determinant of these properties9. Mucus is discharged from the secretory granules of the cell as densely packed, membrane-free boluses 10 which are then hydrated in some unknown way 5. The evidence presented here shows that luteal cow cervical mucus can be hydrated according to the Donnan equilibrium. Within this equilibrium, the changes of pH and salt concentration that effectively control mucus swelling in vitro are in the same range of physiological values reported in cervical mucus of cows and women. Although the actual fluctuations of NaCl in cervical mucus remain a controversial issue^{11,12}, there are indications that the onset of mucus hydration (and ferning formation) is preceded by a decrease in pH which also coincides with the earliest increases in urinary oestrogens 13,14

These findings, together with our previous observations³, point to a new concept in understanding the physiological control of mucus hydration and rheological properties. They predict that mucus boluses consisting of densely packed entanglements of mucins could be swollen in the extracellular medium following a Donnan equilibrium process. Accordingly, the movement of ions (including H⁺), soluble proteins and water across the mucosa, as well as the amount of soluble proteins and ions within the secreted mucus, could be the variables under physiological control which would determine mucus hydration, and thereby its rheological properties. It has been shown that secretions of soluble proteins in the cervix¹⁵ and uterus are responsive to hormonal control (M. K. Postle and R. B. Heap, personal communication). However, the role of the soluble proteins found in the mucus has not been clearly established 15,16 The model we propose suggests an osmotic function for the soluble proteins as potentially entrapped polyions and is consistent with the available evidence, that 'unswollen' luteal mucus contains high amounts of soluble proteins, while hydrated oestrous mucus contains small amounts¹⁵. In the latter case, the mucus molecular network would be expanded allowing soluble proteins to undergo further dilution and escape the gel matrix.

Although there is no direct evidence to validate the predicted hormonal or neurohormonal control of the movement of water, H⁺ and electrolytes across the cervical mucosa, it has been shown that in the respiratory mucosa the movement of water, Na+ and Cl- is responsive to hormonal or neurohormonal

The present experiments were limited to an exploration of the interaction of cow cervical mucus with NaCl at different pH. However, with only minor constraints, the concept that mucus hydration could be regulated by the Donnan equilibrium remains valid for other types of mucus and other ionic influences. For instance, an interesting implication of this concept is the potential existence of a Donnan-equilibriumcoupled system to delay enzymatic catalysis. Indeed, the application of synthetic polymer gels to entrap enzymes (including proteases) is well known in engineering 17 and is beginning to find wide industrial application.

Another significant prediction of the proposed model regards the pathophysiology of cystic fibrosis, where mucus would be permanently unswollen probably due to deficiencies in the control of the swelling medium and/or the polyionic composition of the secreted mucus. Although there is no direct evidence for defective trans-epithelial movement of water and ions in cystic fibrosis, it has been shown that NaCl is markedly increased in exocrine sweat, and that calcium transport seems to be decreased in red cells and fibroblasts and increased in exocrine secretions in these patients 18-20.

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- 1. Gibbons, R. A. & Glover, F. A. Biochem. J. 73, 217-225 (1959)
- Odeblad, E. Acta obstet, pr. A. Biochem. J. 13, 211-223 (1939). Odeblad, E. Acta obstet. gynec. scand. 47, Suppl. 1, 59-79 (1968). Lee, W. I., Verdugo, P., Blandau, R. J. & Gaddum-Rosse, P. Gynec. Invest. 8, 254-266 (1977). Meyer, F. A. Biorheology 13, 49-58 (1976).
- Nadel, J. A., Davis, B. & Phipps, R. J. A. Rev. Physiol. 41, 369-381 (1979).
- Katchalsky, A., Lifson, S. & Eisenberg, H. J. polym. Sci. 7, 571-574 (1951). Donnan, F. G. & Guggenheim, E. A. Z. physik. Chem. A162, 346-360 (1932).

- Clamp, J. R., Allen, A., Gibbons, R. A. & Roberts, G. P. Br. med. Bull. 34, 21-41 (1978). Wolf, D. P., Sokoloski, J., Khan, M. A. & Litt, M. Fert. Steril. 28, 53-58 (1977). Chilton, S. B., Nicosia, S. V., Sowinski, J. M. & Wolf, D. P. J. Cell Biol. 86, 172-180 (1980). Herzberg, M., Joel, C. A. & Katchalsky, A. Fert. Steril. 15, 684-694 (1964). Kopito, L. E., Kososky, H. J., Sturgis, S. H., Lieberman, B. L. & Schwachman, H. Fert. Steril. 24, 409, 506, (1973).
- 13. Moghissi, K. S. in The Uterine Cervix in Reproduction (eds Insler, V. & Bettendorf, G.)
- And The Certain Certain Americans (Casa American)
 And The Certain Americans (Casa American)
 Koreks, M. V. A. M. & Kremer, J. in The Uterine Cervix in Reproduction (eds Insler, V. & Bettendorf, G.) 109-117 (Thieme, Stuttgart, 1977).
- Schumacher, G. F. B. in The Biology of the Cervix (eds Blandau, R. J. & Moghissi, K. S.) 201-233 (University of Chicago Press, 1973).
 Gibbons, R. A. Br. med. Bull. 34, 34-38 (1978).
- 17. O'Driscoll, K. F., Hinberg, I., Korus, R. & Kapoulas, A. J. polym. Sci. Symp. 46, 227-235 (1974).
- Schwachman, H. & Antonowicz, I. Ann. N.Y. Acad. Sci. 93, 600-620 (1962).
- Katz, S. in Perspectives in Cystic Fibrosis (ed. Sturges, J. M.) 3-14 (The Imperial Press, Ontario, 1980).
- 20. Gugler, E., Pallavicini, C. J., Swerdlow, H. & di Sant'Agnese, P. A. J. Pediat. 71, 585-588

The fate of inner cell mass and trophectoderm nuclei transplanted to fertilized mouse eggs

Jacek A. Modlinski*

MRC Mammalian Development Unit, 4 Stephenson Way, London NW1 2HE, UK

When nuclei from 12-18-cell mouse embryos have been injected into fertilized mouse eggs^{1,2}, donor and host chromosomes in those eggs that survived the operation combined to form a single tetraploid nucleus. Some of the resulting embryos developed into morphologically normal blastocysts. At the 8-cell stage, mouse blastomeres are still totipotent3 and are indistinguishable from one another. By the blastocyst stage, inner cell mass and trophectoderm have separated into distinct cell lineages that differ in morphology, physiology and biochemistry. The fetus develops entirely from inner cell mass while the trophectoderm contributes only to placenta and fetal membranes; it is unclear when this is determined. Aggregation experiments suggest that some blastomeres are still labile at the late morula/early blastocyst stage^{6,7}. If this was the case at least some of the donor cells used in my previous nuclear transfers might have been still totipotent. However I report here that nuclei from trophoblast and inner cell mass are different from one another, as judged by the developmental fate of fertilized mouse eggs to which they are transplanted.

Fertilized one-cell eggs were obtained from spontaneously ovulating CR and Q females mated with syngeneic males. Eggs were collected between 10.00 and 12.00 h on the day of mating. Donor cells were from 3.5-4-day old CR, Q and MF1 blastocysts from which the zona pellucida had been removed by pronase. The mural trophectoderm was dissected from the blastocysts microsurgically8; inner cell mass tissue was obtained by either microsurgery or immunosurgery9. The severed mural trophectoderm and inner cell mass tissues were placed for $\sim 30-40$ min in Ca²⁺- and Mg²⁺-free phosphate-buffered saline (a 0.02% solution of EDTA was occasionally also used) and pipetted using a narrow pipette. Single cells taken from the

^{*} Present address: Institute of Zoology, University of Warsaw, Warsaw, Poland

trophectoderm or inner cell mass were drawn into a fine glass micropipette to disrupt the cell membrane; the nucleus and the associated cytoplasm were then injected into fertilized eggs at the early pronuclear stage 1.2. Before and during the operation the host eggs were kept in medium 16 (ref. 10) containing 10 g ml⁻¹ cytochalasin B to facilitate microsurgery^{11,12}. The eggs which survived the operation were cultured in medium 16 for 48-90 h and most of the resulting embryos were examined in air-dried preparations13

Trophectoderm and inner cell mass nuclei looked intact and were similar in appearance at the time of injection, and therefore there was no reason to believe that trophectoderm nuclei suffered more damage than inner cell mass nuclei during recovery; the proportion of eggs degenerating immediately after injection was similar after transplanting either type of nucleus. However, Table 1 shows that the fate of the two classes of eggs was different. Eggs that had received inner cell mass nuclei developed similarly to those injected with morula cell nuclei2. Of the 16 that were cleaved, eight developed to the late morula and blastocyst stage, and appeared normal. In six embryos (one blastocyst, two morulae, one 4-cell and two 6-cell embryos), tetraploid metaphase plates were found. This shows that nuclei originating from these two types of cells (that is, morula and inner cell mass nuclei) can contribute their chromosomes to those of an egg, thus both sets can form a common metaphase plate. The embryo carrying such a tetraploid hybrid nucleus can develop at least to the blastocyst stage. Thus, although the degree of activity of the transferred genome is unknown, one may suppose that the nuclei of such cells do not change irreversibly (if they do so at all) during preimplantation development and their redifferentiation is possible in the egg cytoplasm. In contrast, when the donor nuclei were trophectodermal in origin, donor and host chromosomes still integrated into a single nucleus but development was arrested after two to three cleavages, and no late morulae or blastocysts were formed.

The failure of trophectodermal nuclei to support normal development agrees with the recent findings of Illmensee and Hoppe¹⁴. Mouse eggs enucleated after fertilization and injected with inner cell mass nuclei developed not only into normal blastocysts but, after transfer to pseudo-pregnant foster mothers, into normal fertile mice. In contrast, eggs which received trophectoderm nuclei never developed beyond the first few cleavage divisions.

One of the characteristics of mural trophectoderm (that is, that part of trophectoderm not overlying the inner cell mass) is that it undergoes little or no further cell divisions after the blastocyst stage. DNA accumulation continues by a process of endoreduplication¹⁵, resulting in trophoblast giant cells with large nuclei that may contain several hundred times the haploid DNA content. To determine whether endoreduplication occurred after injection of trophectoderm nuclei into fertilized eggs, the embryos arrested in cleavage (see Table 1) were fixed, air-dried and stained with Feulgen, together with a control series of embryos recovered from the uterus on the fourth day of gestation; DNA determinations were then carried out on the nuclei of experimental and control embryos by microdensitometry. There was no evidence of endoreduplication

Table 1 In vitro development of fertilized mouse eggs injected with inner cell mass or trophectoderm nuclei

	No. of	No. of		after 4 n cultu		1	
Origin of donor cells	eggs injected	surviving (%)	1 œll	2–4 ceII	5–8 cell	М	В
Inner cell mass Trophectoderm 12–18-cell	142 167	21 (14.7) 18(10.7)	5 5	3 7	5 6	5 0	3 0
embryos, data from Modlinski ²	166	15(9.0)	5	2	0	3	5

M, morulae; B, blastocysts.

Table 2 DNA content of the nuclei in mouse embryos arrested in cleavage after injection of a trophectoderm nucleus into the fertilized CQQ

	No. of	Mean cell			uciei w		
Group	embryos	number	2C	4C	4-8C	8C	Total
Trophectoderm nuclear transfer	r 4	6.5	1	10	6	9	26
Control	7	32.7	60	62	0	2	124

Embryos were fixed and air-dried; a cut surface of the mouse liver was pressed on to the end of the slide, and then Feulgen-stained. DNA determinations were made using an M85 Vickers integrating microdensitometer. Liver nuclei gave discrete peaks for 2C, 4C, 8C, 16C, and 32C DNA values, where C is the haploid DNA content; embryo nuclei were assessed against these standards. In the experimental series, each nucleus was measured three to four times; the readings obtained were very consistent. In the control series, where cell number was greater, some nuclei overlapped and were therefore not measured.

(Table 2): the DNA contents of the nuclei were in the 4-8 C (where C is the haploid DNA content) range expected in tetraploid cells arrested in the G₁ or G₂ stage of the cell cycle, respectively, while the diploid controls were almost all in the 2-4 C range.

Although the transplanted trophectoderm nuclei did not undergo endoreduplication as they would during normal development, the observation that cleavage in this series was arrested after two or three cell divisions suggests that these nuclei not only have a restricted potential for further division themselves, but that, following their own programme, they may impose it on the host cell and block its divisions as well. Alternatively, some factor in the trophectoderm cytoplasm, which in this technique is injected into the fertilized egg along with the donor nucleus, may inhibit fur her development. Also, the lack of endoreduplication in such embryos might be the result of the influence of egg cytoplasm. Thus it is likely that the behaviour of trophectoderm transplants is the result of complicated interactions between host and donor nuclei and cytoplasm.

In Amphibia, nuclei even from differentiated cells have, after transplantation to enucleated eggs, been able to support development up to the late tadpole stage, which consists of a wide variety of differentiated tissues 16. The present observation does not necessarily indicate any difference between Amphibia and mammals. Trophectoderm is a highly specialized tissue, with a limited lifespan¹⁷ and no share in forming the embryo itself. It may be that nuclei from tissues differentiated within the embryo will prove better able to participate in normal development.

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- Modlinski, J. A. thous, Warsaw Univ (1976)
- Modimski, J. A. Nanev 273, 466-467 (1978)
 Kelly, S. F. J. exp. Zool. 200, 365-376 (1977)
- serdner, R. L. & Papasonmou, V. E. m. The Berly Development.
 & Wild, A. E.) 107-132 (Cambridge University Press, 1975)
- & Wild, A. E.) 107-132 (Cambridge University Priss, 1975)

 5. Handyside, A. H. & Johnson, M. H. J. Embryol. exp. Morph. 44, 191-199 (1978)

 6. Mants, B. in Premplanismon Stages of Pregionics (sc. Wolstenholms, G. E. W.) 194-207 (Cherchill, London, 1965)

 7. Stern, M. A. & Wilson, I. B. J. Embryol. exp. Morph. 28, 247-254 (1972)

 8. Gardnor, R. L. & Johnson, M. H. J. Embryol. exp. hierph. 28, 279-312 (1972)

 9. Soltar, D. & Knowles, B. B. Proc. sain: Acnd. Sci. U.S.A. 72, 5099-5102 (1975)

 10. Whittingham, D. G. J. Reprod. Fart. 14, 7-12 (1971).

 11. Hoppe, P. C. & Illmansee, K. Proc. sain: Acnd. Sci. U.S.A. 74, 5657-5661 (1977)

 12. Modlinski, J. A. J. Embryol. exp. Morph. 68, 153-161 (1980)

 13. Tarkowski, A. K. Cytogenesics 3, 394-400 (1966).

 14. Illmansee, K. & Hoppe, P. C. 62, 23, 9-18 (1981).

- Introvence, A. K. Cytogenetics 3, 394-400 (1906).
 Illimonees, K. & Hoppe, P. C. Call 23, 9-18 (1981)
 Chapman, V. M., Amedi, J. D. & McLaren, A. Deol'Biel 29, 48-54 (1972)
 Gurdon, J. B., Liekey, R. A. & Rooves, O. R. J. Emeryol. exp. Morph. 34, 93-112 (1975).
 Sherman, M. I. Differentiation 3, 51-67 (1975)

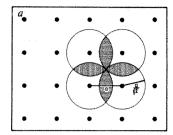
Dendritic territories of cat retinal ganglion cells

H. Wässle*‡, L. Peichl*‡ & B. B. Boycott†

- * Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstrasse 37, D-7400 Tübingen, FRG
- † Medical Research Council Cell Biophysics Unit, King's College, 26-29 Drury Lane, London WC2B 5RL, UK

Ganglion cells have to cover the retina with their dendritic fields so that every point of the visual space is 'seen' by at least one ganglion cell of each physiological type. Using neurofibrillar methods it has now become possible to stain all the α -ganglion cells so that their dendritic network can be analysed¹. Both subpopulations of α -cells, corresponding physiologically to ON-brisk-transient cells²-5 and OFF-brisk-transient cells, achieve a uniform and independent coverage of the retina. The cell bodies are arrayed in a regular mosaic¹. δ and the dendritic fields adapt to the available space. It is suggested that during development interaction between neighbouring ganglion cells of the same functional type regulates their dendritic field sizes.

There are more or less economical ways in which nerve cells can overlay a planar surface with their dendrites, given the requirement that every point should be covered by at least one dendritic field. For cells arrayed in a hexagonal or square lattice with spacing 'a', circular dendritic fields of radius $a/\sqrt{3}$ or $a/\sqrt{2}$ would provide at least onefold coverage (Fig. 1). Were the cells distributed at random, onefold coverage could be achieved either by rather large dendritic fields of uniform size or by interaction between neighbouring cells, where adjustment of the dendritic field size to the local intercellular separation would give rise to a broad and irregular range of dendritic fields.



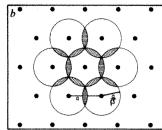


Fig. 1 a, Square lattice of cell bodies. The smallest circular dendritic fields, which would provide a onefold coverage, have a radius of $a/\sqrt{2}$. In the hatched area the dendrites of two cells overlap. b, Hexagonal array of cell bodies. In this instance at least onefold coverage is ensured by dendritic fields of radius $a/\sqrt{3}$.

Ganglion cells of the retina are a good model system for study of the organization of such a sheet of neurones because their cell bodies and their dendritic fields approximate to a single plane. In the cat retina several different morphological 7 and physiological classes 8 of ganglion cells have been defined. With neurofibrillar staining methods all α -ganglion cells were stained to their whole extent, that is, cell body and dendritic tree 1 . It was shown that for all OFF-centre cells the dendritic branches lie close to the inner nuclear layer, whereas for ON-centre cells the branching plane is $\sim\!10~\mu\mathrm{m}$ closer to the ganglion cell layer $^{1.9}$. This then constitutes a functionally homogeneous population of neurones which is accessible to quantitative investigation.

Figure 2a isolates the ON-population of α -cells and shows the field homogeneously covered by the dendrites. That the cell bodies are distributed nonrandomly was shown by analysis of their nearest-neighbour distribution, which is gaussian^{1,6}. The degree of dendritic overlap is rather small; an area centred on

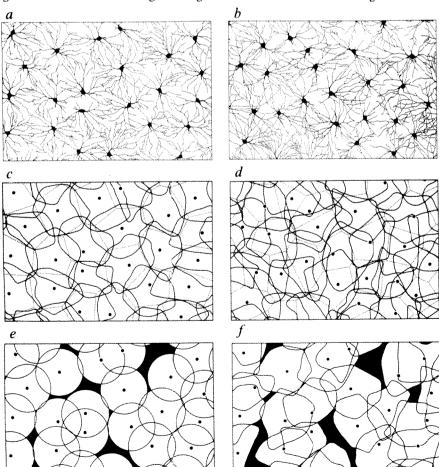


Fig. 2 a, The population of $ON-\alpha$ -ganglion cells taken from an area 1.7×1.2 mm of a Bodian-stained cat retinal whole mount (eccentricity 4 mm). b, Same field as in a, but only the OFF- α -ganglion cells are shown. c, Solid curves are the contours of the dendritic fields of the ON- α -cells shown in a and the dots indicate the cell body locations. The dotted polygons are Dirichlet domains, which were constructed for each cell body as described in the text. d, Same diagram as in c but for the OFF- α -cells. e, Coverage of the retina with hypothetical circular dendritic fields based on the mosaic of $ON-\alpha$ -cells; the black areas are not covered. f, Every ON-α-cell dendritic field is substituted by its right-left mirror image with the cell body unchanged.

‡ Present address: Max-Planck-Institut f
ür H
örnforschung, Deutschordenstr. 46, D-6000 Frankfurt/M, 71, FRG.

the cell body of each neurone is not covered by the dendrites of neighbours. OFF-cells show the same arrangement (Fig. 2b). Dendritic fields were defined by connecting the outermost dendritic tips of every cell by a smooth closed curve (Fig. 2c, d). The dendritic fields are irregular. In some cases there is an apparent correlation between the dendritic tree of a cell and the space not occupied by neighbours.

The concept of Dirichlet domains¹⁰, which cover the area by contiguous convex polygons, is introduced in Fig. 2c and d for comparison. Around every cell body a circle of 500 µm diameter was constructed and the intersecting lines of neighbouring circles were drawn. Dirichlet domains subdivide the area into small territories, one for each cell, with the property that every point in a given territory is closer to its own cell body than to any other. Thus, a dendritic growth model in which the dendrites grow out of the cell bodies at the same time and rate and stop growing when they touch dendrites of neighbouring cells can be described in terms of Dirichlet domains, although they do not account for overlapping dendritic trees. The Dirichlet domains are a rather good approximation to the dendritic fields. Basic features, such as the orientation of the long axis, nearly always agree as between the dendritic field and its Dirichlet domain. This implies that there might be some mechanism of mutual regulation of the dendritic growth of neighbouring ganglion cells of the same type.

Another test for the correlation between the dendritic field dimensions and the available space is shown in Fig. 2e. Circular fields of the average dendritic field diameter (399 µm) were constructed around each ON-cell perikaryon. Such uniform domains leave gaps and the coverage is consequently incomplete and not very homogeneous (Fig. 2e). When, with the cell body in its original position, every dendritic field is replaced by its mirror image, the coverage is again very inhomogeneous, uncovered gaps are found next to areas where the dendritic fields of several ganglion cells overlap (Fig. 2f).

We infer from these observations that some kind of local regulation which defines the shape and size of the dendritic fields must prevail during development. This process must be highly specific because ON-centre cells influence neighbouring ONcentre cells but not OFF-centre cells and vice versa. The same mechanism could regulate the increase of the average dendritic field size with distance from the central area and so balance the decreasing α -cell density. A similarly specific process must be active at the cell body level to regulate nearest-neighbour distances1.

Every point in the field represented by Fig. 2c is covered by an average of 1.4 ON- α -cells (average dendritic field area \times density). For cells arrayed in a square lattice with circular dendritic fields, the geometrical constraints shown in Fig. 1a lead to an average coverage of 1.57 to ensure at least a onefold coverage everywhere. Arrangement of the ganglion cells in a hexagonal lattice would require an average coverage of 1.21. This shows that although the ganglion cells are in fact distributed less precisely than in a perfect lattice, their dendritic trees cover the given field of Fig. 2a more economically than in a square array and close to the theoretical limit possible in a hexagonal lattice.

The size and shape of an α -cell's receptive field centre, although slightly larger, closely corresponds to its dendritic field³. Therefore, these morphological results imply a complete and economical coverage of either functional subclass. Twodimensional sheets of neurones are not only found in the retina; arrangement in layers is common in many parts of the central nervous system. The multilamellar structure of the cerebral cortex is an example and it would be interesting to see whether identical functional units there exhibit the same kinds of territorial attributes and regular intercell spacings.

Received 27 March; accepted 11 June 1981.

- Wässle, H., Peichl, L. & Boycott, B. B. Proc. R. Soc. B212, 157-175 (1981).
- Cleland, B. G., Levick, W. R. & Wässle, H. J. Physiol., Lond. 248, 151-171 (1975).
 Peichl, L. & Wässle, H. Proc. Roc. Soc. B212, 139-156 (1981).
 Cleland, B. G. & Levick, W. R. J. Physiol., Lond. 240, 421-456 (1974).

- 5. Levick, W. R. Nature 254, 659-662 (1975).
- Wässle, H. & Riemann, H. J. Proc. R. Soc. B200, 441-461 (1978) Boycott, B. B. & Wassle, H. J. Physiol., Lond. 240, 397-419 (1974)
- Cleland, B. G. & Levick, W. R. J. Physiol., Lond. 240, 457-492 (1974).

 Nelson, R., Famiglietti, E. V. Jr & Kolb, H. J. Neurophysiol. 41, 472-483 (1978).
- 10. Honda, H. J. theor. Biol. 72, 523-543 (1978).

Androgen increases formation of behaviourally effective oestrogen in dove brain

Th. Steimer* & J. B. Hutchison

MRC Unit on the Development and Integration of Behaviour, University Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA, UK

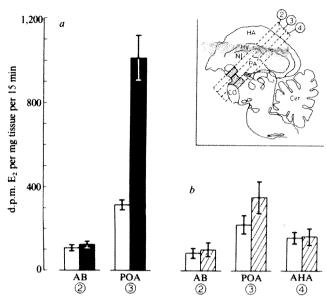
Oestradiol-17ß (E2) formed from testosterone in the brain is thought to be involved in the hormonal control of male sexual behaviour in some mammal species 1-3. In the male dove, Streptopelia risoria, the aromatase system in the preoptic area (POA) is very active in converting testosterone to E2 (ref. 4). This oestrogenic metabolite has specific effects on male nest-orientated courtship patterns, which are mediated by an oestrogensensitive system in the preoptic-anterior hypothalamic area of the brain⁵. The preoptic aromatase system is likely to be important in the regulation of androgen action on male courtship behaviour, as no circulating oestrogen can be detected in the blood plasma of the male⁶. We report here that the level of aromatase activity in the POA depends on androgenic stimulation. Conversion of testosterone to E2 is markedly increased in castrated males injected intramuscularly (i.m.) with testosterone propionate (TP). Increased aromatase activity seems to be due to induction of the enzyme and is specific to the POA These findings indicate that the formation of behaviourally effective oestrogen in a specific brain target area is under hormonal control and suggest a role for this regulatory mechanism in the integration of courtship behaviour in the male dove.

The brain system underlying nest-soliciting display in the male dove can be activated in short-term castrates by either testosterone or E₂ (ref. 7). However, while E₂ effectively restores nest-soliciting to pre-castration levels in long-term castrates, testosterone is virtually ineffective8, suggesting that capacity for aromatization declines with long-term androgen deficit. Prolonged treatment with TP increases nest-soliciting display to some extent in long-term castrates, suggesting that aromatase activity might increase due to the action of androgen⁸. Here we examine the possibility that androgen influences the preoptic aromatase system to increase the formation of E2.

In experiment 1, aromatization of testosterone was measured using a previously described microassay. Two groups of birds (n = 8 for each group) were castrated and 30 days later they were injected on 12 successive days either with androgen or avian saline according to previously described procedures⁷. The dose of androgen (300 µg TP in 0.3 ml avian saline) used is known to induce nest-soliciting behaviour in 30-day castrates⁷. Males were tested daily for courtship⁷ and nest-soliciting behaviour was restored to pre-castration levels in the TP-treated group. Two brain areas were examined, the POA and a non-androgen target area, the area basalis (AB). Aromatase activity in the TP-treated group was increased about threefold as compared to control levels (Fig. 1a). This difference was highly significant (P < 0.001, Student's t-test). Aromatase activity was not affected in the AB, which is immediately adjacent to the POA but is not an androgen target area and shows much lower rates of testosterone conversion4

To determine whether the effect of TP on aromatase activity is dose-dependent, a lower dose (30 µg per day) and shorter

^{*} Present address: Département de Biochimie Médicale, Ceatre Médical Universitaire, 1 rue Michel-Servet, CH-1211 Genève, Switzerland



a, Experiment 1: aromatase activity was markedly increased (P < 0.001, Student's t-test) in the POA of short-term castrates injected i.m. for 12 days with 300 µg per day of TP (solid bars) compared with saline-injected controls (open bars). Aromatase activity in the area basalis (AB) was not affected. b, Experiment 2: treatment with a lower dose of TP (30 µg per day) for 4 days (cross-hatched bars) was more effective than a single 30 µg injection (open bars) in increasing aromatase activity in the POA. It had no effect on either AB or the anterior hypothalamus (AHA): n = 4 in each group. Aromatase activity was measured in homogenates obtained from individual brain samples incubated at 40 °C, pH 7.4 for 15 min with $10 \text{ nM} [1\alpha, 2\alpha(n)^{-3}\text{H}]$ testosterone (specific activity 53 Ci mmol⁻¹, Radiochemical Centre, Amersham) and 1 mg ml⁻¹ NADPH₂ (Sigma, type III). Conversion rates were linear with respect to time and amount of brain tissue used for the incubation⁴. Inset: parasagittal view of the brain showing coronal sections 2, 3 and 4 from which samples AB, POA and AHA (stippled areas) were obtained. See ref. 18 for further details and nomenclature.

periods of treatment were used in experiment 2. In addition to AB and POA the anterior hypothalamus (AHA) was examined. This area is androgen-sensitive, but does not seem to concentrate E_2 to the same extent as the POA. Aromatase activity was higher in the POA after 4 days of treatment than after a single injection (Fig. 1b), although the increase was not as great as that induced by the higher dose (expt 1). Again, this effect seems to be specific to the POA, no change being detectable in AB or AHA. The results of these experiments show that conversion of testosterone to E_2 is stimulated specifically in the POA of castrated male doves by systemic TP treatment. This effect is related to dose and duration of treatment.

In experiment 3, aromatase activity was measured in TPtreated (300 µg per day for 6 successive days) and untreated long-term castrates (n = 4 for each group) using increasing concentrations of the substrate (testosterone) to determine the kinetic properties of the enzyme. Figure 2 shows that the observed increase in activity is probably due to induction of the enzyme: the maximal velocity V_{max} was markedly increased (~fivefold: 250 fmol h⁻¹ per mg tissue as opposed to 48 fmol h⁻¹ per mg tissue) in the POA of castrates after six daily injections. but the Michaelis constant (K_m) was not significantly altered $(2.1 \times 10^{-8} \,\mathrm{M})$ in controls as opposed to $2.7 \times 10^{-8} \,\mathrm{M}$ after TP treatment). Moreover, K_m in the AB was similar in either treated or untreated birds $(2.1 \times 10^{-8} \,\mathrm{M})$ and $2.2 \times 10^{-8} \,\mathrm{M}$, respectively), indicating that the same enzyme is present in both areas, although its tissue concentration is much higher in the POA

These results indicate that the aromatase complex found in the dove brain has a high affinity for its natural substrate, testosterone and that it can be specifically induced in the POA by androgenic stimulation. An earlier study¹⁰ has suggested that

aromatase activity in the rabbit CNS may be influenced by hormonal condition. However, unlike our findings in the dove, aromatase activity was increased nonspecifically both by castration and systemic E₂ administration to intact male rabbits: testosterone had the same effect, but only when administered to intact females. The effect was not localized to a specific area of the brain known to be involved in mediating hormone action on a well-defined biological response, and there was no evidence for induction of the enzyme. Here we have demonstrated a specific induction by androgen on aromatase activity in the POA, a target area known to be associated with oestrogendependent behavioural patterns in the male dove. At this stage, we cannot exclude the possibility that systemically administered testosterone is acting via metabolites formed peripherally or centrally. The enzyme could be induced by E2 itself, because basal rates of aromatization occur in the castrate brain in the absence of androgenic stimulation (Fig. 1). Irrespective of whether testosterone or a metabolite derived from it induces the enzyme, the effect is likely to involve both binding of the active steroid to a specific 'receptor' and genomic activation resulting in de novo synthesis of the aromatase complex.

The active steroid may, however, have an indirect action. Aromatase activity is stimulated in cultured brain cells¹¹ and choriocarcinoma cells¹² by cyclic AMP, and follicle-stimulating hormone (FSH) is a well-known regulator of gonadal aromatase

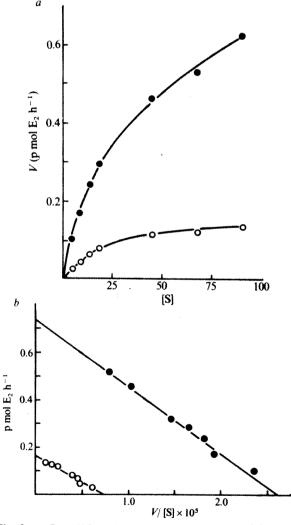


Fig. 2 a, Rate (V) against substrate concentration [S] (nM of testosterone) for aromatase activity in the POA of saline-treated (\bigcirc) or TP-treated (300 µg per day for 6 days, \blacksquare) castrates. b, Eadie–Hofstee plot of the above data, from which the following values were calculated for the apparent kinetic parameters using unweighted linear regression. Saline-treated: $K_{\rm m} = 2.75 \times 10^{-8}$ M, $V_{\rm max} = 250$ fmol h⁻¹ per mg tissue; androgen-stimulated: $K_{\rm m} = 2.04 \times 10^{-8}$ M, $V_{\rm max} = 48$ fmol h⁻¹ per mg tissue.

activity¹³. Moreover, it has been shown recently¹⁴, using Sertoli cell-enriched cultures obtained from immature rats, that some adrenergic neurotransmitter agonists, notably L-isoprenaline and noradrenaline, are as effective as FSH or dibutyryl cyclic AMP in stimulating conversion of testosterone to E_2 (ref. 14). Therefore, testosterone or its active metabolite could act by altering neurotransmitter levels in the POA. Further studies are now being carried out to elucidate the exact mechanism by which systemic TP treatment affects aromatase activity in the dove POA

We suggest that aromatase activity and the production of behaviourally effective oestrogen in the dove POA are modulated by circulating androgens. Because there is evidence for oestrogen-sensitive mechanisms associated with behaviour in the male dove, this finding is likely to be of physiological significance. Thus, oestrogen acting within the brain induces a transition from predominantly aggressive to nest-oriented

Received 20 January; accepted 18 May 1981.

- McEwen, B. S., Davis, P. G., Parsons, B. & Pfaff, D. W. A. Rev. Neurosci. 2, 65-112 (1979). Naftolin. F. & Ryan, K. J. J. Steroid Biochem. 6, 993-997 (1975). Perez-Palacios, G., Larsson, K. & Beyer, C. J. Steroid Biochem. 6, 999-1006 (1975).

- Steimer, Th. & Hutchison, J. B. Brain Res. 192, 586-591 (1980).
- Steinlet, H. & Hutchison, J. B. Jan. 11, 15-41 (1970). Hutchison, J. B. J. Reprod. Fert. 11, 15-41 (1970). Korenbrot, C. C., Schomberg, D. W. & Erickson, C. J. Endocrinology 94, 1126-1132
- 7. Hutchison, J. B. J. Endocr. 50, 97-113 (1971)
- Hutchison, J. B., Steimer, Th. & Duncan, R. J. Endocr. (in the press).

 Martinez-Vargas, M. C., Stumpf, W. E. & Sar, M. J. comp. Neurol. 167, 83-104 (1976).
- 10. Reddy, V. R., Naftolin, F. & Ryan, K. J. Endocrinology 92, 589-594 (1973).

courtship interactions with the female¹⁵. Plasma levels of testosterone reach a peak at the stage of the reproductive cycle when the behavioural transition normally occurs¹⁶. Increased aromatase activity in the POA induced by the testosterone peak may therefore mediate this transition. Moreover, oestrogen derived from testosterone in the POA, in addition to its specific effects on nest-orientated courtship, may also play a part in the regulation of testosterone dependent brain mechanisms of behaviour¹⁷. Therefore, the regulatory action exerted by androgen on aromatase activity in the POA is likely to have more than one effect on brain mechanisms involved in the integration of behaviour during courtship in the male dove.

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- Callard, G. V. Brain Res. 204, 461-464 (1981).
 Bellino, F. I., Hussa, R. O. & Osawa, Y. Steroids 32, 37-44 (1978).
 Armstrong, D. T. & Dorrington, J. H. in Regulatory Mechanisms Affecting Gonadal Hormone Action (eds Thomas, J. A. & Singhal, R. L.) 217-258 (HM & M, Aylesbury,
- Verhoeven, G., Dierickx, P. & De Moor, P. Molec. cell. Endocr. 13, 241-253 (1979).
- Hutchison, J. B. in Advances in the Study of Behaviour (eds Rosenblatt, J. F., Hinde, R. A., Shaw, F. & Beer, C.) 159-200 (Academic, New York, 1976).
- 16. Feder, H. H., Storey, A., Goodwind, D., Reboulleau, C. & Silver, R. Biol. Reprod. 16,
- Hutchison, J. B. & Steimer, Th. Science (in the press).
 Steimer, Th. & Hutchison, J. B. Brain Res. 209, 189-204 (1981).

Benzodiazepines reduce stressaugmented increase in rat urine monoamine oxidase inhibitor

Vivette Glover, S. K. Bhattacharya & M. Sandler

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London W6 0XG, UK

Sandra E. File

Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, UK

Normal human urine inhibits monoamine oxidase (MAO)1. This inhibitory activity, which is also present in rat urine, cannot be accounted for by a large range of known urinary constituents, including certain monoamines or their metabolites, but is caused by an unidentified low-molecular-weight compound(s). Endogenous MAO inhibitors may be important as physiological regulators; there have been reports of a decrease in platelet MAO activity, conceivably deriving from their presence, in a variety of human disease states2. We show here that cold immobilization stress markedly increases the output of rat urine MAO inhibitor, and that this increase is attenuated by benzodiazepine drugs.

Figure 1 shows that control urine samples from unstressed rats produced comparatively little MAO inhibition. Cold immobilization stress increased fivefold the degree of MAO inhibition induced by rat urine, with no concomitant effects on urinary pH or creatinine concentration. Pretreatment of rats with lorazepam [0.25 and 1.25 mg per kg intraperitoneally (i.p.)] gave rise to a statistically significant decrease in the concentration of stress-induced urinary MAO inhibitor. The output of inhibitor at a dosage of 1.25 mg per kg lorazepam was less than that at 0.25 mg per kg. Similarly, acute doses of chlordiazepoxide (5, 10 and 50 mg per kg) also significantly reduced the output which was less at higher dosage.

Acute administration of benzodiazepines causes sedation whereas, after chronic administration, tolerance develops. Output of inhibitor was also examined, therefore, after 5 days' administration of 10 and 50 mg per kg chlordiazepoxide and found to remain significantly less than that of the stressed group

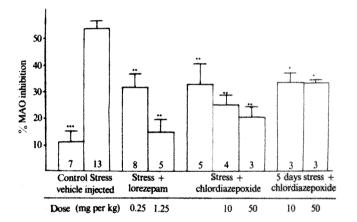


Fig. 1 Effect of cold stress in rats, with and without various benzodiazepine dosage schedules, compared with control animals. Values are means \pm s.e. No. of animals is given at the base of each bar. *** P < 0.001 compared with vehicle-injected stressed group (2-tailed Student's t-test). ** P < 0.01, different from vehicleinjected stressed group (Neuman-Keul's test). * P < 0.05, different from vehicle-injected stressed group (Neuman-Keul's test). The data from the stressed group were subjected to analysis of variance and showed a significant drug effect (F = 8.76, d.f. = 7.35); P <0.0001. The test system consisted of 100 µl phosphate buffer (100 mM, pH 7.4), 20 µl of urine, 20 µl MAO preparation and ¹⁴C-tyramine, final concentration 83 μM (for assay system, see ref. 1). All assays were carried out blind and in duplicate. Male hooded rats (Olac UK) (250-350 g) were starved overnight but allowed water. Controls were subjected to minimal handling and allowed to move freely in their cages. Stress was induced by placing rats in a restraint cage for 2 h at 4°C. Rats were decapitated with a guillotine and urine removed from the bladder by direct puncture. The urine was frozen and stored at -20 °C before assay. Drugs were given by i.p. injection immediately before restraint. Chlordiazepoxide hydrochloride (Roche) was dissolved in distilled water to various concentrations to give an injection volume of 2 ml per kg. Lorazepam (John Wyeth) was dissolved in a vehicle of 2% benzyl alcohol, 18% polyethylene glycol and 80% propylene glycol in a concentration of 4 mg ml⁻¹. It was then diluted with distilled water to give injection volumes of 2 ml per kg. Stressed but drug-free animals received equivalent vehicle or water injections. For the chronic experiments, injections were given once per day for 5 days.

Table 1 Effect of stress and benzodiazepines on pH, creatinine levels and total bladder urine

	pН	Creatinine (mmol I ⁻¹)	Total bladder urine (mg)
Unstressed control (7)	6.6 ± 0.1	4.6 ± 1.2	199 ± 30
Stressed: Vehicle-injected (13) Lorazepam (0.25 mg per kg) (8) Lorazepam	6.5 ± 0.1 6.4 ± 0.1 6.6 ± 0.2	3.4 ± 0.5 2.5 ± 0.4 3.4 ± 1.0	154 ± 30 107 ± 10 169 ± 44
(1.25 mg per kg) (5) Chlordiazepoxide (5 mg per kg) (5)	6.6 ± 0.2	2.1 ± 0.7	103 ± 13
Chlordiazepoxide (10 mg per kg) (8)	6.5 ± 0.2	2.5 ± 0.8	125 ± 18
Chlordiazepoxide (50 mg per kg) (3)	6.4 ± 0.2	2.5 ± 0.7	131 ± 31
Chlordiazepoxide (10 mg per kg× 5 days) (3)	6.8 ± 0.2	4.9 ± 0.8	159±18
Chlordiazepoxide (50 mg per kg× 5 days) (3)	6.6 ± 0.2	4.8 ± 0.6	164 ± 35

Numbers in parentheses indicate number of urine samples pooled.

(Fig. 1). Given acutely, chlordiazepoxide at 10 and 50 mg per kg caused sedation to 58% and 6% of control levels on an activity scale, whereas after 5 days the levels were 89% and 51% respectively3. These effects do not seem to correlate with inhibitor output, which remained significantly reduced after 5 days of 10 mg per kg chlordiazepoxide, suggesting that the benzodiazepine effect was not due solely to sedation.

Inhibitory potency was measured in a standard volume of urine. However, there was no significant difference in urinary pH, urine mass or creatinine concentration between the stressed and control groups, nor between stressed and drug-treated groups (Table 1). There was no correlation between any of these values and output of urinary MAO inhibitor. It therefore seems most unlikely that a difference in urinary flow could account for these findings.

Ethyl acetate extracts of acidified pooled urine samples produced results similar to those produced by unextracted urine; the stressed group exhibited more inhibition than control, with intermediate values in benzodiazepine-treated groups, but with an approximate doubling of percentage inhibition in each (Table 2). This extraction behaviour points to a compound(s) of acidic or neutral nature and indicates that it is not a monoamine. The inhibitor is not generated spontaneously; activity remains constant when fresh rat urine is left at room temperature for 24 h.

Table 2 MAO inhibition by ethyl acetate extracts

	% Inhibi	tion of MAO
	Pooled	Ethyl acetate
	urine	extract
Unstressed control (7)	17.4	47.1
Stress vehicle-injected (13)	48.2	72.4
Lorazepam (13)	29.9	63.4
Chlordiazepoxide (12)	33.9	62.3

Numbers in parentheses denote number of urine samples pooled. Controls for this experiment consisted of four Wistar and three hooded rats. Equal volumes of urine from each rat in the stress and acute drug-treated groups in Table 1 were pooled. The urinary inhibitor was extracted into ethyl acetate as follows: pooled urine samples were diluted 1 in 10 with saturated NaCl acidified to pH 0.9 with HCl. This was shaken with an equal volume of ethyl acetate and centrifuged to separate the layers. The upper layer was aspirated off, the ethyl acetate removed by passing nitrogen through it and the remaining solids taken up to the original urinary volume with 100 mM phosphate buffer, pH 7.4. Blanks, in which water replaced urine, gave no MAO inhibition.

	Table 3 MAO inhibition by carbolines						
Drug	Structure		% MAO inhibition				
		10^{-3}	10^{-4}	10^{-5}	10^{-6}M		
Ethyl β-carbo (FG 7098)	line-3-carboxylate	27.3	13.7	6.0	0.7		
	CO ₂ C ₂ H ₄						
2,9-Dimethyl carboline	1,2,3,4-tetrahydro-β	66.8	54.0	35.4	14.3		
	NCH,						
1,2,3,4-Tetrah	ydro-β-carboline	45.6	21.1	7.8	0		
	NH NH						
Ethyl 3,4-dihy 3-carboxylate	dro-1-methyl-β-carboline-	29.4	14.2	0	0		
	СО ₂ С ₂ Н,						
3,4-Dihydro-B	d-carboline-3-carboxylic acid	32.4	26.5	24.3	0.8		
	Соон						

The drugs were dissolved in distilled water and the pH adjusted to 7.0. 100 µl of each concentration was used in the reaction mixture instead of

The differing patterns of output between the groups investigated seems unlikely to reflect any variation in dietary intake. for all urine samples were obtained after an 18-22-h fast. Nor is it likely to mirror any differences in gut flora; we have found previously that the excretion of urinary inhibitor is similar in control and germ-free rats1.

It is possible that this urinary inhibitor derives from catecholamines or corticosteroids, the output of both of which is increased in this type of stress⁴. However, we have previously shown that none of the catecholamines or their major metabolites is present in human urine in sufficient concentration to account for its MAO inhibitory properties¹. In addition, there is no evidence that corticosteroids or their derivatives can inhibit MAO. We have, therefore, speculated about its possible identity in terms of a third chemical system involved in stress, possibly related to the β -carboline group of compounds⁵. Barker et al. have recently detected three tetrahydro- β -carbolines as normal constituents of rat brain and have also found particularly high concentrations in the adrenal gland. The molecular weight of the urinary inhibitor is of the appropriate order of 200 and carbolines such as harmine and harmaline are very potent MAO inhibitors⁷. Braestrup et al.⁸ have extracted a β-carboline from human urine which is a high-affinity ligand for benzodiazepine receptors. We have, therefore, tested Braestrup's compound (FG 7098) and four other structurally related compounds and found that they inhibit MAO in our test system only at concentrations much higher than the 10^{-8} M range in which FG 7098 was found in human urine (Table 3). However, Braestrup et al. indicate that the ester group of their compound was probably an artefact of the extraction procedure. As slight alterations in structure may markedly affect pharmacological properties, it remains possible that the MAO inhibitor or inhibitors we are studying are related to this compound or are endogenous compounds with a similar range of pharmacological actions.

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- Glover., V., Reveley, M. A. & Sandler, M. Biochem. Pharmac. 29, 467-470 (1980).
- Sandler, M., Reveley, M. A. & Glover, V. J. clin. Path. 34, 292-302 (1981).
- File, S. E., Mabbutt, P. S. & Pearce, J. B. Br. J. Pharmac. 73, 2280-2290 (1981)
- 4. Kvetnansky, R. in Catecholamines and Stress: Recent Advances (eds Usdin, E., Kvetnansky, R. & Kopin, I. J.) 7-18 (Elsevier, New York, 1980).

 5. Sandler, M., Glover, V., Elsworth, J. D., Lewinsohn, R. & Reveley, M. A. in Mono
- Oxidase: Structure, Function, and Altered Functions (eds Singer, T. P., von Korff, R. W. & Murphy, D. L.) 447-456 (Academic, New York, 1979).
- Barker, S. A., Harrison, R. E. W., Monti, J. A. Brown, G. B. & Christian, S. T. Biochem. Pharmac. 30, 9-27 (1981).
- Buckholtz, N. S. & Boggan, W. O. Biochem. Pharmac. 26, 1991–1996 (1977).
 Braestrup, C., Nielsen, M. & Olsen, E. Proc. natn. Acad. Sci. U.S.A. 77, 2288–2292 (1980).

Modulation of affinity of postsynaptic serotonin receptors by antidepressant drugs

G. Fillion & M. P. Fillion

Laboratory of Pharmacology, Pasteur Institute, F75724 Paris Cédex 15, France

Antidepressant drugs are generally thought to act by changing aminergic neurotransmission. Thus, according to indoleamine hypothesis1, antidepressant drugs increase the amount of serotonin (5-HT) available at the receptor level by inhibition either of 5-HT uptake or of its degradative enzyme (monoamine oxidase)2. Various experimental observations have, however, suggested that the effects of antidepressant drugs may be located at the postsynaptic receptor level³⁻⁷. We report here that antidepressant drugs alter the structural conformation of the postsynaptic serotoninergic receptor in synaptosomal membranes isolated from rat and horse brains in such a way as to inactivate them.

Serotonin postsynaptic receptors are regulated by a mechanism involving several conformational states of the recognition site for 5-HT⁸, with different affinities for 5-HT. The transition of the receptor protein from one state to another modulates the activity of the effector system, a 5-HT-sensitive adenylate cyclase^{9,10}. In the resting state, the recognition site binds 5-HT with a low affinity. Exposure to an agonist (but not to an antagonist) induces the structural transition of the receptor to an intermediate state stabilized by GTP; this state corresponds to the activation of the adenylate cyclase. The transition process ends with a final high-affinity state which can no longer activate the effector (desensitized state).

Our data consist of measurements of the affinity of ³H-5-HT for synaptic membranes pre-treated with various drugs (Fig. 1). Pre-exposure of the membranes to imipramine (10 nM) changed the affinity of the receptor for 5-HT; the affinity constant, $K_{\rm D}$, was ~12 nM in the control and ~2 nM after pre-exposure to the antidepressant (Fig. 1). This effect is comparable with that observed after pre-exposure of the membranes to 5-HT itself (Fig. 1). Imipramine is also effective at a lower concentration (1 nM) where a similar increase in affinity is observed (Fig. 2a) and in this case, the total number of binding sites is almost unchanged (<15%). The drug also produced an increase in affinity at a higher concentration (0.1 µM), but there was a marked reduction in the number of sites binding ³H-5-HT (40-60%). Note that this effect is not the result of a direct binding of the imipramine to the serotoninergic site as the $K_{\rm I}$ (10 μ M) of the drug (measured using imipramine concentrations which competitively inhibit ³H-5-HT binding) is several orders of magnitude greater than those which produce an increase in affinity. Furthermore, the presence of a competitive inhibitor would be expected to decrease the apparent affinity, not increase it as seen here.

The affinity change induced by imipramine is not observed in the presence of GTP (10^{-4} M, Fig. 2b). Other tricyclic antidepressant drugs have also been tested with similar results (see Table 1). Other non-antidepressant substances tested, chlorpromazine (10 nM and 10 µM, Fig. 2b), antiserotonins, cinan-

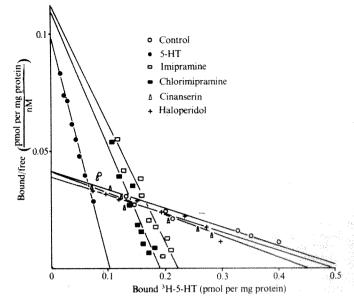


Fig. 1 Scatchard plot of ³H-5-HT binding to synaptic membranes preexposed to various drugs. Assays were done using membranes isolated from rat brains. Sprague-Dawley rats were decapitated and their brains isolated on ice and the whole forebrain dissected. These tissues were homogenized and treated as described elsewhere¹² to obtain enriched preparations of synaptosomal membranes by differential and density gradient centrifugation. Synaptosomal membranes were preincubated at 37 °C for 10 min to eliminate the endogenous 5-HT which could still be present and which could interfere with the binding of ³H-5-HT as shown previously ^{18,19}. These were then diluted 10 times in Tris-HCl buffer, 50 µM, pH 7.4, containing nialamide (100 μM) and ascorbic acid (0.1%). The membranes were then pre-exposed to 5-HT (10 nM) at 37 °C for 10 min or 15 min or to the substance being investigated at the indicated concentration (generally 10 nM); controls were incubated in buffer. The samples were washed either by centrifugation (20,000g, 10 min) and dilution in the initial volume of buffer, or by simple dilution (20-fold). Aliquots of these washed membranes were incubated at 22 °C for 8 min in the presence of various concentrations of ³H-5-HT (2-30 nM). Specific binding was determined as previously described using a rapid filtration technique. Results for membranes isolated from striatum or hippocampus of horse brain were not significantly different. In the experiment shown, rat brain membranes isolated from striatum were pre-exposed to buffer or 10 nM of the indicated drug and washed. After incubation in the presence of ${}^{3}H-5-HT$, the K_{D} observed after preincubation in buffer, 5-HT, imipramine, chlorimipramine, cinanserin and haloperidol was 12.4, 1.07, 2.08, 1.56, 12.7 and 13.5 nM, respectively

serin, methysergide, methergoline, methiothepin (10 nM), neuroleptics, haloperidol (10 nM) and fluphenazine (10 nM) had no effect.

Interestingly, the change in affinity induced by 5-HT is inhibited by methysergide (10 µM) added together with the amine during pre-exposure of the membranes; a similar inhibition of the effect of imipramine is observed in the same experimental conditions.

The change in the affinity for ³H-5-HT induced by preexposure of the membranes to 5-HT itself occurs in rat brain synaptosomal preparations and in membranes isolated from striatum8 or from hippocampus. This phenomenon seems to be widespread for serotoninergic sites as we also observed it in membranes isolated from horse brain (striatum and hippocampus). As we have previously shown¹¹, these sites are located on postsynaptic membranes. The affinity constant observed for 5-HT binding to membranes which had not been pre-exposed is ~15 nM, whereas membranes pre-exposed to 5-HT and washed bind 5-HT with a higher affinity $(K_D = 1.8 \pm 0.4 \text{ nM})$. This increase in affinity is induced by serotoninergic agonists and not by antagonists. As previously reported^{9,10}, these affinity changes might correspond to structural changes of the receptor protein as they do not occur after pretreatment of the membranes with a sulphydryl group reagent.

In experimental conditions which produce a high-affinity binding for 5-HT, no adenylate cyclase activation is observed, whereas in the presence of GTP (or GppNHp) the low-affinity binding is stabilized and adenylate cyclase activation occurs^{9,10}. These observations and other information on binding kinetics

Fluphenazine

Haloperidol

Table 1 Affinity constant of ³ H-5-	HT binding to synaptic membranes			
Pre-exposure	$K_{\mathbf{D}}$ (nM)			
None	15.3 ± 1.26 (14)			
Serotoninergic agonists				
5-HT	1.8 ± 0.42 (8)			
Bufotenine	3.23			
5-MeO-N,N-dimethyltryptamine	2.08			
Tricyclic antidepressants				
Imipramine	2.42 ± 0.22 (6)			
Chlorimipramine	1.72 ± 0.99 (3)			
Amitriptyline	4.2 ± 0.35 (3)			
Trimeprimine	$4.0 \pm 0.3 (3)$			
Desipramine	4.9 ± 0.69 (3)			
Doxepin	4.5			
Atypical antidepressants				
Iprindole	3.9 ± 1.02 (3)			
Mianserine	2.9 ± 0.88 (3)			
Fluoxetine	2.45 ± 0.22 (3)			
Fenfluramine	2.1			
Serotoninergic antagonists				
Methysergide	12.43 ± 0.48 (3)			
Methergoline	9.8			
Cinanserin	12.7			
Methiothepin	$15.0 \pm 2.9 (3)$			
Other drugs	*			
Chlorpromazine	14.24 ± 1.95 (5)			

Affinity changes of 3 H-5-HT binding induced by various drugs. Membranes were pre-exposed to the drug being investigated (10 nM) at 37 °C for 10 min, diluted 20-fold in Tris-HCl buffer (50 mM, pH 7.4) and incubated at 22 °C for 8 min, in the presence of various concentrations of 3 H-5-HT. The radioactivity specifically bound was determined by a rapid filtration technique. The affinity constants are the means \pm s.e. of 3-6 assays. For the drugs tested twice only, the values observed for individual assays did not differ by >30% of the mean.

 10.7 ± 1.9 (3)

led us to propose the regulatory mechanism summarized above.

All the drugs tested which have clinical properties of antidepressants, except the group of the inhibitors of monoamine oxidase (IMAO), are able to increase the affinity of the 5-HT receptor for the amine itself. Active drugs include tricyclic antidepressants (imipramine, chlorimipramine, amytriptyline, trimeprimine, desipramine) which are also inhibitors of 5-HT or noradrenaline (NA) uptake, other antidepressants which are only weak inhibitors of amine uptake (iprindol or mianserin) and doxepin which is considered to be a weak antidepressant. The changes in affinity induced by these drugs do not therefore seem to be related to their capacity to inhibit the uptake of 5-HT or NA. Furthermore, many of the drugs, are used at concentrations which have no effect on the monoamine uptake¹². The clinical properties of fluoxetine and fenfluramine as antidepressants have not been established.

Note that the antidepressants of the IMAO group are ineffective. They are known to act on the degradative enzyme of 5-HT, resulting in an increase in the availability of 5-HT in the synaptic cleft. These drugs do not increase the affinity of the binding site for ³H-5-HT. The assay could be performed in the presence or absence of 100 μ M nialamide without any alteration to the ability of the binding site to be either in a conformational state corresponding to a high or a low affinity for ³H-5-HT.

Chlorpromazine, which is not considered to possess true antidepressant properties although it has a chemical structure very like that of imipramine, is unable to increase the affinity of the 5-HT receptor for its ligand even at concentrations up to 10^{-6} M; haloperidol and fluphenazine, neuroleptic drugs which lack antidepressant properties, are also ineffective.

Thus, this effect of the antidepressant drugs on the post-synaptic serotoninergic receptor seems to be specific. It does not occur directly at the serotoninergic site by competition with 5-HT as the affinity constants of these drugs for the 5-HT site correspond generally to a rather low affinity in the range $1-10\,\mu\text{M}^{13}$. Several hypotheses might be proposed to explain this effect. The antidepressant drugs might act by binding to a specific site corresponding to the existence of an endogenous imipramine-like ligand. In that case, the occupation of the site would modulate the conformation of the serotoninergic recep-

tors possibly through an allosteric process. Binding sites for ³H-imipramine have already been described in synaptosomal membranes from rat brain¹⁴. On the other hand, these drugs might act by changing some physical characteristics of the membranes in a manner analogous to that described by Hirata and Axelrod¹⁵ in the close vicinity of the serotoninergic receptor.

The drugs which induce the transition of the site to high affinity are either 5-HT agonists or antidepressants. We have shown that the binding of 5-HT to this conformation of the site is unable to activate the adenylate cyclase and therefore produces a desensitized state of the receptors; antidepressant drugs thus

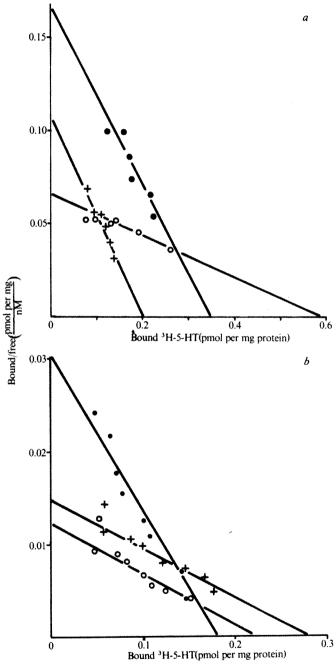


Fig. 2 a, Effect of the pre-exposure of membranes to imipramine on the binding of ${}^{3}\text{H-5-HT}$ to its specific sites. Scatchard plot of the binding curves for membranes not pre-exposed to imipramine (control, \bigcirc); and pre-exposed to 1 nM (+) and 10 nM (\bigcirc) imipramine. Experimental conditions were the same as for Fig. 1. The affinity constants for ${}^{3}\text{H-5-HT}$ were 11, 2.2 and 2.04 nM for control, 1 and 10 nM imipramine, respectively. b, Lack of effect of imipramine pre-exposure on purified synaptosome membranes treated with GTP. The same experimental conditions were used as for Fig. 1 except that the test membrane preparation contained GTP during pre-exposure to imipramine. The corresponding affinity constants were: $K_D = 5$, 19.1 and 17.2 nM after pre-exposure of the membranes to buffer (control, \bigcirc), imipramine (10 nM, \bigcirc) and imipramine + GTP (10 μ M, +), respectively.

seem to inactivate the postsynaptic 5-HT receptor. Because drugs which lack antidepressant properties do not have this capacity, we suggest that the clinical properties of tricyclic antidepressants are related to their capacity to desensitize the postsynaptic 5-HT receptor.

The structural changes induced by tricyclic antidepressants might represent a critical step in the receptor modifications observed after long-term administration^{7,16,17}. Our results support new concepts of the mechanism of action of antidepressants and indicate that the clinical properties of these drugs might involve an effect on the postsynaptic 5-HT receptor.

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- van Praag, H. M. Pharmacopsychiatry 7, 281-292 (1974). Sulser, F. Trends pharmac. Sci. Rev. 1, 92-94 (1979).

- Suiset, F. Irenas pratriae: Sci. Rev. 1, 72-74 (1975).
 Takahashi, R. et al. Folia Psychiat. neurol, jap. 30, 207 (1976).
 Aprison, M., Takahashi, R. & Tachiki, K. in Neuropharmacology and Behaviour (eds Haber, B. & Aprison, M. H.) 23 (Plenum, New York, 1978). 5. Tachiki, K. et al. Biochem. Psychiat. 13, 429 (1978).

- Jacoms, N. et al. Biocnem. 139cmai. 13, 427 (1270). Jones, R. & Roberts, M. Br. J. Pharmac. 65, 501-510 (1979). Segawa, T., Mizuta, T. & Nomura, Y. Eur. J. Pharmac. 58, 75-83 (1979).
- Fillion, G., Fillion, M. P. Eur. J. Pharmac. 65, 109-112 (1980).
 Fillion, G., Fillion, M. P. & Rousselle, J. C. Neurosci. Lett. 5, 869 (1980).
 Fillion, G., Fillion, M. P. & Rousselle, J. C. J. Physiol., Paris (in the press).
 Fillion, G. et al. J. Neurochem. 33, 567-570 (1979).

- Shaskan, E. & Snyder, S. J. Pharmac. exp. Ther. 175, 404-418 (1970).
 Fillion, G. et al. Molec. Pharmac. 14, 50-59 (1978).
- Raisman, R., Briley, M. & Langer, S. Eur. J. Pharmac. 61, 373 (1980).
 Hirata, F. & Axelrod, J. Nature 275, 219-220 (1978).
- de Montigny, C. & Aghajanian, G. Science 202, 1303 (1978). Peroutka, A. & Snyder, S. Science 210, 88-90 (1980).
- Nelson, D. L., Herbet, A., Bourgoin, S., Glowinski, J. & Hamon, M. Molec. Pharmac. 14, 983-995 (1978).
- 19. Peroutka, A. & Snyder, S. Molec. Pharmac. 16, 687-699 (1979)

Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour

William J. Freed, John M. Morihisa, Eleanor Spoor, Barry J. Hoffer*, Lars Olson†, Ake Seiger† & Richard Jed Wyatt

Adult Psychiatry Branch, Division of Special Mental Health Research, Intramural Research Program, National Institute of Mental Health, Washington DC, 20032, USA

Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA

† Department of Histology, Karolinska Institute 10401, Stockholm, Sweden

Rats with unilateral lesions of substantia nigra pars compacta (SN), the area of the brain containing most dopamine-containing neurones, are a widely recognized animal model of Parkinson's disease¹⁻⁴. When given dopamine agonists such as apomorphine, such rats rotate in a direction contralateral to the lesion, presumably because of the development of supersensitive dopamine receptors in the striatum ipsilateral to the lesion. When grafts of embryonic SN are placed in the lateral ventricle^{5,6}, or into a transplant cavity⁷ adjacent to the striatum in animals with SN lesions, this rotational behaviour has been shown to decrease. Histochemical examinations have shown that axons from the grafts have grown into the striatum⁵⁻⁷, and biochemical measurements indicate that dopamine concentrations are increased in areas of the striatum adjacent to the SN grafts6. Nevertheless, an obvious problem with this technique, both for basic research and possible clinical applications, is the requirement for fetal central nervous donor tissue. We now describe how grafts of adrenal medulla can be used with similar effects, involving chromaffin cells.

There are several reasons for considering the adrenal medulla as a potential replacement for fetal SN grafts. First, the normal

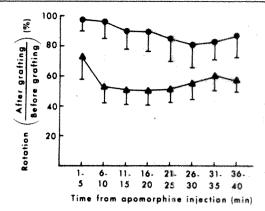


Fig. 1 Contralateral apomorphine-induced turning after grafting. expressed as a percentage of turning before grafting, is shown as a function of time after apomorphine injections. To derive these data, the 40-min testing sessions were divided into eight 5-min segments. For each of these segments, a mean pregrafting rotation rate was determined, and the rotation rate after grafting was expressed as a percentage of this pregrafting rotation rate. Triangles indicate animals that received adrenal medulla grafts (n = 13); circles indicate control animals that received sciatic nerve grafts (n = 15). Vertical lines indicate s.e.m. A two-way analysis of variance for one repeated measure showed a significant main effect of treatment (adrenal versus sciatic nerve grafts [F(91,26) = 4.40]; P = 0.043]. The main effect of measures and measures × treatment interactions were not significant (F(7.182) = 1.71; P = 0.109) and (F(7,182) = 0.81; P = 0.417, respectively].

adrenal medulla produces dopamine as an intermediary in the synthesis of adrenaline^{8,9}. Second, adrenal chromaffin cells, which are normally rounded in shape, become angular and develop processes when grown as grafts in the anterior eye chamber¹⁰⁻¹² or when grown in culture in the absence of corticosteriods¹³⁻¹⁵. Finally, processes originating from intraocular adrenal medulla grafts can innervate intraocular grafts of cerebral cortex, as demonstrated by histochemical studies14

Unilateral SN lesions were produced^{5,6} in male Sprague-Dawley rats by stereotaxic injections of 6-hydroxydopamine hydrobromide into the right SN. Approximately 2 months later, the animals were tested for apomorphine-induced (0.1 and 0.25 mg per kg subcutaneously) rotational behaviour using an automated apparatus. The smaller of the two doses that produced at least 80 counterclockwise rotations in 40 min was used for each animal. Animals turning at less than this rate were not used. After the initial screening, rotational behaviour was re-examined for five to seven additional sessions to obtain a stable baseline for each rat.

Adrenal glands were removed from young adult Sprague-Dawley rats (125-200 g) and the medulla dissected free. The dissected medulla was then bathed in sterile lactated Ringers at room temperature. A cut was made in the surface of the medulla to enable it to be opened, and the interior was removed. This method was used to avoid inclusion of the adrenal cortex in the graft. Four to six grafts were implanted in the lateral ventricle on the 6-hydroxydopamine-lesioned side in each of 13 rats (age at least 5 months) using Pellegrino and Cushman¹⁷ coordinates of 1.5 mm lateral and 1.5 anterior to bregma, 3.5-4.0 mm below the dura, as previously described. Control (n = 15) animals received grafts of sciatic nerve instead of adrenal medulla.

Adrenal medulla (n = 13) or sciatic nerve (control) grafts (n = 15) were implanted and 2 months allowed for survival and growth of the graft tissue. At this time, rotational behaviour was examined for five 40-min sessions, and some of the animals killed for histochemical studies.

When the rats were tested 2 months after transplantation, rotational behaviour was significantly less in the animals with adrenal medulla grafts than in those with sciatic nerve grafts (Fig. 1). Histochemical fluorescence studies of six randomly selected rats showed the denervations of the caudate nucleus to

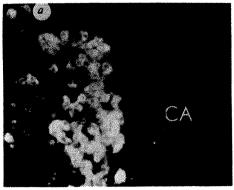




Fig. 2 Falck-Hillarp fluorescence microscopy of intraventricular adrenal medulary grafts. a, Overview of cluster of strongly fluorescent chromaffin cells within a graft. Some cells are elongated or polygonal with short coarse processes (upper left), but very few thin nerve fibre-like processes have formed (CA, caudate). ×160. b, Close-up of two chromaffin cell clusters. Three nuclei are seen in the lower cluster: one cell has a tapering nerve fibre-like process (arrow). ×290.

be essentially complete, with only a few fine terminals remaining in one rat. Grafts with catecholamine-containing chromaffin cells were found in five of the six animals. As shown in Fig. 2, these cells had developed polygonal shapes and were usually grouped in one or more small clusters within the grafts. Two types of fluorescent cells were found in the adrenal grafts, similar to the noradrenaline (very strongly fluorescent) and adrenaline (moderately fluorescent) cells present in normal adrenal medulla^{21,22}. Some of these cells had fine elongated processes, but very few fibres were seen to leave the grafts and enter the host brains. The total numbers of chromaffin cells found in the grafts counted by methods previously described⁵, were 880, 4,080, 66, 517 and 2,134, respectively, for the five animals. Adrenal cortex was also found in the graft that contained 4,080 chromaffin cells. Rotational behaviour was not reduced in this animal, nor in the animal that had only 66 chromaffin cells.

The observations suggest that adrenal medulla grafts can reduce lesion-induced rotational behaviour, even though there was no clear evidence that the grafts actually reinnervated the host caudate nucleus. Although the transplanted chromaffin cells were elongated and produced fine fibres, these fibres were found almost entirely within the grafts, and rarely penetrated into the caudate nucleus. This suggests that catecholamines or other substances diffused from the grafts to receptor sites in the caudate nucleus in sufficient quantity to reduce caudate dopaminergic supersensitivity and consequently, apomorphineinduced rotation.

Adult adrenal medullary cells show a remarkable phenotypic plasticity in response to environmental changes. Thus, when pieces of adrenal medulla are grafted into the anterior chamber of the eye, chromaffin cells will migrate into the sympathetically denervated host iris and form catecholamine-containing nerve fibres that reinnervate the iris10. Tissue culture experiments show that chromaffin cells of rodents and man express the endocrine phenotype in the presence of glucocorticoids and the sympathetic adrenergic neurone phenotype in the absence of glucocorticoids and the presence of nerve growth factor (NGF)^{12-15,23}. Thus, histochemical changes in grafted adrenal medullary tissue in the eye may be explained by the removal of the normal adrenal cortical environment and increased amounts of NGF in the sympathetically denervated host iris (see ref. 24). We have recently shown that chromaffin cells grafted into the eye chamber can also innervate adjacent grafts of brain tissue16. In this case, fine varicose fibres, typical of telencephalic adrenergic afferents, were found. In contrast to rat chromaffin cells, bovine chromaffin cells show little or no evidence of fibre

production when cultured or transplanted to the anterior eye chamber or brain 15,25

Although the results reported here clearly demonstrate longterm survival of grafted chromaffin cells and reduction of apomorphine-induced rotational behaviour, there was only partial transformation of chromaffin cells towards the neuronal phenotype. Brain tissue does not seem to contain or produce any appreciable amount of NGF^{26,27}. Therefore, these results agree well with the findings from tissue culture that chromaffin cells will survive and produce small numbers of nerve fibres without NGF, but require NGF for extensive fibre formation (see refs. 15,23). Because of the sparsity of innervation of host caudate, the present behavioural results are probably best explained by a release of catecholamines from the grafts and subsequent diffusion of the amines to supersensitive dopamine receptors. Fluorescence microscopy demonstrated that both noradrenaline and adrenaline cells were present in at least some of the grafts. although the proportion of noradrenaline cells seemed higher in grafts than in normal adrenal medulla. The relative role of dopamine, noradrenaline and adrenaline and other possible neurotransmitters or peptides released by the grafts, such as enkephalin²⁸⁻³⁰, remains to be determined. Experiments are in progress to measure the concentrations of various catecholamines in the graft and adjacent host brain. Conceivably, release might also be influenced by any fibres from the host brain which innervate the grafted tissue, analogous to the normal regulation of medullary catecholamine release by cholinergic afferent input. A further question would be the presence of all-or-none action potentials and impulse-dependent transmitter release. Action potentials have been reported in the NGF-transformed phaeochromocytoma line PC-12 Greene³¹

Although many questions remain unanswered, the data reported here suggest that it may be possible to use adrenal medulla in place of fetal SN as a source of tissue for catecholamine-containing grafts. If further studies are successful, peripheral autografts could be potential replacements for destroyed or damaged central neurones. We are now atempting to produce adrenal autografts in subhuman primates and to develop protocols to increase fibre ingrowth from adrenal grafts to the caudate nucleus.

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- 1. Andén, N. E. in Advances in Parkinsonism (eds Birkmayer, W. & Hornikiewicz, O.)
- 169-177 (Hoffman-LaRoche & Co. Ltd. Basel, 1975). Ungerstedt, U. in *The Neurosciences Third Study Program* (eds Schmitt, F. O. & Worden,
- Ongerstedt, U. 11 The Neurosciences I thra study Program less Schimit, P. O. & F. G.) 695-703. (MIT Press, Cambridge, 1974).
 Glick, S. D., Jerussi, T. P. & Fleisher, L. N. Life Sci. 18, 889-896 (1976).
 Costall, B., Naylor, R. J. & Pycock, C. J. Pharm. Pharmac. 27, 943-946 (1975).
 Perlow, M. J. et al. Science 204, 643-647 (1979).
- Freed. W. J. et al. Ann. Neurol. 8, 510-519 (1980)
- Bjorklund, A., Dunnett, S. B., Stenevi, U., Lewis, M. E. & Iverson, C. D. Brain Res. 199, 307-333 (1980).
- Molinoff, P. B. & Axelrod, J. A. Rev. Biochem. 40, 465-500 (1971)
- Blaschko, H. & Muscholl, E. Handb. Exp. Pharmac. 33, (1972). Olson, L. Histochemie 22, 1-7 (1970).
- 11. Olson, L., Seiger, A., Ebendahl, T. & Hoffer, B. Adv. Biochem. Pharmac. 25, 27-34 (1980).
- Unsiker, K., Tschechne, B. & Tschechne, D. Brain Res. 152, 334-340 (1978).
 Unsicker, K., Krisch, B., Otten, U. & Thoenen, H. Proc natn. Acad. Sci. U.S.A. 75, 3498-3502 (1978).
- 14. Unsicker, K. & Chamley, J. H. Cell Tissue Res. 177, 247-268 (1977)
- Unsicker, K., Rieffert, B. & Ziegler, W. Adv. Biochem. Pharmac., 25, 51-59 (1980).
 Olson, L., Seiger, A., Freedman, R. & Hoffer, B. Expl Neurol. 70, 414-426 (1980).
- 17. Pellegrino, L. J. & Cushman, A. J. A Stereotaxic Atlas of the Rat Brain (Meredith, New York, 1967).
- Corrodi, H. & Jonsson, G. J. Histochem. Cytochem. 15, 65-78 (1967).
 Falck, B., Hillarp, N.-A., Thieme, G. & Torp, A. J. Histochem. Cytochem. 10, 348-354
- Olson, L. & Ungerstedt, U. Histochemie 22, 8-19 (1970). Piezzi, R. S. & Cavicchia, J. C. Anat. Rec. 175, 77-86 (1973)
- Wood, J. G. & Barnett, R. J. Anat. Rec. 145, 301-301 (1963)
- Tischler, A. S. & Greene, L. A. Adv. Biochem. Pharmac. 25, 61-68 (1980).
 Ebendal, T., Olson, L., Seiger, A. & Hedlund, K. O. Nature 286, 25-28 (1980).
 Perlow, M. J., Kumakura, K. & Guidotti, A. Proc. natn. Acad. Sci. U.S.A. 77, 5278-5281
- 26. Olson, L., Ebendal, T. & Seiger, A. Devl Neurosci. 2, 160-176 (179)
- Freed, W. J. Brain Res. Bull. 1, 393-412 (1976).

- Freeu, W. J. Brain Res. Batt. 1, 393-412 (1970).
 Lundberg, J. M. et al. Proc. natn. Acad. Sci. U.S.A. 76, 4079-4083 (1979).
 Yang, H.-Y. T., Hexum, T. & Costa, E. Life Sci. 27, 1119-1125 (1980).
 Viveros, O. H., Dilberto, E. J., Hazum, E. & Chang, K.-J. Molec. Pharmac. 16, 1101-1108
- 31. Greene, L. A. Adv. Pharmac. Ther. 10, 197-206 (1978).

Immunological enhancement of tumour allografts following treatment of mice with TNP-conjugated alloantigen and anti-TNP antibody

L. V. Hutchinson & L. Brent

Department of Immunology, St Mary's Hospital Medical School, London W2 1PG, UK

Immunological enhancement—the prolonged survival of tumour or organ allografts brought about by passive immunization of the host with alloantibody directed against the graft's antigens—has not yet been applied successfully to clinical organ transplantation. There are many reasons for this, among them the difficulty of preparing the required battery of alloantisers and the danger of causing hyperacute rejection of kidney allografts. We show here that it should be possible to circumvent both these problems by using a xenogeneic antibody directed against a well defined hapten rather than against histocompatibility antigens.

The mechanism of immunological enhancement is still a matter for debate¹⁻⁵. One hypothesis advanced to account for antibody-mediated suppression of specific immune responses is that graft antigen and anti-graft antibody combine to form complexes which are bound specifically by the antigen receptors of lymphocytes, by virtue of free antigenic determinants. In this way, antigen-specific cells (ARCs) of the recipient are coated with antibody indirectly bound to the lymphocyte membrane through an antigen bridge. It is postulated that these opsonized ARCs are subsequently destroyed by Fc-receptor-bearing host cells such as macrophages⁶⁻⁹. The most direct evidence for the hypothesis of ARC opsonization (ARCO) (reviewed in ref. 4) comes from experiments in which syngeneic radiolabelled ARCs were taken up by the reticuloendothelial system when injected intravenously (i.v.) into rats bearing enhanced kidney transplants^{6,7} or mice bearing enhanced skin allografts¹⁰. This is strongly supported by experiments in which mice were pretreated with complexes between sheep red blood cells (SRBCs) and anti-SRBC antibodys or with allogeneic cells and alloantibody9.

Following the ARCO hypothesis, one possible prediction is that the antigenic determinant in the complex bound by receptors of ARCs need not be identical with that reacting with the

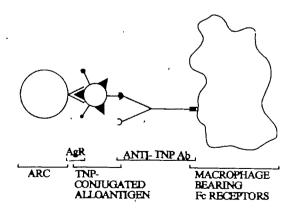


Fig. 1 The concept of opsonization of alloreactive ARCs by TNP-conjugated alloantigen and anti-TNP antibody. Free alloantigens determinants (A), in complexes formed between TNP-conjugated alloantigens (where ♥ signifies TNP determinant) and anti-TNP antibody, are specifically bound by the antigen receptor (AgR) on the antigen-reactive cells (ARCs) In this way ARCs are opsonized by antibody indirectly bound to the cell surface through an antigen bridge, to be destroyed by Fc -receptor-bearing host cells.

Table 1 a, Enhancement of P815 (H-2*) tumour in A strain (H-2*) mice by anti-H-2* serum; b, enhancement of EL-4 (H-2*) tumour in A strain mice by anti-H-2* serum

Serum (μJ)	MTA±s e.(mm²)	P
a None	28.0±3.4	
10	66 4±8.8	< 0 01
25	84.0 ± 11.4	< 0 01
50	58.6 ± 7.0	< 0.01
100	40.7 ± 3.0	< 0.05
b None	34.7 ± 1.1	
12.5	69.2 ± 7.7	< 0 01
25	87.5±90	< 0 001
50	76.1 ± 11.6	< 0.01

MTA, mean tumour area estimated on day 7. Values are for groups of six mice

enhancing antibody (Fig. 1). It has indeed been shown that, to achieve the most efficient opsonization of ARCs, the ARC receptors and the antibody should kind to different determinants in the complex9, thus avoiding blocking of the determinants required for the linkage to cell receptors. Sinclair and Law11 have recently shown that complexes formed between trinitrophenyl (TNP)-conjugated alloantigen and anti-TNP antibody can suppress in vitro alloimmune responses, and that anti-TNP antibody enchances the in vivo growth of TNPconjugated allogeneic tumour cells. We too have found that alloantigens coupled to TNP retain alloantigenic activity, that they can be specifically bound by alloreactive ARCs, and that ARCs which have bound TNP-alloantigen/anti-TNP antibody complexes are specifically diverted to the liver when injected i.v. into normal mice12. Our present experiments confirm that anti-TNP antibody enhances TNP-conjugated tumour allografts and we have extended these observations by showing that complexes between TNP-conjugated alloantigen and anti-TNP alloantibody specifically enhance the growth of unmodified tumour cells.

The mice used were from inbred strains BALB/c (H-2^d). DBA/2 (H-2 $^{\circ}$), C57BL/6 (H-2 $^{\circ}$) and A/J (H-2 $^{\circ}$) bred by the Animal Department of St Mary's Hospital Medical School, London. They were 6-10 weeks old at the start of each experiment. Anti-H-2^d and anti-H-2^b allogntisers were raised by injecting five doses of 2×10^7 allogeneic (BALB/c or C57BL/6) spleen cells intraperitoneally (i.p.) into female A strain mice at 2-week intervals. Anti-TNP serum was produced in female New Zealand White rabbits by the injection of 500 µg TNP-KLH (keyhole limpet haemocyanin) antigen in complete Freund's adjuvant on days 0 and 21, followed by i.v. booster inoculi of 1 mg of the same antigen in saline on days 45 and 59. The rabbits were bled on day 70 and the freshly prepared sera were stored at -20 °C. P815 mastocytoma and EL-← leukaemia cells were maintained by i.p. passage in DBA/2 and C57BL/6 mice, respectively. Conjugation of cells with TNP¹³ was achieved by incubating splenic lymphocytes or tumour cells at a concentration of 2.5×10⁴ per ml in 10 mM trinitrobenzene sulphonic acid (TNBS, Sigma) dissolved in phosphate-buffered saline (PBS), pH 7.2, at 37 °C for 10 min. The cells had been washed in PBS before incubation and were washed four times after incubation. The cell membrane antigen was prepared by a modification of the technique described by Standring and

Table 2 Enhancement of P815 tumour cells by TNP-BALB/c antigen and anti-TNP serum

TNP-BALB/c antigen (spleen cell equivalents)	Anti-TNP serum (ml)	MTA±s.c. (mm²)	P
None	None	33.1 ± 53	
3.2×10^7	0.32	81.5 ± 7.0	< 0.001
10 ⁷	0.1	59.1 ± 9.6	< 0.05
3.2 ± 10^{4}	0.032	53.1±9.0	NS
10 ⁴	0.01	51.6 ± 7.0	NS

MTA, mean tumour area estimated on day 7. Values are for groups of five mice NS, not significant.

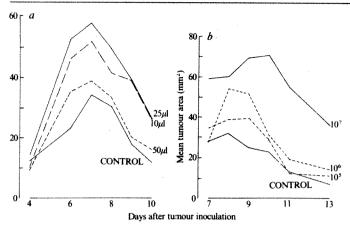


Fig. 2 a, Enhancement of TNP-conjugated tumour by anti-TNP serum. P815 mastocytoma cells (H-2^d) were hapten-conjugated by incubation in 10 mM TNBS (see text). 10⁷ viable TNP-P815 cells were injected s.c. into A strain (H-2^a) mice and 0-50 μl rabbit anti-TNP-KLH serum was injected i.p. Control mice received no serum. Tumour size was measured at intervals after inoculation and the mean tumour area (MTA) calculated as described in the text. b, Enhancement of tumour allografts by TNP-conjugated alloantigen and anti-TNP serum. 10⁷ viable P815 mastocytoma cells (H-2^d) were injected s.c. into A strain (H-2^a) mice. TNP-conjugated BALB/c (H-2^d) cell membrane antigen (see text), derived from 10⁵-10⁷ cells, was injected i.v. and 0.1 ml rabbit anti-TNP-KLH serum was given i.p., both at the time of tumour inoculation. Control mice received no treatment. Tumour size was measured on days 7-13 and the MTA calculated.

Williams 14 . Thus, TNP-conjugated or unmodified spleen cells were pelleted, resuspended in 2% Tween 40 (Sigma) in 0.1 M Tris-HCl buffered saline (pH 8.0) and stirred at 0 °C for 60 min. The cells were then homogenized by 20 strokes of a Dounce glass homogenizer and the suspension was centrifuged at 2,000g for 15 min. The supernatant was centrifuged again, this time at 100,000g for 45 min, and the sedimented membrane material was resuspended in PBS and stored in aliquots at -80 °C.

The experimental design was as follows. 10^7 tumour cells in 0.1 ml balanced salt solution (BSS) containing 10% fetal calf serum (FCS) were injected subcutaneously (s.c.) into A strain mice on day 0. Different doses of antigen $(0-10^8$ cell equivalents) and alloantiserum or anti-TNP serum (0-0.5 ml) were injected i.v. and i.p., respectively, within 1 h of tumour cell inoculation. The resulting tumours were measured from day 6 onwards. The diameter (d) of the tumours was measured in two planes at right angles to each other and the tumour surface area (A) was calculated according to the formula: $A = \pi(d_1 \times d_2)/4$. The statistical significance of the data was assessed using Student's t-test.

A-anti-BALB/c (anti-H- 2^d) serum enhanced the growth of the P815 (H- 2^d) tumour in A strain (H- 2^a) mice (Table 1a). The optimal effect was obtained using 25 μ l per mouse; presumably, higher doses were cytotoxic to the grafted cells (see below). Similar results were obtained using the EL-4 (H- 2^b) tumour cells and an anti-C57BL/6 (H- 2^b) serum (Table 1b). The enhanced growth was clearly strain specific in that A-anti-BALB/c serum did not enhance the growth of EL-4 and anti-C57BL/6 serum did not enhance the growth of the P815 tumour (results not shown).

Similarly, TNP-antiserum enhanced the growth of TNP-conjugated tumour cells (TNP-P815; Fig. 2a), thus confirming the work of Sinclair and Law¹¹. Again, 25 μ l proved to be optimal although 10 μ l were here almost as effective; 50 μ l had no

Table 3 Effect of TNP-BALB/c antigen on P815 tumour growth in A strain mice

MTA ± s.e. (mm ²)
24.6 ± 5.3
23.7 ± 6.7
23.8 ± 2.4
29.7 ± 6.7
23.1 ± 4.9

MTA, mean tumour area estimated on day 8. Values are for groups of six mice.

significant effect. TNP-P815 cells are, in fact, readily lysed in vitro by anti-TNP serum and complement (I.V.H., unpublished), a finding that almost certainly accounts for the lack of success with the higher dose.

Figure 2b shows that unmodified P815 tumour implants were enhanced through the use of TNP-BALB/c membrane antigen and anti-TNP antibody. Strain A mice were given 10⁷ unmodified tumour cells s.c., 0.1 ml anti-TNP serum i.p., and TNP-modified cell membrane antigen at 10^5-10^7 cell equivalents per mouse i.v. Tumour growth was considerably enhanced in animals treated with TNP-conjugated membranes obtained from 10⁷ cells and with 0.1 ml antiserum, whereas doses representing 105 or 106 cells were less effective. When the dose of antigen and antiserum was increased whilst maintaining the ratio of 107 cell equivalents to 0.1 ml serum, the level of enhancement was correspondingly greater (Table 2). TNP-BALB/c cell membrane alone, at doses of 10⁵-10⁸ cell equivalents, had no effect (Table 3). Finally, it was shown that the enhanced state was strain specific. Strain A mice treated with TNP-BALB/c (H-2^d) or TNP-C57BL/6 (H-2^b) cell membrane antigen and anti-TNP serum were inoculated with P815 (H-2d) or EL-4 (H-2b) tumour cells. TNP-BALB/c membrane enhanced P815 but not EL-4 and, conversely, TNP-C57BL/6 membrane enhanced EL-4 but not P815 (Table 4).

The data therefore show that anti-TNP antibody enhances the growth of TNP-conjugated tumour cells, thus confirming the finding by Sinclair and Law¹¹, and further, that it enhances the

Table 4 Specificity of tumour enhancement by TNP-conjugated antigen and anti-TNP serum

	P815 tur	nour	EL-4 tumour		
Treatment	MTA±s.e. (mm²)	P	MTA±s.e. (mm²)	P	
Control	16.9 ± 2.2		34.7 ± 1.1		
TNP-BALB/c antigen	18.4 ± 2.8	NS	40.3 ± 4.9	NS	
TNP-C57BL/6 antigen	20.2 ± 4.0	NS	29.5 ± 3.1	NS	
Anti-TNP serum TNP-BALB/c antigen	18.2 ± 3.8	NS	33.3 ± 6.8	NS	
+ anti-TNP serum TNP-C57BL/6 antigen	49.5 ± 7.1	< 0.01	39.2 ± 3.5	NS	
+anti-TNP serum	16.3 ± 3.5	NS	66.8 ± 3.9	< 0.001	

Tumour allograft recipients were injected i.v. with cell membrane antigen derived from 10⁷ TNP-conjugated BALB/c or C57BL/6 cells with or without 0.1 ml rabbit anti-TNP-KLH serum i.p. Mice received 10⁷ tumour cells s.c. MTA, mean tumour area estimated on day 7. Values are for groups of 7-10 mice.

growth of an unmodified tumour allograft, the enhancement being specific and mediated by TNP-alloantigen/anti-TNP antibody complexes. Because the mice were injected only once with antigen and antibody it is perhaps not surprising that growth of the enhanced tumours was generally not progressive. The proposed mechanism of this enhancement has been outlined above. Although it is possible that more than one mechanism is operative in immunological enhancement, there is substantial evidence for the participation of antigen-reactive cell opsonization by antigen-antibody complexes⁴. We have, furthermore, found that TNP-modified H-2 antigens bind specifically to lymphocytes activated specifically in mixed lymphocyte culture and that ARCs to which complexes have been bound are taken up promptly by the reticuloendothelial system when injected i.v. into normal mice¹². The experiments reported here establish the direct immunosuppressive action of such complexes in vivo.

These findings may have special significance in two fields—in tumour immunology and in organ transplantation. Tumours often express neo-determinants that are either products of their own disturbed genome or of viral origin. The experiments making use of TNP-modified antigen provide an experimental model for the latter, the hapten taking the place of virally derived determinants. The ARCO phenomenon has already been illustrated in a syngeneic tumour system, using a spontaneous reticulum cell sarcoma (RCS) in SJL/J mice^{15,16}.

Although the viral aetiology of the RCS is uncertain it is possible that antibodies against viral products were involved.

As far as tissue and organ transplantation is concerned, preliminary data indicate that the survival of normal allografts, too, can be prolonged by the administration of TNP-modified donor antigen and anti-TNP antibody (I.V.H., unpublished). This approach may be indispensable if enhancement is to be attempted in clinical organ transplantation, for the preparation of the donor membrane antigen is quick $(2\frac{1}{2}-3 \text{ h})$ and simple, and only a single antiserum directed against a well defined hapten such as TNP would be required to enhance grafts from any donor. Two further advantages would be that the antiserum can be prepared in animals or in monoclonal cell culture, thus eliminating the problem of having to prepare alloantisera in human volunteers; and that an anti-haptenic antibody, because it does not combine directly with the graft, cannot cause hyperacute rejection. Further studies on these lines are in progress.

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- Stuart, F. P., Weiss, A., & Fitch, F. W. in Immunological Tolerance and Enhancement (eds Stuart, F. P. & Fitch, F. W.) 29-60 (MTP, Lancaster, 1979).
- Voisin, G. A. Immun. Rev. 49, 3-59 (1980).
 Morris, P. J. Immun. Rev. 49, 93-125 (1980)

- Hutchinson, I. V. Immun. Rev. 49, 167-197 (1980).
 Brent, L. & Batchelor, J. R. in Clinical Aspects of Immunology (eds Lachmann, P. J. & Peters, D. K.) (Blackwell, Oxford, in the press).

 Hutchinson, I. V. & Zola, H. Transplanta Proc. 9, 961-963 (1977).

 Hutchinson, I. V. & Zola, H. Transplantation 23, 464-469 (1977).

 Hutchinson, I. V. & Zola, H. Cell. Immun. 36, 161-169 (1978).

- Hutchinson, I. V. & Bonavida, B. Transplantn Proc. 11, 919-922 (1979).
 Hutchinson, I. V., Rayfield, L. S. & Brent, L. Transplantation (submitted).
- Sinclair, N. R. St. C. & Law, F. Y. J. Immun. 123, 1439-1444 (1979).
 Hutchinson, I. V., James, N. D. & Brent, L. Transplantation (submitted).

- Forman, J., Vitetta, E. S., Hart, D. A. & Klein, J. J. Immun. 118, 797-802 (1977).
 Standring, R. & Williams, A. F. Biochim. biophys. Acta 508, 85-96 (1978).
 Hutchinson, I. V., Roman, J. M. & Bonavida, B. Adv. exp. Biol. Med. B121, 553-562 (1980).
- 16. Bonavida, B., Roman, J. M. & Hutchinson, I. V. Transplantn Proc. 12, 59-64 (1980).

Specific suppressor cells in graft-host tolerance of HLA-identical marrow transplantation

Mang-So Tsoi, Rainer Storb, Sherrie Dobbs & E. Donnall Thomas

Fred Hutchinson Cancer Research Center and Department of Medicine, Division of Oncology, University of Washington School of Medicine, Seattle, Washington 98104, USA

Graft-versus-host disease (GvHD) is a cell-mediated immunological reaction, and there is evidence in the rodent system that long-term survival without GvHD is associated with the presence of lymphocytes which specifically suppress the GvH reaction. We have accordingly developed an assay for the influence of circulating cells from patients with HLA-identical marrow grafts on the reaction of donor cells against host lymphocytes (collected before transplantation and modified by trinitrophenyl (TNP)). We find that patients without GvHD have radiation-sensitive circulating cells which specifically suppress the in vitro model of the GvH reaction. Our findings seem to have a bearing on graft-host tolerance and the causation of GvHD in vivo.

Most long-term patients after human marrow transplantation from HLA-identical siblings are healthy stable chimaeras who need no further immunosuppressive therapy^{1,2}. However, 30% of the chimaeras have mild to severe chronic GvHD characterized by lesions resembling those seen in autoimmune collagen vascular diseases^{3,4}, immunodeficiency⁵ and frequent bacterial infections⁶. We have described deposits of IgM and complement C'3 at the dermo-epidermal junction, raising the possibility that humoral anti-host immunity is involved in chronic GvHD7. The participation of cell-mediated immunity was suggested by the

	Table 1	Suppressor cell assay	
Culture	Responder cells	Stimulator cells* (irradiated)	'Suppressor' cells
Test Control A Control B	Donor Donor Chimaera	TNP-host TNP-host TNP-host	Chimaera† Donor Chimaera

Mononuclear leukocytes were separated in lymphocyte separation medium (Ficoll-Hypaque, Bionetics)^{8,10}. Host lymphocytes used as stimulating cells were cryopreserved before transplantation and thawed before use8. TNP-coupling with 10 mM trinitrobenzenesulphonic acid in phosphate-buffered saline at pH 7.4 for 10 min was done by published methods¹⁹⁻²¹. Stimulator cells were treated with 1,600 rad irradiation. Responder and suppressor cells were prepared from freshly drawn blood. The cells were finally suspended in RPMI medium with 20% male AB or pooled human male serum, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. From each cell suspension, 0.05 ml containing 5 × 104 lymphocytes was plated on to round-bottom plates (Linbro 76-013-05) as responder, stimulator or suppressor cells in ratios of 1:1:1. Cultures were set up in quadruplicate and incubated for 6 days at 37 °C in a humidified 5% CO $_2$ atmosphere. For the last 16 h of incubation, 2 μ Ci of ³H-thymidine (NEN) were added. The cultures were then collected and lymphoctye incorporation of the isotope-labelled thymidine was measured in a liquid scintillation counter.

% Suppression =
$$1 - \frac{\text{median c.p.m. of test cultures}}{0.5(\text{median c.p.m. of control cultures }(A + B))} \times 100$$

This formula has been described and justified¹⁰. A suppression of ≥30% was considered significant.

- * Other stimulator cells were also used (see Tables 2,3).
- † Chimaera cells = donor lymphocytes living in the marrow recipients.

	Table 2 A	n example	of results in	patient 9	87					
Responder	Suppressor									
		***************************************	TN	P-modif	ied					
		Auto- logous*	Host	Donor	Un- related	Un- related				
		Median c.p.m. of quadruplicate cultures								
Test Donor	Chimaera		327 (5,912)*	6,320	11,352	7,602				
Controls A, Donor	Donor	1,830	9,573 (4,931)†	9,509	11,677	14,649				
B, Chimaera Mean of A+B	Chimaera	208	1,227 5,401	2,593 6,051	4,683 8,180	4,174 9,412				
% Suppression			94 (-20)	-4	-39	19				

A test was considered not evaluable when the MLC response of donor cells to a stimulator population was less than twice that of the autologous control. Test cultures consisted of 5×10^4 donor lymphocytes as responders, 5×10^4 stimulator cells and 5×10^4 chimaera cells as suppressors. Control culture A consisted of 2 (5×10^4) donor lymphocytes and 5×10^4 stimulator cells. Control culture B consisted of 2 (5×104) chimaera lymphocytes and 5×104 stimulator cells. % Suppression was calculated according to the formula in Table 1 except for cultures indicated (*†). The high negative suppression which occurred less frequently than positive suppression resulted from an enhanced MLC response in the mixed culture. Chimaera cells alone often showed a lower MLC response than donor cells alone. The enhancement seen in mixed culture is probably due to an increased response of chimaera cells in the presence of donor cells. Chimaera cells may lack helper cells necessary for an adequate MLC response, and these are provided by the donor cells.

- In these cultures, the chimaera cells were irradiated with 1,600 rad.
- † In these cultures, 5×10⁴ irradiated donor cells were added to the responder (donor) cells and stimulator cells. No control B was set up because irradiated suppressor cells gave no response to the stimulators.

observation of unidirectional reactivity of lymphocytes (of donor origin) from most chimaeras with chronic GvHD in response to cryopreserved host lymphocytes in mixed leukocyte culture (MLC)^{8,5}. It was tempting to speculate that this finding represented the in vitro equivalent of a continuing immunological reaction which manifested itself in vivo as chronic GvHD. Conversely, chimaeras without GvHD lacked in vivo evidence for immunological reactions, and their lymphocytes in general did not display MLC reactivity to host cells8. We have also reported the presence in patients with chronic GvHD of circulating cells that suppressed the ability of lymphocytes from the marrow donor to proliferate in response to unrelated lymphocytes and/or to concanavalin A^{10,11}. Others described abnormal

Table 3 Suppressor cell activities in long-term patients

	Underlying UPN disease		***************************************				
Patients			Test day post-grafting	Host	Donor	Unrelated	Unrelated
					% Supr	pression	
Without GvHD $(n = 14)$	656	AML	732	36	ND	20	-3†
W			1,460	55	ND	ND	-6
	674	AML	1,012	47	21	7	20
	* 679	ALL	764	50	ND	NR	-29
			923	38	-12	3	10
			1,066	33	ND	-18	-38
	732	AA	349	45	ND	-24	-23
	737	AA	831	66	86	-65	7
	741	AA	842	57	-100	78	17
	771	AA	996	64	NR	-23 0 -36 -24 14	-50 9† 22 -41 -59 19 ND
	776	AML	371	34	ND		
	777	AA	822	56	23 75 ND -4 ND		
	896	AA	364	79			
	959	AML	381	71			
	987	ALL	362	94		-39	
	1,019	AML	331	-51		ND	
	1,019	AML	374	36	NR	-18	-4
Chronic GvHD $(n = 23)$	612	AA	1,080	-100	-71	27	-5
	630	AA	911	27	25	18	12
	644	ALL	959	68	-12	-15	30
	705	AML	745	-34	-35	NA	83
	723*	AMML	868	-27	3	11	-4
	734	AA	830	53	-35	11	36
	800	CML	652	1	53	23	42
	804	AMML	699	-22	ND	-23	-10
	838	AA	734	-29	ND	59	30
	886	ALL	350	69	16	NA	22
	889	AMML	372	48	-23	0	-15
	909	AML	535	10	70	-100	NA
	911	ALL	368	1	38	4	-53
	957	CML	354	-15	NR	-25	62
	962	AML	336	57	68	44	-21
	993	AML	394	-57	14	-9	-37
	995	AML	364	-88	-100	NA	78
	1,002*	CML	357	-100	NR	NR	2
	1,002*	AMML	357 390	21	ND	ND	95
		AMIL	211	84	NR	11	35
	1,023		238	-30	ND	60	-98
	1,068	AMML		44	NR NR	NR	74
	1,083	Hodgkin's lymphoma	171	44	NK	NK	149
	1,233	AMML	131	1	ND	ND	-19

UPN, unique patient number. Underlying disease: AA, aplastic anaemia; ALL, acute lymphoblastic leukaemia; AML, acute myelogenous leukaemia; AMML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia. Suppression of $\ge 30\%$ is indicated by bold figures. ND, not done; NA, not applicable, when chimaera MLC response (control B) \ge donor MLC response (control A)¹⁰; NR, no response, when donor MLC response to the stimulators (control A) $\le 2\times$ that of the autologous control.

numbers of TH₂⁺ cells in some patients with GvHD¹². Studies of *in vitro* immunoglobulin synthesis showed a lack of helper cells and the presence of suppressor cells in chimaeras with chronic GvHD¹³. Such cellular imbalance may explain the severe immunodeficiency seen in patients with chronic GvHD. Stable long-term chimaeras generally did not show evidence for similar immunological deficiencies.

The mechanisms maintaining stable graft-host tolerance in healthy long-term survivors after marrow transplantation have been recently investigated. A role for serum-blocking factors has been ruled out in both canine and human recipients of marrow grafts^{14,15}. Although the involvement of specific suppressor cells in the maintenance of transplantation tolerance has been established by many investigators¹⁶, in clinical marrow transplantation, such evidence is lacking due to the difficulty of studying a model with HLA identity. In the animal models, we have presented circumstantial evidence for an active suppressor mechanism in stable canine radiation chimaeras¹⁷, and Tutschka et al. suggested that the development of specific and nonspecific suppressor cells in rat chimaeras after the resolution of GvHD accounted for the establishment of specific graft-host tolerance¹⁸.

In the present study, the in vitro model was devised to test

chimaera cells for their ability to suppress the response of donor lymphocytes to TNP-modified host cells stored before transplantation. TNP was used because donor lymphocytes did not respond to unmodified host cells in the MLC due to HLA identity. The TNP moiety of trinitrobenzene sulphonate covalently links to ε -amino groups of the lysines of cell-surface proteins. Studies in mice have shown that cytotoxic cells generated in vitro after sensitization with TNP-modified autologous cells recognize not only the TNP moiety but also self components coded for by genes mapping in H-2 (ref. 19). Studies on the human cytotoxic response to TNP-modified cells have suggested that almost half of the cytotoxicity generated was directed against polymorphic determinants thought to be HLA linked20. Some of the cytotoxic activity seemed to be directed against TNP in association with relatively nonpolymorphic antigens widely shared among humans²¹. These findings were interpreted as evidence for considerable diversity of the types of cell-surface determinants that human cytotoxic cells can recognize in association with TNP.

The hypothesis underlying the present study was that polymorphic determinants not linked with HLA can be recognized in association with TNP in a primary MLC assay and that these determinants have a role in human GvHD. We presumed that

[†] Results previously reported10.

^{*} These patients had extremely limited chronic GvHD.

suppression of the MLC reactivity by lymphocytes from the chimaeras without GvHD would be specific for recognition of TNP-associated host determinants but would not impair recognition of TNP-associated determinants shared between the siblings or with unrelated individuals. Conversely, recognition of TNP-associated determinants by donor lymphocytes would not be abrogated by lymphocytes from chimaeras with chronic GvHD.

Thirty-seven patients with aplastic anaemia, leukaemia or Hodgkin's lymphoma (Table 3), 8-50 yr old, were treated by high-dose cyclophosphamide, either alone or in combination with 1,000 rad total body irradiation, in preparation for a marrow graft from a genotypically HLA-identical sibling^{1,2}. All were given immunosuppression with intermittent methotrexate for no longer than 3 months post-grafting. Fourteen were healthy chimaeras without GvHD and 23 had minimal to severe chronic GvHD. Tests were carried out between 131 and 1,460 days post-grafting. At the time of testing six patients were under immunosuppressive treatment for chronic GvHD⁴ while the remainder were untreated.

Details of the lymphocyte separation, TNP modification and the assay system are described in Table 1 legend. Lymphocytes from 37 marrow donors were stimulated by TNP-modified lymphocytes from their recipients collected before transplantation (TNP-modified host cells) with stimulation indices (SI) ranging over 2-15.8 (mean 4.3). Mononuclear cells from the peripheral blood of the chimaeras were tested for their ability to suppress the reactivity of the donor lymphocytes to TNPmodified host lymphocytes. In addition, we tested the effect of chimaera lymphocytes on the response of the donor lymphocytes to unmodified, unrelated stimulating cells from healthy individuals ('nonspecific' suppression)10. To test for specificity, the effect of chimaera lymphocytes on the responses of the donor lymphocytes to TNP-modified donor and TNP-modified unrelated lymphocytes was also studied.

Table 2 shows an example of the results in a patient without GvHD (UPN 987) studied 362 days after transplantation. The circulating lymphocytes from the chimaera, when mixed with donor lymphocytes in an MLC, suppressed the response to TNP-modified host cells by 94%. The suppressor activity was abrogated by exposure of chimaera cells to 1,600 rad irradiation. (Radiosensitivity of the suppressor cells was also shown in four out of four other cases studied.) The chimaera's lymphocytes did not suppress the response of donor lymphocytes to TNP-modified donor nor unrelated TNP-modified or unmodified cells.

Table 3 summarizes the results in the 37 patients studied. Lymphocytes from 13 of 14 healthy chimaeras suppressed the response of donor lymphocytes to TNP-modified host lymphocytes compared with those of only 5 of 23 chimaeras with chronic GvHD (P < 0.0005, χ^2 test). Lymphocytes from none of the healthy chimaeras suppressed the ability of their marrow donor lymphocytes to respond to unmodified unrelated lymphocytes compared with those of 9 of the 22 chimaeras with chronic GvHD tested (P < 0.01). We were able to test two patients (UPNs 656 and 679) from the former group repeatedly and show comparable results. Suppression of donor lymphocyte responses to TNP-modified self and TNP-modified unrelated lymphocytes was seen in 2/7 and 1/14 cases among healthy chimaeras and in 4/14 and 3/16 cases among chimaeras with chronic GvHD (differences not significant).

The results of the study are in agreement with our hypothesis. Most patients without GvHD had circulating cells 'specifically' suppressing donor lymphocyte reactivity to TNP-modified host cells while most patients with chronic GvHD lacked specific suppressor cells. This suggests that stable graft-host tolerance after human marrow grafting is maintained by specific suppressor cells. Conversely, the lack of specific suppressor cells in patients with chronic GvHD allows an immunological reaction to occur which manifests itself in vivo as GvHD and in vitro as unidirectional reactivity of chimaera lymphocytes (that is, donor lymphocytes in the patient) against cryopreserved host lymphocytes in MLC8. The nature of these suppressor cells and the time of their appearance in the blood after transplantation are unknown.

The present study also confirms a previous observation 10,11 on the existence of another class of suppressor cells, present in patients with and absent in those without chronic GvHD, which nonspecifically suppresses the response of marrow donor lymphocytes to unmodified unrelated lymphocytes in MLC. These nonspecific suppressor cells may be one explanation for the severe immunodeficiency⁵ and the recurrent infectious complications⁶ characteristic of patients with chronic GvHD.

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- Thomas, E. D. et al. New Engl. J. Med. 301, 597-599 (1979). Storb, R. et al. Ann. intern. Med. 92, 30-36 (1980).
- Shulman, H. M. et al. Am. J. Path. 91, 545-570 (1978). Sullivan, K. M. et al. Blood 57, 267-276 (1981).
- Noel, D. R. et al. Blood 51, 1087-1105 (1978). Atkinson, K. et al. Blood 53, 720-731 (1979).
- Arkinson, A. et al. J. Immun. 120, 1485–1492 (1978). Tsoi, M. S., Storb, R., Dobbs, S., Medill, L. & Thomas, E. D. J. Immun. 125, 2258–2262
- 9. Tsoi, M. S., Storb, R., Dobbs, S., Santos, E. & Thomas, E. D. Transplantn Proc. 13, 237-240 (1981). 10. Tsoi, M. S. et al. J. Immun. 123, 1970–1976 (1979)
- Tsoi, M. S., Storb, R., Dobbs, S., Sullivan, K. M. & Thomas, E. D. in Biology of Bone Marrow Transplantation (eds Gale, R. P. & Fox, C. F.) 119-125 (Academic, New York, 1980).
- Reinherz, E. L., Parkman, R., Rappeport, J., Rosen, F. S. & Schlossman, S. F. New Engl. J. Med. 300, 1061–1068 (1979).
- Lum, L. G., Seigneuret, M. C., Storb, R., Witherspoon, R. P. & Thomas, E. D. Transplantn Proc. 13, 1231-1236 (1981).
- Proc. 13, 1231-1236 (1981).
 14. Tsoi, M. S. et al. J. Immun. 114, 531-539 (1975).
 15. Tsoi, M. S., Storb, R., Weiden, P. L. & Thomas, E. D. J. Immun. 118, 1799-1805 (1977).
 16. Hilgert, I. Immun. Rev. 46, 27-53 (1979).
 17. Weiden, P. L., Storb, R., Tsoi, M. S., Graham, T. C., Lerner, K. G. & Thomas, E. D.
- J. Immun. 116, 1212-1219 (1976).
 Tutschka, P. J., Hutchins, G. M. & Santos, G. W. in Experimental Hematology Today (eds.)
- Baum, S. J. & Ledney, G. D.) 113-121 (Springer, New York, 1979) Shearer, G. M. Eur. J. Immun. 4, 527-533 (1974).

- Shaw, S. & Shearer, G. M. J. Immun. 121, 290-299 (1978).
 Shaw, S., Nelson, D. L. & Shearer, G. M. J. Immun. 121, 281-289 (1978).

Human Ia molecules carrying DC1 determinants differ in both α - and β -subunits from Ia molecules carrying DR determinants

Giorgio Corte*, Franco Calabi*, Guido Damiani*, Antonio Bargellesi*, Roberto Tosit & Rosa Sorrentino‡

* Istituto di Chimica Biologica, Università di Genova, Viale Benedetto XV, 1-16132 Genoa, Italy Laboratorio di Biologia Cellulare, CNR, Rome, Italy ‡Gruppo di Microbiologia e Patologia Generale, Università di Roma, Rome, Italy

Several genetic markers of human la molecules, each recognized specific alloantisera, have been defined: 1,2,3,4,5,w6,7,w8,w9,w10. They are controlled by a single locus, HLA-DR. In addition alloantigenic specificities have been identified which are associated, at the population level, with two or more of the above DR specificities. They have been called 'supertypic specificities'. DC1 supertypic specificity (nomenclature equivalents: MB1, MT1, LB12) has attracted particular attention, because although exhibiting a population association with DR1, 2 and w6, DC1 is carried by a different molecular species¹⁻³. Possibly, DC1 may represent the first genetic marker of a locus different from the DR locus but in strong linkage disequilibrium and therefore probably closely linked to it. Here we report the development of a monoclonal reagent specifically directed against DC1 and its utilization in a structural analysis of DC1 molecules as compared with Ia molecules carrying different DR determinants.

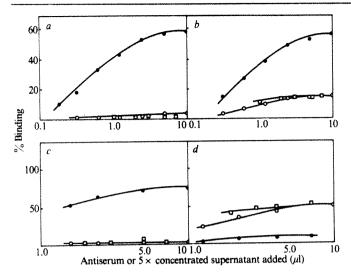


Fig. 1 A detergent-solubilized Ia preparation from the Daudi cell line was ¹²⁵I-labelled by the chloramine T method. Limited papain digestion was performed at 1:40 enzyme to protein ratio (bovine serum albumin as carrier protein) for 30 min at 37 °C. Papainunsplit' and 'papain-split' fractions were separated on a column of Biogel A 1.5 and further purified by LcH affinity chromatography. Details about the whole procedure have been previously reported8. Tests of the two fractions with the different antisera were performed by the double precipitation direct binding test1 Aliquots of antigen containing 15,000 c.p.m. were incubated with different antiserum dilutions for 16 h at 20 °C in 0.075 M Tris-HC1 buffer, pH 7.8, containing 0.2% Renex-20 detergent and 0.02% bovine serum albumin. Precipitating antiserum (either sheep antihuman or rabbit anti-mouse immunoglobulin) was then added at equivalence and the radioactivity in the washed precipitate determined. Per cent binding was expressed relative to the binding of the same antigenic preparation by a rabbit antihuman Ia antiserum. Further details are given elsewhere2. Absorption of the papain-split fraction was achieved by reacting 200,000 c.p.m. of antigen with 20 µl of either Fe131/4 or Fe88/37 alloantiserum for 16 h at 20 °C. The supernatant recovered after precipitation with sheep anti-human immunoglobulin was then further tested with the different antisera at varying dilutions. O, Fe131/4; •, Fe88/37; \square , BT3/4. a, Daudi unsplit; b, Daudi split (not absorbed); c, Daudi split (absorbed with Fe 131/4, anti-DC1); d, Daudi split (absorbed with Fe 88/37, anti-DRw6).

Table 1 Reactivity of monoclonal BT3/4 with lymphoblastoid B-cell lines and peripheral blood B cells

	DR phenotype	Reactivity with BT3/4		
Cell lines				
LG2	1, 1	+		
WT18	2, 2	+		
LG17	2, 2	+		
LG38	5, 5			
WT48	5, 5			
WT52	w6, w6	+		
LG28	w6, w6	+		
Daudi	w6, -	+		
Raji	3, w6	+		
M. W.	2, 4	+		
Donors				
A. B	2, w6	+		
G. D.	4, 5			
M. F.	1, 4	+		
F. C.	5, 7	-		
A. M.	5, -			
R. A.	2, 4	+		

Lymphoblastoid B-cell lines LG2, LG17, LG28 were provided by Dr W. Leibold and were derived as previously described²⁷. All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (Seromed). B cells were purified from peripheral blood by already described techniques²⁸. Indirect immunofluorescence was carried out as previously described²⁸.

The monoclonal antibody BT3/4 is secreted by a hybridoma obtained by the method described by Gefter et al.4. Briefly, BALB/c mice were immunized intraperitoneally at 10-day intervals with 107 E-rosetting cells obtained from a secondary mixed lymphocyte culture (MLC). A mouse was killed 4 days after the third injection and its spleen was fused with P3/X63-Ag8-U1 myeloma cells. Fourteen days after fusion the supernatant of each cell was assayed by indirect immunofluorescence on activated T cells. Positive cultures were cloned on soft agar essentially as described by Coffino et al.5, and the clone BT3/4 passed into ascites form for mass production of monoclonal IgG1. When tested on a panel of cell lines and peripheral blood lymphocytes by indirect immunofluorescence, BT3/4 invariably reacted only with lymphoblastoid B-cell lines and B lymphocytes from donors with a DR phenotype including DR 1, 2 or w6 (Table 1). The specificity of BT3/4 is similar to that previously reported^{6,7} for the monoclonal antibody Genox 353.

At the molecular level, the specificity of BT3/4 was tested on purified Ia preparations from the Daudi cell line (Drw6⁺,DC1⁺). As reported previously, after limited papain digestion, two fractions of Daudi Ia can be separated by gel filtration: 'papainunsplit' fraction, retaining the approximate size of untreated molecules, and a 'papain-split' fraction, with decreased molecular weight. DC1 molecules were present in increased proportion in the 'papain-split' fraction8. This provided a convenient test of the specificity of the monoclonal BT3/4, which, as shown in Fig. 1 (a and b) did not react with the 'papain unsplit' fraction, while reacting similarly to the reference anti-DC1 alloantiserum Fe131/4 with the 'papain-split' fraction. Moreover, the reactivity of BT3/4 was completely abolished by absorption with Fe 131/4, whereas absorption with Fe 88/37 (anti-DRw6) induced a parallel increase of binding of Fe 131/4 and of monoclonal BT3/4 (Fig. 1, c and d). This shows that the molecules which carry the DC1 determinant as recognized by a specific anti-DC1 alloantiserum are the same molecules which carry the determinant recognized by the BT3/4 monoclonal antibody.

DC1 antigen was purified by immunoabsorption to Sepharose-bound BT3/4 from the lymphoblastoid cell lines

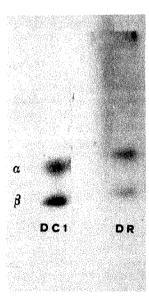


Fig. 2 Autoradiograph of a 12.5% SDS-PAGE of unreduced DC1 and DR antigens. 10^7 WT52 cells were lysed with 0.5% Nonidet P40 and centrifuged to remove nuclei and cell debris. DC1 and DR molecules were purified from the lysate by the immunoabsorption method described in detail in ref. 9. BT3/4 and PTF29 were coupled to Sepharose 4-B by the cyanogen bromide technique. A Sepharose-bound monoclonal antihuman IgE was used as control. The antigens were eluted with 5% SDS and labelled using the chloramine T method according to Greenwood et al. 29 . SDS-PAGE was carried out in slab gels using the discontinuous Tris buffer system as previously reported Non-reducing conditions were used to increase separation of α -and β -chains.

LG2 and WT52, carrying DR allospecificities 1 and w6, respectively. After iodination, the bound material was analysed in SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 2).

DC1 antigen is composed of two chains with an apparent molecular weight very similar to that of DR α - and β -chains. However, unreduced DC1 β -chain moves slightly faster than DR β -chain at an apparent molecular weight of 23,000.

The structure of DC1 antigens isolated from the two lines was further compared by two-dimensional peptide mapping with that of DR 2, 5, w6 and 7 similarly purified with the monoclonal PTF 29.12 (ref. 9). Figure 3 shows the comparison between peptic digests obtained from α chains of the two DC1 preparations and of the four DR antigens; DC1 α -chains isolated from DR 1 and DR w6 cell lines are identical, but bear no resemblance to any of the four DR α -chains. In Fig. 4 the equivalent comparison is reported for DC1 and DR β -chains. As previously reported, the maps of the β -chains from the four different DR alleles show, besides the expected degree of allotypic variability, a core of 12 invariant peptides; none of these peptides is present in the DC1 β -chain. Again β -chains of DC1 purified from the DR 1 and DR 6 cell lines are identical.

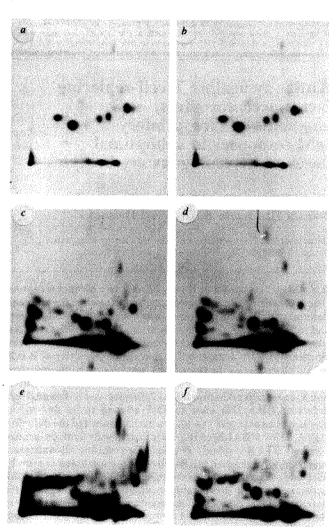


Fig. 3 Autoradiographs of peptide maps of ¹²⁵I-labelled α-chains of DC1 and DR antigens. α- And β-chains were eluted from the gel and processed for peptide mapping as previously described. Briefly, after complete reduction with dithiothreitol and alkylation with iodoacetamide, α- and β-chains were digested with pepsin (1;50 enzyme to protein ratio, 18 h at 37 °C in 100 μl of formic acid/acetic acid/water (1;4;45)). Two-dimensional peptide maps were obtained by a technique based on the method of Feinstein α, DC1 α-chain from LG2 (DR1, 1); b, DC1 α-chain from WT52 (DRW6, W6); c, DR α-chain from WT52; d, DR α-chain from IBW9 (DR7, 7); e, DR α-chain from LG17 (DR2, 2); f, DR α-chain from WT48 (DR5,5).

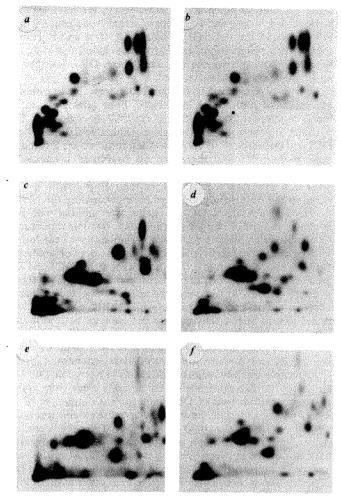


Fig. 4 Autoradiographs of peptide maps of 125 I-labelled β chains of DC1 and DR antigens. a, DC1 β -chain from LG2 (DR1, 1); b, DC1 β -chain from WT52 (DRw6, w6); c, DR β -chain from WT52; d, β -chain from IBW9 (DR7, 7); e, DR β -chain from LG17 (DR2, 2); f, DR β -chain from WT48 (DR, 5). The detailed comparison of DR chains from different alleles is given elsewhere. DR peptide maps similar to those shown in ref. 9 have been included here to facilitate comparison with the peptide maps of DC1 chains.

It should be stressed that this type of technique, involving comparison of molecules labelled in a single amino acid, underestimates the degree of homology of their primary sequences. In fact, a single amino acid substitution is sufficient to cause a peptide to move to a different position. However, it can be safely concluded that DR and DC1 differ both in their α - and β -chains and that the minimum number of different amino acids corresponds to the number of spots present in the maps (α and β respectively). A precise quantitation must await sequence data.

The data presented are better discussed in their possible analogies to the murine Ia system. Murine Ia includes at least two subsets of molecules with similar basic structures: I-A molecules and I-E molecules. Based on recent data 10,11 , the A α - and A β -subunits as well as the E β -subunit are controlled by genes in the I-A subregion, whereas the E α -subunit maps in the I-E subregion. Polymorphic structural differences can be detected mainly on the three I-A subregion-controlled subunits. Several structural and serological findings, including amino acid sequence data, indicate homology between murine I-E molecules and 'human Ia molecules' 12,13 . These data were obtained before the recognition of separate subsets of Ia molecules probably controlled by different loci 1,14,15 . Therefore it is very likely that the major molecular subset, that is, the DR locuscontrolled molecules, were actually tested. In fact, DC1 molecules constitute a minor fraction as compared with DR molecules and their presence would not have affected the

sequence analysis much above 'noise level'. We have actually shown that a mouse anti I-E^k alloantiserum could actively bind DR molecules but not DC1 molecules (R. Tosi and P. Ivanyi, unpublished observation). It must be concluded that only the homology between murine I-E and human DR-controlled Ia molecules is clearly demonstrated.

Thus it is possible that DC molecules may be the human equivalent of murine I-A molecules. Four analogies can be found to support this hypothesis: (1) DC1 β -chains and DR β -chains are controlled by very closely linked loci. In fact, both DC1 and DR serologically detected determinants are located on their β -chains^{1,15} and there are no confirmed instances of DC/DR recombination. (2) A β -chains have been shown to possess a lower molecular weight than E β -chains¹⁶. This parallels the observation reported here concerning the migration in SDS-PAGE of DC1 β-chains as compared with DR β -chains. (See ref. 15 for a discussion of the possible significance of this size difference.) (3) Extensive variations have been found, both by limited sequence analysis 12,17 and by peptide analysis 18 between murine A α and E α . Our data show that DC1 and DR molecules differ not only, as expected, in their β -chains, but also in their α -chains. (4) A monoclonal antibody directed against an allotypic specificity of rat Ia molecules, MRC OX3, was also found to recognize a polymorphic determinant both in mouse and in man^{19,20}. In man, it reacted only with DR 1, 2 or w6 positive cell lines, thus behaving as an anti-DC1. In the mouse, it recognized an I-A subregion-controlled determinant.

The above are only indirect indications of a possible DC/I-A homology. A direct demonstration can only come from amino acid sequence analysis of DC1 molecules. Comparison of α chains will be especially informative as these exhibit less polymorphic variations than do β -chains¹². Whatever the homology between single human and murine Ia loci, the data suggest a basic similarity between the two systems. Both murine and human Ia molecular pools can be subdivided into at least two subsets which substantially differ from each other both for their α - and β -chains. A similar conclusion has been reached for rat In antigens²¹. The association of DC1 β -chains with a distinctive species of α -subunit definitely rules out the possibility that the DC1 supertypic specificity is controlled by the HLA-DR locus. In fact, the different DR allelic products have been shown to possess marked structural variations in their β -subunits but not in their α -subunits ^{9,22,23}, while the structure of the α -subunit associated to the DC1 molecule has clearly distinctive features. This confirms previous immunochemical and genetic data which assigned DC1 to a separate, non-DR, locus.

An interesting point, as in the mouse system, is the noninterchangeability of α -chains in their association with β -chains encoded by different loci. This is probably due to a selective post translational association, as coordinated translation of α -and β -chains from the same messenger seems unlikely because separate messenger RNAs for the two chains can be isolated²⁴, and because A β -chains can associate with A α -chains transmitted by the homologous chromosome²⁵. Markert and Cresswell²⁶ have recently reported the existence of at least two species of β -subunits and at least three species of non-interchangeably associated α -subunits. Apart from the interpretation given by the authors on the localization of alloantigenic determinants on the different chains, the above data, in agreement with ours, also imply the existence in man of Ia molecular subsets differing for both α - and β -chains and carrying alloantigenic determinants which are transmitted in strong linkage disequilibrium with DR determinants.

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- 1. Tosi, R., Tanigaki, N., Centis, D., Ferrara, G. B. & Pressman, D. J. exp. Med. 148, 1592-1597 (1978).
- 2. Duquesnoy, R. J. Transplantn Proc. 12, 148-151 (1980).
- Termijtelen, A., Boettcher, B., Bradley, B. A., D'Amaro, J. & van Rood, J. J. Tissue Antigens 16, 140-146 (1980).
- Gefter, M. L., Margulies, D. H. & Scharff, M. D. Somatic Cell Genet. 3, 231-235 (1977).
- 5. Coffino, P., Baumal, R., Laskov, R. & Scharff, M. D. J. cell. Physiol. 79, 429-440 (1972).

- 6. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. Immun.
- 7. Brodsky, F. M., Parham, P. & Bodmer, W. F. Tissue Antigens 16, 30-48 (1980)
- Brodsky, F. M., Farham, F. & Bodmer, W. F. Itssue Antigens 16, 30-48 (1980).
 Tanigaki, N., Tosi, R., Koyama, K. & Pressman, D. Immunology 39, 615-620 (1980).
 Corte, G., Damiani, G., Calabi, F., Fabbi, M. & Bargellesi, A. Proc. natn. Acad. Sci. U.S.A. 78, 534-538 (1981).
 Silver, J. & Russel, W. A. Immunogenetics 8, 339-341 (1979).
- 11. Cook, R. G., Capra, J. D., Bednarczyk, J. L., Uhr, J. W. & Vitetta, E. S. J. Immun. 123, 2799–2803 (1979).
- 12. Cook, R. G., Siegelman, M. H., Capra, J. D., Uhr, J. W. & Vitetta, E. S. J. Immun. 122, 2232-2237 (1978)
- 13. Delovitch, T. & Falk, J. I. Immunogenetics 8, 405-410 (1979).
- Katagiri, M. et al. Immunogenetics 9, 335-351 (1979).
 Tanigaki, N., Tosi, R., Pressman, D. & Ferrara, G. B. Immunogenetics 10, 151-169 (1980).
- Cook, R. G., Uhr, J. W., Capra, J. D. & Vitetta, E. S. J. Immun. 121, 2205-2212.
 MacMillan, M., Cecka, J. M., Murphy, D. B. & McDevitt, H. O. Proc. natn. Acad. Sci. U.S.A. 74, 5135-5139 (1977).
- 18. MacMillan, M., Cecka, J. M., Hood, L., Murphy, D. B. & McDevitt, H. O. Nature 277, 663-665 (1979).
- McMaster, W. R. & Williams, A. F. Eur. J. Immun. 9, 426-433 (1979).
 McMaster, W. R., Wineares, B. C. & Parham, P. Tissue Antigens 14, 453-458 (1979).
- 21. Blankenhorn, E. P., Cecka, J. M., Frelinger, J., Gotze, D. & Hood, L. Eur. J. Immun. 10, 145-151 (1980).
- 22. Kaufman, J. F., Anderson, R. L. & Strominger, J. L. J. exp. Med. 152, 37s-53s (1980).
- Charron, D. & McDevitt, H. O. Proc. natn. Acad. Sci. U.S.A. 76, 6567-6569 (1979).
- Korman, A. J., Ploegh, H. L., Kaufman, J. F., Owen, M. J. & Strominger, J. L. J. exp. Med. 152, 65s-82s (1980).
- 25. Silver, J., Swain, S. L. & Hubert, J. J. Nature 286, 272-274
- 26. Markert, M. L. & Creswell, P. Proc. natn. Acad. Sci. U.S.A. 77, 6101-6104 (1980).
- Gatti, R. A. & Leibold, W. Tissue Antigens 13, 35-44 (1979)
- Corte, G., Moretta, L., Damiani, G., Mingari, M. C. & Bargellesi, A. Eur. J. Immun. 11,
- Greenwood F C. Hunter W. M. & Glover, J. S. Biochem, J. 89, 114-123 (1963)
- Feinstein, A., Richardson, N. E. & McIhinney, R. A. J. in Cells of Immunoglobin Synthesis (eds Pernis, B. & Vogel, F.) 165-188 (Academic, New York, 1979).

Antibody against T cell-replacing factor acceptor site(s) augments in vivo primary IgM responses to suboptimal doses of heterologous erythrocytes

Kiyoshi Takatsu, Yoshimi Sano, Shohken Tomita, Noboru Hashimoto & Toshiyuki Hamaoka

Department of Oncogenesis, Institute for Cancer Research, Osaka University Medical School, Osaka 553, Japan

It is widely accepted that B cells possess two distinct receptors. One is the surface immunoglobulin that recognizes the antigenic determinant; the other transmits the signals for proliferation and differentiation of the B cells after receiving differentiation stimuli mediated by a soluble factor derived from helper T cells. Previously¹⁻⁴, we demonstrated that the responsivity of B cells to T cell-replacing factor (TRF) was under X-chromosome control, as determined by genetic analysis of the TRF-nonresponsiveness of B cells from DBA/2Ha mice. An X-linked B-cell defect of DBA/2Ha mice to TRF seemed to be due to the absence of an acceptor site(s). We have immunized the defective $(DBA/2Ha9 \times BALB/c\delta)F_1$ male mice with antigen-primed parental BALB/c splenic B cells. The resulting alloantiserum preferentially inhibited the B-cell response mediated in vitro by TRF added continuously to the culture. We report here that this antiserum, which presumably contains antibody against the putative TRF-acceptor site, augments primary IgM responses to simultaneously administered suboptimal doses of heterologous erythrocytes injected into TRF high-responder animals. These augmented IgM-PFC (plaque -forming cell) responses were also observed in other X-linked B-cell defective CBA/N mice, but not in TRF low-responder DBA/2Ha mice, suggesting that the putative TRF-acceptor site(s) functionally detectable by this antiserum is distinct from the Lyb 3 and Lyb 5 components.

To prepare alloantiserum containing anti-TRF-acceptor site(s) antibody, (DBA/2Ha×BALB/c)(DC)F₁ male mice responder animals) were immunized intra-(TRF-low peritoneally (i.p.) with splenic B cells (2×107) from DNP (dinitrophenyl)-KLH (keyhole limpet haemocyanin)-primed BALB/c mice of a high TRF-responder parental strain, with

Table 1 Enhancing activity of (DC)F₁ male anti-BALB/c B-cell antiserum on PFC responses in BALB/c and BALB/c nu/nu mice stimulated with graded doses of SRBC

0.1	Control	337 (1.63)	
		33/(1.03)	2,42 (1,50)
	(DC)F ₁ (d)anti-BALB/c	4,808 (1.38)	24.1 (1.50)
1.0	Control	1,682 (1.47)	12.1 (1.49)
	(DC)F ₁ (d)anti-BALB/c	6,832 (1.40)	53.0 (1.04)
10.0	Control	29,337 (1.23)	190.0 (1.19)
	(DC)F ₁ (&)anti-BALB/c	48,245 (1.07)	290.0 (1.09)
1.0	Control	477 (1.20)	2.17 (1.13)
	(DC)F ₁ (d)anti-BALB/c	4,604 (1.19)	21.5 (1.25)
10.0	Control	3,342 (1.06)	49.2 (1.14)
	(DC)F ₁ (d)anti-BALB/c	9,997 (1.04)	113.3 (1.25)
	10.0	1.0 Control (DC)F ₁ (\$\delta\$)anti-BALB/c 10.0 Control (DC)F ₁ (\$\delta\$)anti-BALB/c 1.0 Control (DC)F ₁ (\$\delta\$)anti-BALB/c 10.0 Control	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$

BALB/c or BALB/c nu/nu mice, aged 5-7 weeks, were immunized i.v. with various amounts of SRBC in combination with $(DC)F_1$ male anti-BALB/c antiserum (1:40) or control serum, in a volume of 0.2 ml. PFC responses 4 days after antigen stimulation and antiserum injection are shown. Results are expressed as geometric means \pm s.e.m.

4 mg aluminium hydroxide gel and Bordetella pertussis vaccine (1×10^9) , followed by i.p. injection of DNP-primed B cells without adjuvant once a week for 5 weeks. Seven days after the final immunization, each mouse was partially bled once a week for 4 weeks and the serum pooled. The activity of the antiserum was determined by measuring the inhibition of TRF-mediated antibody response according to methods used elsewhere.

To determine whether the reaction of the TRF acceptor on the surface of the B cells with anti-TRF acceptor antibody altered the immune reactivity of these cells, we examined the PFC responses of BALB/c mice on stimulation with 1×10^6 sheep red blood cells (SRBC) alone or in combination with graded doses of anti-TRF acceptor site antiserum or control serum. As shown in Fig. 1, mice stimulated with SRBC alone or in combination with control serum produced ~ 500-1,500 PFCs per spleen (5-10 PFCs per 10⁶ cells), whereas mice injected with SRBC and an optimal concentration of anti-TRF acceptor site antiserum produced a 5- to 10-fold increase in anti-SRBC PFCs. Studies of the time course of this enhancing effect of the antiserum revealed that the peak response occurred on day 4 after antiserum injection in combination with a suboptimal dose of SRBC, and that by day 8 this response had declined to control serum levels (data not shown). Mice injected with (DC)F₁ male anti-BALB/c B-cell antiserum alone without antigen also produced much fewer (1.5-1.8 times the background) but significant PFC responses to SRBC. This increase in PFCs due to the antiserum was observed in anti-HRBC (horse red blood cell) plaques as well (data not shown). These findings suggest that the anti-TRF acceptor antiserum acts synergistically with antigen to produce enhanced antigen-specific PFC responses. However, this does not exclude the possibility that anti-TRF-acceptor antibody alone induces polyclonal B-cell activation.

To demonstrate that the enhancing effect of (DC)F₁ male anti-BALB/c B-cell antiserum on the PFC response of BALB/c mice is particularly evident when the triggering signals provided by both antigen and helper T cells are relatively low, we examined the effect of anti-TRF-acceptor antiserum: (1) in the presence of decreased concentrations of antigen, and (2) in the absence of T cells. Addition of antiserum (Table 1) markedly enhanced the PFC response to low doses of SRBC from 1×10^3 to 1×10^6 . In contrast, the enhancing effect of antiserum on PFC responses of those mice stimulated with large doses of SRBC was marginal. In a further experiment, the ability of (DC)F₁ male anti-BALB/c B-cell antiserum to substitute for helper T-cell activity in the induction of a primary PFC response was tested in nu/nu mice. BALB/c nu/nu mice produced a low number of anti-SRBC PFCs after priming with 1×106 SRBC plus control serum, compared with the IgM anti-SRBC PFC response of BALB/c mice in the same experimental conditions. However, the administration of (DC)F₁ male anti-BALB/c B-cell antiserum with SRBC to these nu/nu mice resulted in a striking increase in anti-SRBC PFC responses (Table 1).

It has been reported that CBA/N mice lack a subpopulation of B cells that appears late in ontogeny⁵⁻⁷ and responds to

thymus-independent type 2 antigens, such as Ficoll and dextran^{2,9}. As the defect is X-linked and recessive, an alloantiserum denoted anti-Lyb 3 that would react exclusively with this subset of B cells in all mouse strains except CBA/N was raised by immunizing (CBA/N×BALB/c)F₁ male mice with BALB/c spleen cells³. Huber et al.⁵ demonstrated that this anti-Lyb 3 antiserum acts synergistically with antigen to produce enhanced antigen-specific PFC responses in various strains of mice except CBA/N. We decided to test whether this augmenting effect of (DC)F₁ male anti-BALB/c B cell antiserum is due to an interaction between antibody and TRF-acceptor sites on the surface of B cells, and also to test whether or not the TRF-acceptor sites detected by this antibody are identical to or closely associated with the Lyb 3 molecules. We examined the effect of (DC)F₁ male anti-BALB/c B-cell antiserum on primary anti-SRBC

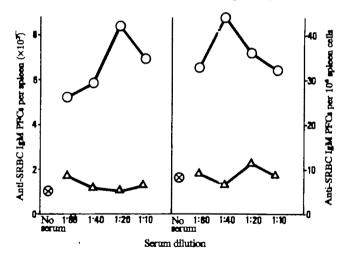


Fig. 1 Dose-related augmenting effect of (DC)F₁ male anti-BALB/c B-cell antiserum on anti-SEBC PFC responses. (DBA/2Ha×BALB/c)(DC)F₁ male anti-3ALB/c B-cell alloantiserum containing anti-TRF-acceptor site antibody was prepared by the method described elsewhere². Briefly, after treatment with monocional anti-Thy 1.2 antiserum and complement, splenic B cells from DNP-KLH-primed B cells of mice of a high TRFresponder strain (BALB/c) were used as immunizing cells. Low TRF-responder (DC)F₁ male mice were injected i.p. with these DNP-primed B cells (2×10^7) , with 4 mg aluminium hydroxide gel and B. pertussis vaccine (1×10^7) , followed by i.p. injections of DNP-primed B cells without adjuvant once a week for 6 weeks. Seven days after the final immunization, partial bleeding was performed on each mouse once a week for 4 weeks and the serum pooled. Six-week old BALB/c mice were immunized i.v. with 1×10^6 SRBC alone (\otimes) or in combination with varying dilutions of (DC)F₁ male anti-BALB/c B-cell antiserum (O) or normal (DC)F₁ mouse serum (Δ) as a control, in a volume of 0.2 ml. After 4 days the mice were killed and the number of IgM plaque-forming cells (PFC) to SRBC was determined per spleen and per 10° spleen cells. Each point represents the mean PFC response of six mice from a representative experiment of a series of five.

Table 2 The effect of (DC)F₁ male anti-BALB/c B-cell antiserum on various strains of mice

Strain	Serum	Direct PFCs per spleen	Direct PFCs per 10 ⁶ spleen cells
BALB/c	Control	518 (1.09)	4.31 (1.17)
•	(DC)F ₁ (3)anti-BALB/c	7,590 (1.23)	42.5 (1.24)
C57BL/6	Control	649 (1.28)	7.10 (1.27)
•	(DC)F ₁ (3)anti-BALB/c	3,934 (1.09)	39.0 (1.02)
DBA/2Ha	Control	104 (1.44)	1.48 (1.22)
•	(DC)F ₁ (3)anti-BALB/c	326 (1.33)	1.78 (1.29)
CBA/N	Control	165 (1.37)	3.30 (1.44)
•	(DC)F ₁ (3)anti-BALB/c	3,030 (1.03)	73.6 (1.12)

Various strains of mice, aged 6-8 weeks, were immunized i.v. with 1×10^6 SRBC in combination with (DC)F₁ male anti-BALB/c antiserum (1:40) or control serum, in a volume of 0.2 ml. Results are expressed as geometric means ± s.e.m.

responses in various strains of mice. As shown in Table 2, enhancement of anti-SRBC responses was observed not only in BALB/c but also in C57BL/6 and CBA/N mice. In contrast, intravenous (i.v.) injections of varying doses of anti-TRF acceptor serum failed to augment the anti-SRBC PFC response in DBA/2Ha mice, indicating that the augmenting effect was clearly due to a specific reaction of this antiserum with a component which is absent from the DBA/2Ha mice (Table 2). The fact that injection of CBA/N mice with SRBC in combination with antiserum produced more than a 10-fold increase in anti-SRBC PFCs indicated that enhancement of the anti-SRBC responses in CBA/N mice is clearly due to a specific reaction of this serum with a component that is different from Lyb 3 and Lyb 5, which are absent from CBA/N mice.

Our results clearly demonstrate that passive administration of anti-TRF acceptor site antiserum in combination with suboptimal doses of SRBC in TRF high-response animals can induce augmented primary anti-SRBC IgM responses (Fig. 1 and Table 2), and that stimulation of the acceptor site(s) on antigenreactive B cells with antibody allows more efficient triggering of B cells by antigen. The ability of anti-TRF acceptor site antibody to substitute for helper T-cell activity in the induction of a primary response is obvious from the fact that anti-TRF acceptor site antiserum administered to nu/nu mice resulted in the production of a significant PFC response (Table 1). Although the positive response of CBA/N mice to (DC)F₁ male anti-BALB/c B-cell antiserum supports the notion that the TRFacceptor site(s) which we detected here is different from Lyb 3 (and -5 and -7) components, another possibility is that there are several (at least more than one) kinds of TRF-acceptor site molecules on B cells, especially in the light of reports by Parker et al. 10 and Ahmed et al. 11 on the inability of unprimed B cells from CBA/N mice to respond to TRF (concanavalin A (Con A) supernatant). As anti-Lyb 3 antiserum was also able to augment a primary anti-SRBC response in vivo to suboptimal doses of antigen, and thus showed the same functional characteristics as our antiserum, Lyb 3 could be another acceptor molecule for the TRF originated from Con A supernatant. The interrelation between the TRF acceptor site(s) detected here and its Lyb 3 components awaits further investigation.

Note that there is a discrepancy between the triggering effect of anti-TRF acceptor antibody as described here and the inhibitory effect of the same antibody on B cell-triggering mediated by TRF as described previously²⁻⁴. Recently, we found that antigen-primed B cells could be triggered in vitro by anti-TRFacceptor antibody when the cells were pulsed with the antibody before the culture, whereas continuous presence of the antibody never triggered the B cells, and resulted in the inhibition of B-cell triggering mediated by TRF (to be published elsewhere). Therefore, this discrepancy in the effect of the antiserum may be explained by the dose of antiserum present in the critical period of B-cell triggering. Figure 1 shows that, in in vivo conditions the B-cell responses were greater at lower concentration (1:20) than at higher concentration (1:10), and an optimal concentration of antibody was required to trigger the B cells. This may be due to the generation of similar experimental conditions in vivo to those in the antibody-pulsing of B cells in vitro. However, this notion does not necessarily exclude other possibilities such as difference in effect of antibodies of various classes contained in the antiserum or difference in threshold of various B-cell subsets (antigen-primed B cells as opposed to non-primed B cells) to be stimulated by the antibody.

Although further investigation is required, such an alloantiserum containing anti-TRF-acceptor antibody may provide us with the experimental strategy necessary for characterization of the TRF-acceptor site on B cells and for analysis of the fine mechanism of B-cell triggering through this site.

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- Takatsu, K., Tominaga, A. & Hamaoka, T. J. Immun. 124, 2414-2422 (1980).
 Tominaga, A., Takatsu, K. & Hamaoka, T. J. Immun. 124, 2423-2429 (1980).
 Takatsu, K., Tanaka, K., Tominaga, A., Kumahara, Y. & Hamaoka, T. J. Immun. 125, 2646-2653 (1980)
- 4. Takatsu, K., Tanaka, K., Tominaga, A. & Hamaoka, T. in B Lymphocytes in the Immune Response: Functional, Developmental, and Interactive Properties (eds Klinman, N. R., Mosier, D. E., Scher, I. & Vitetta, E. S.) 331-338 (Elsevier, Amsterdam, 1981).
- Huber, B., Gershon, R. K. & Cantor, H. J. exp. Med. 145, 10-20 (1977). Ahmed, A. et al. J. exp. Med. 145, 101-110 (1977).

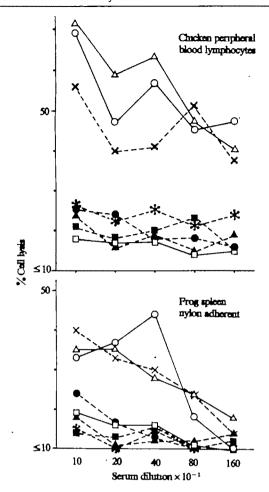
- Subbarao, B. et al. J. exp. Med. 149, 495-506 (1979). Mosier, D. E., Mond, J. J. & Goldings, E. A. J. Immun. 119, 1874-1878 (1977)
- Mond, J. J., Scher, I., Mosier, D. E., Baese, M. & Paul, W. E. Eur. J. Immun. 8, 457-463
- 10. Parker, D. C., Fothergill, J. J. & Wadsworth, D. J. Immun. 123, 931-941 (1979)
- Ahmed, A. & Sher, I. in B Lymphocytes in the Immune Response (eds Cooper, M., Mosier, D., Sher, I. & Vitetta, E.) 117 (Elsevier, Amsterdam, 1979).

Phylogenetic tracing of Ia genes

Nobukata Shinohara*, David H. Sachs†, Noritaka Nonaka* & Hiroshi Yamamoto*

- * Department of Immunology, School of Medicine, Chiba University, Chiba City, Chiba 280, Japan
- † Transplantation Biology Section, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The I region of the mouse H-2 complex has been subdivided into five subregions (A, B, J, E, C) on the basis of serological and functional analyses of intra-MHC (major histocompatibility complex) recombinant strains1. However, no serologically detectable specificities nor Ir genes have been localized to the I-E subregion of the $H-2^b$ and $H-2^s$ haplotypes. Attempts to identify I-E gene products of these haplotypes by chemical analyses have also been unsuccessful^{2,3}. Thus, the H-2 complex of these haplotypes seems not to express I-E subregion genes. In this case, alloantisera raised in 'I-E-negative' strains of mice against 'I-E-positive' mouse cells might contain, in addition to the usual alloantibodies, antibodies reactive with conserved portions of I-E molecules inherited from a hypothetical primordial gene $(I-E_0)$ which may have existed long before the speciation of contemporary animals. Such antibodies would show extensive cross-reactions with the I-E homologues of various species of animals. Interspecies cross-reactions of anti-Ia mouse alloantibodies have indeed been observed. Although such a cross-reaction was first found between mouse I-A and rat Ia-homologues, subsequent studies indicated much broader and more frequent interspecies cross-reactions of anti-I-E antibodies4-8. We have now expanded the demonstration of such interspecies cross-reactions to submammalian vertebrates. The possible basis of these unusual cross-reactions and its phylogenetic implication will be discussed.



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Fig. 1 Cross-reaction of anti-Ik mouse alloantibody with lymphocytes of submammalian species. Reactivity of the antiserum was assessed by two-stage Trypan blue cytotoxicity assay using rabbit complement. When necessary, rabbit complement was preabsorbed with crythrocytes of the testing animal. Absorption of the antiserum with mouse spleen cells was performed by incubating the 1:10 dilution of the serum with 1×10^9 cells per ml for 1 h on ice. The completion of the absorption was confirmed by cytotoxicity assay on cells of the absorbing strain. Frog spleen cells were selected for adherence to nylon fibre in an attempt to enrich Ia-positive cells. Cytotoxicity curves of an A.TH anti-A.TL antiserum absorbed with: no cells (○), B10.BR (□), B10.D2 (●), B10 (△), B10.A (▲), B10.A(2R) (■), B10.A(3R) (*) and $B10.A(4R)(\times)$.

The present study used an A.TH anti-A.TL antiserum (serum 201) which is specific for the entire I region of the k haplotype. This antiserum showed cross-reactivity on lymphocytes of various mammalian species (data not shown). Further examination of the cross-reactivity of serum 201 on chicken and frog (Rana catesbelana) lymphocytes revealed that the cross-reac-

Table 1 Mapping of the cross-reactive antigen in the mouse by absorption study

		_		_					
Mouse strain of absorbing cells	H-2 complex								Absorption of the cross-reactive antibody
B10.BR	k	k	k	k	k	k	k	k	+
B10.D2	d	d	d	d	d	d	d	d	+
B10	b	b	b	b	b	ь	b	b	****
B10.A	k	k	k	k	k	d	d	d	+
B10.A(2R)	k	k	k		k.		d	Ь	+
B10.A(3R)	ь	ь	b	b	Tk.	ď	d	d	+
B10.A(4R)	k	k	1	Ъ	ь	ь	Ъ	ь	

See Fig. 1 legend for details.

tion extended even to submammalian species (Fig. 1). The cytotoxicity of the antiserum on these cells was not due to contaminating natural antibodies, as B10.BR (H-2k) cells completely absorbed the cytotoxic antibodies in both cases (Fig. 1). Likewise, B10.D2 and B10.A cells absorbed the antibodies whereas B10 cells did not. Absorption of serum 201 with B10.A(2R) and with B10.A(3R) cells eliminated the crossreactive antibodies, placing the gene encoding the cross-reactive antigen between the I-J and D regions (Fig. 1, Table 1). This result was corroborated by the failure of B10.A(4R) cells to absorb the cross-reactive antibodies. These results were identical to those observed for a variety of mammalian lymphocytes.

To confirm these findings, mouse antisera raised against various non-mammalian vertebrates such as chicken, frog and fish (Carassius carassius) were tested for reactivity on mouse lymphocytes. As shown in Table 2, all three antisera raised in I^* mice against non-mammalian lymphocytes showed cross-reactions on B10.BR cells. Reactivity of these antisers on A.TL and B10.S(9R) cells located the gene encoding the cross-reactive antigen between the I-B and D regions. These results not only confirmed the specificity of the cress-reactions detected by serum 201 but also indicated that even fish lymphocytes express molecules cross-reactive with $I-E/C^{I}$ gene products.

It has already been shown in several mammalian species that the cross-reactive antigens are homologues of murine Ia antigens—glycoproteins consisting of two polypeptide chains (molecular weights 35,000 and 28,000) which are encoded by MHC-linked genes⁶⁻¹⁰. Thus, it seems quite likely that the

Table 2 Cross-reactions of mouse xenoantisers raised against submammalian lymphocytes on allogeneic mouse spleen cells

	H−2 complex								Killing by			
Target cells	K	Α	В	J	E	С	s	D	A.TH α-chicken	A.TH α-frog	A.TH α-fi s h	
A.TH							3		-*	_	-	
A.TL	2	k	k	k	k	k	k	d	+ +	+	+	
B10.S			\$	\$		\$	1	1		-	_	
B10.BR	k	k	k	k	k	k	k	k	+	+	+	
B10.A	k	k	k	k.	k	d	d	d	+	+	+	
B10.S(9R)	8		?	k	k	d	ď	d	+	+	+	

^{* &}lt;10% above complement background.

cross-reactive antigens of other species are also homologues of the murine Ia molecules. The I-A- and the I-E-subregion genes in the mouse seem to have essentially overlapping functions. Although we do not know the exact nature of the functions of these genes, the I-E gene seems to be functionally dominant in the mouse, as judged from the extent of the alloantigeneic polymorphism and the number of Ir genes mapped to these subregions. Such functional dominance of the I-A subregion in this species may account for the apparent non-catastrophic loss of I-E antigens in some mouse strains. From these studies, the origin of the $I-E_0$ gene has been traced back at least to fish. This cross-reactive anti-Ia antibody may thus prove to be a powerful probe with which to study the evolutionary significance of Ia molecules and Ia-bearing cells in the immune system.

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^{†&}gt;25% above complement background.

¹ Klein, J., Flaherty, L., VandeBerg, J. L. & Shreiffer, D. C. Jennussegmenter 6, 489-512

Jones, P. P., Murphy, D. B. & McDevitt, H. O. J. exp. Med. 148, 925-939 (1978)

Jones, P. P., Mimphy, D. B. & McDevitt, H. O. J. arp. Mod. 148, 923-939 (1978)
Ozato, K., Linney, J. K., El-Gamd, M. & Sacha, D. H. J. Imenus. 125, 940-945 (1980)
Sacha, D. H., Humphrey, G. W. & Linney, J. K. J. arp. Mod. 146, 381-393 (1977)
Shmobara, N., Linney, J. K. & Sacha, D. H. J. Imenus. 121, 637-640 (1978)
Linney, J. K., Mann, D. L. & Sacha, D. H. J. Imenus. 126, 633-627 (1979)
Linney, J. K. & Sacha, D. H. J. Imenus. 126, 623-627 (1979)
Shmobara, N. & Sacha, D. H. J. Imenus. 126, 934-937 (1981)
David, C. S. Transplence Res. 30, 299-322 (1976).
Whitehold, E. K. & Willey J. Imenus. 23, 23 (1977).

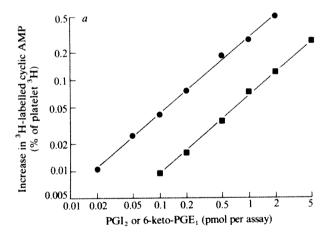
Wakeland, E K & Klem, J. Incommencements 8, 27-39 (1979).

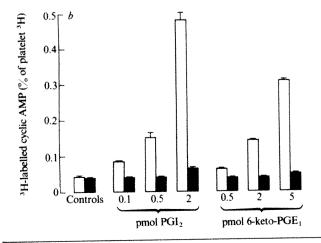
Measurement of circulating prostacyclin

R. J. Haslam & M. D. McClenaghan

Department of Pathology, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

Prostacyclin (PGI₂) is a potent inhibitor of platelet aggregation and a vasodilator that is synthesized from prostaglandin endoperoxides by vascular endothelial and smooth muscle cells^{1,2}. Its action on platelets is mediated by cyclic AMP3-5. Circulating PGI₂ has been detected through its inhibitory effect on the accumulation of platelets on collagen strips superfused with blood from anaesthetized and heparinized cats⁶ and rabbits⁷ and this led to the suggestion that PGI2 is released continuously into blood passing through the lungs and functions as a circulating hormone that regulates platelet aggregability 6-9. Because of its chemical instability, no more sensitive method of detecting circulating PGI₂ has been developed and there is doubt over whether PGI₂ is present in more physiological conditions^{10,11}. Although 6-keto-PGF_{1 α}, the stable non-enzymatic breakdown product of PGI₂, has been assayed in human^{12,13} and rabbit^{14,15} plasma, uncertainty over the fraction present as PGI2 in the circulation has limited the value of the results obtained. Finally, it has been suggested that 6-keto-PGE1, a metabolic product of PGI_2 or 6-keto- $PGF_{1\alpha}$ that can inhibit platelet aggregation 16,17, may also function as a circulating hormone 18. To resolve these uncertainties, we have now developed a sensitive bioassay for PGI2-like compounds in blood. The results indicate that fresh arterial blood from physiologically normal rabbits contains insufficient PGI2 or 6-keto-PGE1 to affect platelet function.





Our assay is based on measurement of the increases in platelet ³H-labelled cyclic AMP that occur on addition of platelets containing ³H-labelled adenine nucleotides to blood. The ³Hlabelled cyclic AMP formation attributable to PGI3-like compounds in the blood was identified using an antibody that bound them. Inclusion of 1 mM 3-isobutyl-1-methylxanthine (IBMX) in the reaction mixture to inhibit platelet cyclic AMP phosphodiesterase enhanced the sensitivity of the assay about 10-fold. After addition of labelled platelets to citrated rabbit blood containing 1 mM IBMX, the increase in platelet ³Hlabelled cyclic AMP was linear with respect to time for 0.5-1.0 min, whether PGI₂ was present or not, thus providing a measure of platelet adenylate cyclase activity. When blood was preincubated at 37 °C for >1 h to remove any endogenous PGI₂, IBMX increased the platelet ³H-labelled cyclic AMP content from a basal value of $0.024 \pm 0.002\%$ of platelet ³H to $0.070 \pm$ 0.004% in 0.5 min (means \pm s.e.m., n = 30). Double reciprocal plots of the additional increases caused by exogenous PGI₂ against the PGI₂ concentration (10-1,000 pmol ml⁻¹) were linear, suggesting a single population of functional PGI₂ receptors. Computer analysis using a weighted least squares method¹⁹ gave a $K_{\rm m}$ value for PGI₂ of 29.9 ± 2.6 pmol ml⁻¹ and a $V_{\rm max}$ for the PGI₂-stimulated conversion of platelet ³H-labelled adenine nucleotides to cyclic AMP of $3.7 \pm 0.1\%$ in 0.5 min (means \pm s.e.m. from five experiments). For 6-keto-PGE₁, a K_m of 101 ± 25 pmol ml⁻¹ and a V_{max} of $3.2 \pm 0.6\%$ were obtained (means ±s.e.m. from four experiments). However, when using the formation of ³H-labelled cyclic AMP to assay amounts of PGI₂ or 6-keto-PGE₁ less than 5 pmol, we have found it most convenient to calculate experimental values from linear regressions of log $[\Delta^3H$ -labelled cyclic AMP] on log $[PGI_2]$ or log

Fig. 1 Increases in ³H-labelled cyclic AMP in labelled platelets added to blood containing IBMX and either PGI₂ or 6-keto-PGE₁; antagonistic effects of antibody prepared against 6-keto-PGF1a. Washed rabbit platelets were prepared using blood from pentobarbital-anaesthetized animals by a modification of the method of Ardlie et al. 31. After the first wash the platelets were resuspended at 2.5×10^9 ml⁻¹ in Ca²⁺-free Tyrode's solution containing 5 mM PIPES buffer, pH 6.5, and were incubated with 2 μM [2,8-3H]adenine (34 Ci mmol⁻¹, ICN) for 90 min at 20-22 °C. Almost complete uptake of the 3H-adenine occurred. The labelled platelets were then isolated by centrifugation and resuspended at 2.5 × 109 ml⁻¹ in Tyrode's solution containing 0.35% (w/v) bovine serum albumin, 5 mM HEPES buffer, pH 7.4, and an apyrase concentration (~30 µg ml⁻¹) capable of degrading 100 µM ADP at 0.1 µmol min⁻¹ at 37 °C. This suspension was stored at 37 °C in a sealed tube and was usable for up to 3 h. Blood (13.5 ml) for assay of added prostaglandin standards was withdrawn from the central artery of the ear of an unanaesthetized rabbit into a syringe containing 1.5 ml of 3.8% (w/v) trisodium citrate and was incubated in a sealed tube at 37 °C for 1 h to allow time for complete breakdown of any endogenous PGI2. Incubation mixtures (0.5 ml) were then constituted as follows: 0.4-ml samples of preincubated citrated blood were mixed with 0.5 µmol of IBMX in 0.04 ml of 0.154 M NaCl and with 0.02 ml of 0.154 M NaCl with or without y globulin (60 µg) from rabbits immunized against 6-keto-PGF_{1α} (ref. 10). Prostaglandin standards (0-5 pmol) in 0.02 ml of 0.139 M NaCl containing 9.4 mM Na₂CO₃ (PGI₂) or of 0.154 M NaCl (6-keto-PGE₁) were then added and exactly 0.1 min later 0.04 ml of the suspension of labelled platelets (1-1.5 µCi ³H) was introduced. Incubations were continued for 0.5 min at 37 °C, at which time the reactions were stopped by addition of 1.0 ml of ice-cold 15% (w/v) trichloroacetic acid and 1,000 d.p.m. of ¹⁴C-labelled cyclic AMP (28 mCi mmol⁻¹, Amersham). Each incubation was performed in triplicate. The acidified samples were centrifuged and the acid extracts applied to columns (15×0.7 cm) containing 1.5 g alumina (WN-3, Sigma) that had been washed with 10% (w/v) trichloroacetic acid. The columns were then washed with 9 ml of 10% (w/v) trichloroacetic acid, 9 ml of water and 2 ml of 0.2 M ammonium formate (pH 6.0) and cyclic AMP was eluted with a further 3 ml of 0.2 M ammonium formate³². The eluates were applied to columns containing 1.5 ml (packed volume) of Dowex 50 resin (Bio-Rad AG 50W-X8, 100-200 mesh, H* form). These columns were then washed with 6 ml of 1 mM potassium phosphate buffer, pH 7.3, and cyclic AMP was finally eluted in a further 9 ml of the same buffer which was lyophilized and counted for ³H and ¹⁴C by liquid scintillation. After correction for the recovery of ¹⁴C-labelled cyclic AMP (50-70%), platelet ³H-labelled cyclic AMP was expressed as a percentage of the total platelet ³H. a, log/log plots of the increases in platelet ³H-labelled cyclic AMP caused by different amounts of PGI₂ (●) or 6-keto-PGE₁ (■) present in blood samples also containing IBMX. b, ³H-labelled cyclic AMP (mean values \pm s.e.m., n = 3) found in labelled platelets incubated with rabbit blood containing IBMX and the indicated amounts of PGI2 or 6-keto-PGE1 either without (unshaded) or with (shaded) antibody. The results shown in a and bare from different experiments.

Table 1 Arterial concentrations of PGI₂ after intravenous injection of PGI₂ or angiotensin II

Compound injected	Time of PGI ₂ measurement (min)	Arterial PGI ₂ (pmol ml ⁻¹)	PGI ₂ remaining in blood (% of that injected)
PGI, (1 nmol per kg)	-5	$0.06 \pm 0.01(4)$	
	+2	$1.55 \pm 0.18(4)$	9.7 ± 1.1
	+5	$0.19 \pm 0.02(4)$	0.9 ± 0.1
	+10	$0.09 \pm 0.01(4)$	0.2 ± 0.1
Angiotensin II	+2	$7.55 \pm 0.37(4)$	
(5 nmol per kg)	+5	$1.53 \pm 0.37(3)$	
(+10	$0.26 \pm 0.03(3)$	
	+30	$0.03 \pm 0.01(3)$	

Values for PGI₂ are means \pm s.e.m. of the number of unanaesthetized male rabbits indicated in parentheses. The rabbits received single intravenous injections of 3–5 μ M PGI₂ in a solution containing 0.139 M NaCl and 9.4 mM Na₂CO₃ or of 20 μ M angiotensin II in 0.154 M NaCl. Arterial blood (13.5 ml) for measurement of the effects of standard amounts of PGI₂ on labelled platelets (as in Fig. 1a) was taken from each rabbit 60–90 min before injection of compound and further 2.7-ml samples for immediate assay of circulating PGI₂ were taken at the times indicated with respect to injection of compound (from progressively more proximal segments of the central arteries of the ears). The per cent of injected PGI₂ remaining in the blood (means \pm s.e.m.) was calculated on the approximate assumption that this compound was homogeneously distributed throughout a blood volume of 65 ml per kg. In control experiments, injections of vehicle did not affect arterial PGI₂ levels. Addition of angiotensin II (5–50 pmol) to assays had no effect on the increase in platelet ³H-labelled cyclic AMP caused by PGI₂ (2 pmol).

[6-keto-PGE₁] (Fig. 1a); for these regressions r^2 usually exceeded 0.99. The smallest amount of PGI₂ added to 0.5-ml assay mixtures (see Fig. 1 legend) that significantly increased platelet ³H-labelled cyclic AMP above the values obtained with IBMX alone was 0.02 pmol (average increase 0.009% of the platelet ³H). With <5 pmol per assay, amounts of 6-keto-PGE₁ four- to fivefold larger than PGI₂ were required to induce equivalent increases in platelet ³H-labelled cyclic AMP (Fig. 1a).

The y-globulin fraction from rabbits immunized with 6-keto-PGF₁₀ conjugated to bovine serum albumin has been shown to block the inhibition of platelet aggregation by PGI₂ (ref. 10). We found that inclusion of 60 µg of a comparable antibody preparation in assay mixtures containing rabbit citrated blood prevented the increases in platelet 3H-labelled cyclic AMP caused by ≤ 0.5 pmol of PGI₂ and inhibited the effects of 2 pmol of PGI₂ by >90% (Fig. 1b). Antibody to 6-keto-PGF_{1 α} also suppressed the increases in platelet cyclic AMP caused by ≤5 pmol of 6-keto-PGE₁. That these actions of the antibody preparation were due to its ability to bind PGI2-like compounds rather than to a nonspecific effect on platelet adenylate cyclase activity was established by the observation that its effects were blocked by addition of >100 pmol of 6-keto-PGF_{1a} to the assay. To distinguish between PGI2 and 6-keto-PGE1, use was made of a marked difference in their stability in citrated blood. Assays (for example, Fig. 2) showed that the activity of PGI₂ added to citrated rabbit blood (pH 7.6-7.8) decreased exponentially with a half life of 9.3 ± 0.6 min (mean \pm s.e.m., n = 6), whereas that of 6-keto-PGE₁ declined with a half life of >40 min.

To measure PGI₂-like compounds in circulating rabbit blood, 2.7 ml was rapidly withdrawn from the central artery of the ear into a syringe containing 0.3 ml of 3.8% (w/v) trisodium citrate. Citrated blood (0.4 ml) was then pipetted into tubes holding 0.5 µmol of IBMX with antibody (three tubes) and without antibody (three tubes). Labelled platelet suspension was added immediately and the mixtures (0.5 ml) were incubated for 0.5 min at 37 °C before extraction of ³H-labelled cyclic AMP (see Fig. 1 legend). All six incubations were completed within 2.5 min of arterial puncture, so that breakdown of PGI₂ had little effect on the results. The presumptive PGI₂ present in freshly taken blood was calculated from the difference between the mean values for the platelet ³H-labelled cyclic AMP formed in the presence and absence of antibody and that formed in preincubated blood from the same animal on addition of standard amounts of PGI₂ and the same preparation of labelled

platelets. Assays were performed on fresh arterial blood from 27 conscious rabbits (New Zealand White, 2.5-3.5 kg). The observed mean value for the formation of ³H-labelled cyclic AMP in labelled platelets added to the fresh blood was in each case diminished by antibody to 6-keto-PGF_{1a}, but this decrease was only statistically significant in 11 individual animals (P < 0.05, one-sided t-test), indicating that blood concentrations of PGI₂-like compounds were close to the limit of sensitivity of the method. Estimates of the mean arterial presumptive PGI2 were made using groups of animals and the values obtained after correction for dilution by anticoagulant were $0.050 \pm 0.007 \,\text{pmol ml}^{-1}$ in 17 male rabbits and $0.066 \pm 0.066 \pm 0.006 \,\text{m}$ $0.017 \text{ pmol ml}^{-1}$ in 10 female rabbits (means \pm s.e.m.). Both mean values are significantly different from zero (P < 0.001 and <0.0025, respectively, in one-sided t-tests), but no significant difference between the sexes was detected. As addition of antibody did not decrease the cyclic AMP formed when labelled platelets were added to blood samples that had been preincubated at 37 °C for 30 min (P>0.1; t-test, n=6), the small but significant amount of PGI2-like material present before preincubation of these samples was probably PGI₂.

We have also assaved the PGI₂-like compounds in rabbit arterial blood at various times after rapid intravenous injection of PGI₂ at 1 nmol per kg (Table 1). Approximately 90% of the injected PGI₂ had disappeared from the circulation after 2 min and 99% after 5 min. When arterial blood taken 2 min after injection of PGI₂ was incubated at 37 °C (see Fig. 2), the assayable activity disappeared with a half life of 8.3 ± 0.3 min (mean \pm s.e.m., n = 3), indicating the presence of PGI₂ rather than 6-keto-PGE, in the circulation. Intravenous injection of angiotensin II has been shown to lead to the appearance of a circulating PGI₂-like substance in both cats²⁰ and dogs^{21,22}. We have observed the same phenomenon in rabbits; 2 min after injection of angiotensin II at 5 nmol per kg, the arterial concentration of presumptive PGI₂ reached 7.55 ± 0.37 pmol ml⁻¹ and then declined rapidly (Table 1). The in vitro half life of the material present in the circulation after 2 min was 7.4 ± 0.5 min (mean \pm s.e.m., n = 3), indicating that it was probably PGI₂ and certainly not 6-keto-PGE1.

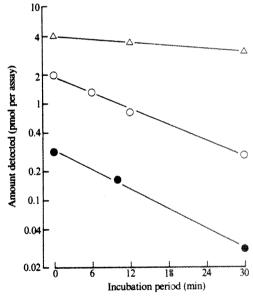


Fig. 2 Stability of PGI₂ and of 6-keto-PGE₁ in rabbit blood. Samples (0.4 ml) of citrated blood (pH 7.7) that had been preincubated for 60 min at 37 °C were mixed with 2 pmol of PGI₂ or 5 pmol of 6-keto-PGE₁. After further incubation at 37 °C for the indicated periods, IBMX and labelled platelets were added and the increase in platelet ³H-labelled cyclic AMP measured over 0.5 min. The PGI₂ or 6-keto-PGE₁ remaining was calculated from the effects of standard amounts of these prostaglandins added to samples of the same blood 0.1 min before the labelled platelets (see Fig. 1a). Samples of freshly drawn arterial blood from a rabbit injected 2 min previously with PGI₂ at 1 nmol per kg were assayed for PGI₂-like activity immediately and after incubation at 37 °C for 10 and 30 min. The decay of PGI₂ (O), 6-keto-PGE₁ (△) and of the PGI₂-like activity present in blood after injection of PGI₂ (●) are plotted on a semi-logarithmic scale.

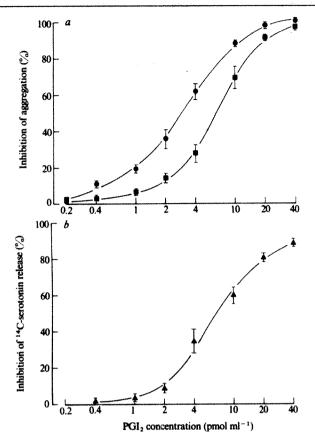


Fig. 3 Inhibition of rabbit platelet function by PGI₂. a, ADP-induced platelet aggregation. Incubation mixtures (1 ml) comprising 0.85 ml of citrated plasma containing 4.25×10⁸ platelets and PGI₂ at the concentrations indicated were stirred for 0.5 min at 37 °C in an aggregometer (Payton Associates) before addition of ADP. The per cent inhibitions by PGI₂ of the decreases in absorbance caused by 0.75 µM ADP (●) and 2 µM ADP (\blacksquare) after stirring for a further 0.5 min are shown (means \pm s.e.m., n = 5and 6, respectively). Control decreases in absorbance (means ±s.e.m.) were 0.134 ± 0.015 with 0.75 μ M ADP and 0.208 ± 0.019 with 2 μ M ADP. b, Collagen-induced release of serotonin. Incubation mixtures (1 ml) comprising 0.8 ml of citrated plasma containing 4 × 108 platelets (labelled by preincubation with 0.5 µM 14C-serotonin and the indicated concentrations of PGI₂ were stirred for 0.5 min before addition of collagen (50 µg ml⁻¹). After stirring for a further 2 min, 0.2 ml of 9% (w/v) paraformaldehyde was added, the samples were centrifuged and the release of platelet tonin into the supernatant was measured; this amounted to $57 \pm 5\%$ in controls without PGI_2 (n = 6). The per cent inhibitions of release (means ±s.e.m.) are shown (A).

To determine whether the small amounts of PGI₂ normally found in rabbit arterial blood could affect platelet function, we studied the sensitivity of the aggregation of platelets by ADP and of the release of platelet serotonin by collagen to inhibition by PGI₂ in rabbit citrated platelet-rich plasma (Fig. 3). As preliminary experiments showed that preincubation with PGI₂ for 0.5 min gave an optimal inhibition, this period was used subsequently. With a concentration of ADP that caused maximum aggregation (2 μM), an IC₅₀ for PGI₂ of 7 pmol ml⁻¹ was obtained (Fig. 3a), similar to the value reported by Whittle et al.²³. Almost the same concentrations of PGI₂ were required to inhibit the release of serotonin by collagen at 50 µg ml⁻¹ (Fig. 3b). With sub-optimal doses of ADP, PGI₂ was more effective (Fig. 3a), as expected in view of the ability of ADP to inhibit platelet adenylate cyclase⁵. However, no inhibition was observed with PGI₂ concentrations < 0.4 pmol ml⁻¹. 6-Keto-PGE₁ was approximately one-fifth as effective as PGI2 in inhibiting ADP-induced platelet aggregation.

Our experiments show that rabbit arterial blood contains trace amounts of a compound that shares three properties with PGI₂, namely the capacity to activate platelet adenylate cyclase, an affinity for an antibody to 6-keto-PGF1a and lability in whole blood. However, the values we have obtained for this presumptive PGI2 are one to two orders of magnitude lower than those implied by the collagen strip superfusion technique or by two studies in which 6-keto-PGF_{1a} levels were measured in rabbit serum¹² and plasma¹⁴. Although the collagen strip method did not allow accurate quantitation of circulating PGI2, antibody to PGI₂ enhanced the deposition of platelets from rabbit arterial blood to about the same extent as infusion of PGI₂ at 1 ng ml⁻¹ (2.8 pmol ml⁻¹) into venous blood depressed platelet deposition7. This suggests that arterial PGI2 concentrations of this order were present. A possible explanation of the discrepancy between these results and ours is that various features of the superfusion technique may stimulate PGI₂ synthesis, for example, the embolization of platelet aggregates from the collagen strip into the lungs. Intravenous injection of an air embolus has been shown to stimulate the release of PGI₂ in the cat⁸. The high 6-keto-PGF_{1a} content of rabbit serum¹² may reflect synthesis of PGI₂ by white cells from PGH₂ released by stimulated platelets²⁴. Although one group¹⁴ has reported a mean 6-keto-PGF_{1 α} concentration of 0.75 ng ml⁻¹ (2 pmol ml⁻¹) in rabbit plasma, another group¹⁵ obtained values for plasma 6-keto- $PGF_{1\alpha}$ of less than 0.1 ng ml⁻¹ (0.3 pmol ml⁻¹).

We believe our technique provides the most sensitive and accurate method yet available for measuring blood PGI2. The results obtained show that in physiologically normal rabbits, the arterial concentration of PGI2 is an order of magnitude too low to affect platelet function. Thus, our findings do not support the concept, based partly on experiments with rabbits⁷, that PGI₂ is a circulating hormone that regulates platelet aggregability course, the absence of significant PGI₂ from the normal circulation does not preclude its release in large amounts and distant action in conditions that provoke its synthesis by a substantial fraction of the pulmonary or peripheral vascular bed, as is apparently observed after injection of angiotensin II. However, in view of the fairly rapid clearance of PGI₂ from the circulation that we and others²⁵ have observed, we suggest that the synthesis and action of PGI2 are most often likely to be part of a local response to a local stimulus. Such PGI₂ synthesis may have important functions in limiting the extension of haemostatic plugs and thrombi²⁶⁻²⁹ and in the dispersal of platelet aggregates trapped by the lungs and other organs³⁰

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- Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. Nature 263, 663-665 (1976). Moncada, S. & Vane, J. R. Br. med. Bull. 34, 129-135 (1978).
- Gorman, R. R., Bunting, S. & Willer, O. V. Prostaglandins 13, 377–388 (1977). Tateson, J. E., Moncada, S. & Vane, J. R. Prostaglandins 13, 389–397 (1977).
- Haslam, R. J., Davidson, M. M. L., Davies, T., Lynham, J. A. & McClenaghan, M. D. Adv. Cyclic Nucleotide Res. 9, 533–552 (1978).
- Gryglewski, R. J., Korbut, R. & Ocetkiewicz, A. Nature 273, 765-767 (1978)
- Moncada, S., Korbut, R., Bunting, S. & Vane, J. R. Nature 273, 767-768 (1978). Gryglewski, R. J. Biochem. Pharmac. 28, 3161-3166 (1979). Moncada, S. & Vane, J. R. Fedn Proc. 38, 66-71 (1979).
- Smith, J. B., Oglettee, M. L., Lefer, A. M. & Nicolaou, K. C. Nature 274, 64-65 (1978).
 Steer, M. L., MacIntyre, D. E., Levine, L. & Salzman, E. W. Nature 283, 194-195 (1980).
- Mitchell, M. D. Prostaglandin Med. 1, 13-21 (1978).
- Hensby, C. N., Fitzgerald, G. A., Friedman, L. A., Lewis, P. J. & Dollery, C. T. Prostaglandins 18, 731-736 (1979).
- 14. Cerskus, A. L., Ali, M. & McDonald, J. W. D. Thromb. Res. 18, 693-705 (1980).
- Bult, H., Beetens, J. & Herman, A. G. Eur. J. Pharmac. 63, 47-56 (1980). Wong, P. Y.-K., Malik, K. U., Desiderio, D. M., McGiff, J. C. & Sun, F. F. Biochem. biophys.
- Res. Commun. 93, 486-494 (1980).
- Res. Commun. 93, 480-494 (1960). Wong, P. Y.-K., McGiff, J. C., Sun, F. F. & Lee, W. H. Eur. J. Pharmac. 60, 245-248 (19 Quilley, C. P., Wong, P. Y.-K. & McGiff, J. C. Eur. J. Pharmac. 57, 273-276 (1979). Cleland, W. W. Adv. Enzym. 29, 1-32 (1967).
- Swies, J., Radomski, M. & Gryglewski, R. J. Pharmac. Res. Commun. 11, 649-655 (1979). Mullane, K. M. & Moncada, S. Prostaglandins 20, 25-49 (1980).
- Dusting, G. J. J. cardiovascular Pharmac. 3, 197-206 (1981).
 Whittle, B. J. R., Moncada, S. & Vane, J. R. Prostaglandins 16, 373-388 (1978).
- Blackwell, G. J. et al. Br. J. Pharmac. 64, 436P (1978). Dusting, G. J., Moncada, S. & Vane, J. R. Br. J. Pharm
- Kelton, J. G., Hirsh, J., Carter, C. J. & Buchanan, M. R. J. clin. Invest. 62, 892-895 (1978). Bougain, R. H. Haemostasis 7, 252-255 (1978).
- Aiken, J. W., Gorman, R. R. & Shebuski, R. J. Prostaglandins 17, 483-494 (1979)
- Aiken, J. W., Gorman, R. R. & Shebuski, R. J. Prostaglandins 17, 483-494 (1979).
 Rosenblum, W. I., El-Sabban, F. & Ellis, E. F. Am. J. Physiol. 239, H220-H226 (1980).
 Hemker, D. P., Shebuski, R. J. & Aiken, J. W. J. Pharmac. exp. Ther. 212, 246-252 (1980).
 Ardlie, N. G., Packham, M. A. & Mustard, J. F. Br. J. Haemat. 19, 7-17 (1970).
 Jakobs, K. H., Böhme, E. & Schultz, G. in Eukaryotic Cell Function and Growth (eds.)
- Dumont, J. E., Brown, B. L. & Marshall, N. J.) 295-311 (Plenum, New York, 1976).

The phosphatidylinositol cycle and the regulation of arachidonic acid production

Eduardo G. Lapetina, M. M. Billah & P. Cuatrecasas

Department of Molecular Biology, The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709, USA

An increase in the metabolism of phosphatidylinositol occurs in a wide variety of tissues by the action of specific ligands¹⁻³. In platelets, the interaction of thrombin with its receptor initiates the degradation of phosphatidylinositol by the action of a specific phospholipase C (refs 4-8). In normal conditions of stimulation, the resultant 1,2-diacylglycerol is rapidly and completely phosphorylated to phosphatidic acid⁴⁻¹¹. The formation of phosphatidic acid precedes the release of arachidonic acid from the phospholipids of stimulated platelets5. This early appearence of phosphatidate might result in the initial production of arachidonic acid and lysophosphatidic acid by the action of a phospholipase A2 specific for phosphatidate12 Phosphatidate/lysophosphatidate could induce calciumgating 13-15 and subsequently stimulate phospholipases of the A2-type8, that degrade phosphatidylcholine, phosphatidylethanolamine and a further fraction of phosphatidylinositol6. Alternatively, the lysophosphatidate produced may serve as a substrate for the transfer of arachidonate directly from other phospholipids 16,17 to form new phosphatidate which in turn can release more arachidonate. Overall, such a sequence would be equivalent to phospholipase A2 activation of other phospholipids. Our present data indicate that when the release of arachidonic acid is completely inhibited by cyclic AMP or quinacrine, phosphatidic acid is redirected entirely to phosphatidylinositol and there is no production of arachidonate. In these conditions, the availability of calcium might be profoundly restricted. The correlation in platelets of a phosphatidylinositol by a specific phospholipase A2 might suggest that these phenomena are applicable to activations in other cell systems.

Phosphatidylinositol is not the only phospholipid that contributes to the production of arachidonic acid in stimulated platelets^{4-10,18}. Phosphatidylcholine and phosphatidylethanolamine also release arachidonic acid by the action of phospholipase A2 activities8. Both lysophosphatidylcholine and lysophosphatidylethanolamine have recently been found in stimulated platelets 10,18. In a similar way, lysophosphatidic acid is also produced in intact platelets that have been prelabelled with ³²P and stimulated with thrombin (Fig. 1). Thrombin is very effective in producing phosphatidic acid^{4-6,19} and lysophosphatidic acid¹⁹, whereas ionophore A23187 forms virtually no phosphatidic acid^{4,5} or lysophosphatidic acid (Fig. 1). Calcium ions enhance the thrombin-induced formation of phosphatidic acid as well as of lysophosphatidic acid (Fig. 1). We have described elsewhere the existence of a specific phospholipase A₂ which is present in platelet membranes and which specifically degrades phosphatidic acid¹². This enzyme activity $(K_m 20 \mu M)$ is most active at pH 7.0, requires Ca²⁺ (10 μM) for maximal activity and is inhibited by quinacrine¹². The existence and specific properties of this enzyme suggest a possible important role in the production of arachidonic acid in stimulated platelets. This phosphatidate-specific phospholipase A₂ has distinctly different properties from those of the phospholipases A2 that degrade phosphatidylethanolamine and phosphatidylcholine8, as its activity does not depend on the presence of detergents, alkaline pH or high concentration of Ca

Phosphatidate is a key intermediate in the phosphatidylinositol cycle¹⁻³. In this cycle, four consecutive enzyme activities are involved in the degradation and resynthesis of phosphatidylinositol (phosphatidylinositol-specific phospholipase C; 1,2-diacylglycerol kinase; CTP-phosphatidate: cytidyl

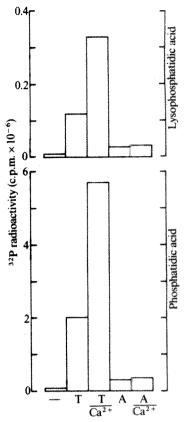


Fig. 1 Effect of thrombin and ionophore A23187 on the formation of phosphatidic acid and lyosphosphatidic acid in platelets. Horse platelets were labelled with ³²P-orthophosphate after separation from one (550 ml) unit of bibod as described previously^{5,6}. Platelets were then resuspended in 10 ml of buffer (134 mM NaCl, 15 mM Tris-HCl pH 7.4, 1 mM EGTA, 5 mM glucose), 5 mCi of ³²P-orthophosphate were added and the platelets incubated at 37 °C for 2 h. After centrifugation and resuspension, the final concentration of platelets was 1×10⁹ per 0.5 ml, which was the volume used for the assays. Samples (0.5 ml) were incubated with thrombin (T) (1 unit ml⁻¹)-or ionophore A23187 (A) (1 μM) ± calcium chloride (3 mM) in both cases, for 10 min at 37 °C. Lipid extraction and chromatographic separation of lipids have been detailed elsewhere^{5,6}. Phosphatidic acid and lysophosphatidic acid were separated by a TLC method which uses oxalate-impregnated plates as described before¹².

phosphatidyl CDP-1,2-diacylglycerol-inositol transferase: transferase). To study the effects of calcium on the phosphatidic acid and phosphatidylinositol of stimulated platelets, platelets were labelled with 32P-orthophosphate and resuspended in an EGTA-containing buffer. If those platelets are then incubated with quinacrine and stimulated with thrombin, the release of arachidonic acid is completely blocked but phosphatidic acid is formed as a consequence of the degradation of phosphatidylinositol⁶. After an initial period, the label in phosphatidate decreases while the labelling of phosphatidylinositol increases (Fig. 2). This increased conversion of phosphatidate to phosphatidylinositol is blocked by the addition of ionophore A23187 plus calcium ions (Fig. 2). In this case, there is a further accumulation of labelled phosphatidic acid while the increased labelling of phosphatidylinositol is completely blocked (Fig. 2). These results indicate that calcium inhibits the resynthesis of phosphatidylinositol from phosphatidic acid after thrombin stimulation (Fig. 2)¹⁰. In fact, Ca²⁺ has a direct inhibitory action on the enzymes involved in the resynthesis process (CTPphosphatidate: cytidyl transferase and CDP-1,2-diacylglycerolinositol phosphatidyl transferase)20,21 These data indicate that the phosphatidylinositol cycle can actively function in the presence of quinacrine, which completely blocks the production of arachidonic acid from all platelet phospholipids⁶. Calcium, on the other hand, interrupts the phosphatidylinositol cycle and phosphatidate accumulates (Fig. 2).

Cyclic AMP inhibits the 'release reaction' of platelets as well as aggregation⁴. The action of cyclic AMP on platelet enzymes has been variously ascribed to the inhibition of the conversion of arachidonic acid to cyclooxygenase metabolites²², the production of arachidonic acid from phospholipids^{4,23-27} and the formation of phosphatidic acid^{4,5}. All these actions ultimately reduce the production of arachidonate or its conversion to active cyclooxygenase products. We previously described the action of cyclic AMP in reducing phosphatidic acid to an inhibition of phospholipase C7. Further studies now reveal that the phosphatidylinositol cycle is not inhibited by cyclic AMP despite the profound reduction in the quantity of phosphatidate produced. Figure 3 describes the action of cyclic AMP on the reactions related to the increased turnover of phosphatidylinositol in platelets prelabelled with ³²P-orthophosphate. Cyclic AMP seems substantially to increase the rate of conversion of phosphatidate to phosphatidylinositol, thereby decreasing the steady state concentration of phosphatidate. As we are proposing that the production of arachidonic acid might be related to the formation of phosphatidate, this could serve as the basis for the cyclic AMP-induced inhibition of arachidonate production. In the presence of quinacrine, which completely blocks the formation of arachidonic acid from various phospholipids^{6,12}, thrombin greatly increases the breakdown and resynthesis (turnover) of phosphatidylinositol as shown by increased labelling of ³²Pphosphatidylinositol (Fig. 3). These data indicate that the integrity of the phosphatidylinositol cycle is maintained in

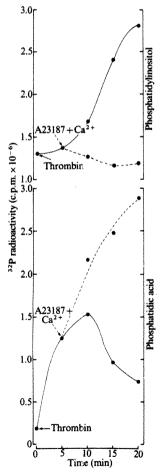


Fig. 2 Effect of ionophore A23187 plus calcium ions on phosphatidylinositol and phosphatidic acid of platelets pretreated with quinacrine and thrombin. Platelets prelabelled with ³²P-orthophosphate were obtained as for Fig. 1. Samples (0.5 ml) were incubated with quinacrine for 5 min at 37 °C and then thrombin (1 unit ml⁻¹) was added for different periods of time as indicated. After 5 min treatment with thrombin, in some assays (----) ionophore A23187 (1 µM) plus calcium chloride (3 mM) were added. Other details as for Fig 1.

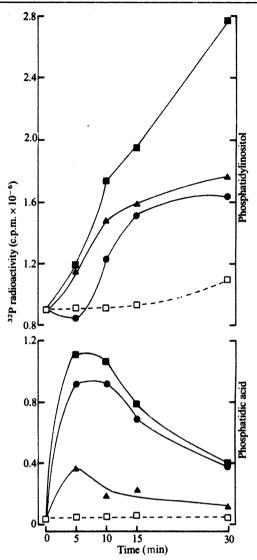


Fig. 3 Effect of thrombin on phosphatidylinositol and phosphatidic acid of platelets pretreated with cyclic AMP or quinacrine. Preincubations with quinacrine (0.75 mM) (or dibutyryl cyclic AMP (1 mM) (A) were for 5 min and thrombin (1 unit ml⁻¹) was then added for different periods of time as indicated. . Assays containing only thrombin; , control assays. Phosphatidic acid was separated on TLC⁵ and phosphatidylinositol was separated on formaldehyde-impregnated paper^{5,6}. Other details as for Fig 1.

conditions (cyclic AMP or quinacrine) in which the specific release of arachidonic acid induced by thrombin is completely blocked.

The information presented here indicates that calcium interrupts the phosphatidylinositol cycle¹⁻³ and leads to accumulation of the intermediate product, phosphatidic acid. In stimulated platelets a specific phospholipase A₂ released arachidonic acid from the phosphatidate produced¹², with the consequent appearence of lysophosphatidic acid. This phosphatidate-lysophosphatidate interconversion might important in the subsequent and specific mobilization of phosphatidylcholine, arachidonic acid from phatidylethanolamine and phosphatidylinositol⁶. In thrombinstimulated platelets cyclic AMP enhances the overall turnover of the phosphatidylinositol cycle by increasing the rate of conversion of phosphatidic acid to phosphatidylinositol, and thus inhibits the release of arachidonic acid from various phospholipids.

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- Lapetina, E. G. & Michell, R. H. FEBS Lett. 31, 1-10 (1973).
 Michell, R. H. Biochim. biophys. Acta 415, 81-147 (1975).
 Michell, R. H. Trends biochem. Sci 4, 128-131 (1979).
 Lapetina, E. G., Chandrabose, K. & Cuatrecasas, P. Proc. natn. Acad. Sci. U.S.A. 75, 818-822 (1978).

- Lapetina, E. G. & Cuatrecasas, P. Biochim. biophys. Acta 573, 394-402 (1979)
- 6. Lapetina, E. G., Billah, M. M. & Cuatrecasas, P. J. biol. Chem 256, 5037-5040 (1981).
 7. Billah, M. M., Lapetina, E. G. & Cuatrecasas, P. Biochem. biophys. Res. Commun. 90, 92-08 (1979)
- Billah, M. M., Lapetina, E. G. & Cuatrecasas, P. J. biol. Chem. 255, 10227-10231 (1980).
- Walenga, R., Vanderhoek, J. Y. & Feinstein, M. B. *J. biol. Chem.* 255, 6024–6027 (1980).
 Broekman, M. J., Ward, M. W. & Marcus, A. J. *J. clin. Invest.* 66, 275–283 (1980).
- Brockman, M. J., Ward, M. W. & Martus, A. J. J. Clin. Intest. 66, 2132-23 (1930).
 Lapetina, E. G., Billah, M. M. & Cuatrecasas, P. in The Regulation of Coagulation (eds Mann, K. G. & Taylor, F. B.) 491-497 (Elsevier, New York, 1980).
 Billah, M. M., Lapetina, E. G. & Cuatrecasas, P. J. biol. Chem. 256, 5399-5403 (1981).
 Gerrard, J. M., Butler, A. M., Peterson, D. A. & White, J. G. Prostaglandins Med. 1,
- 387-396 (1978).
- 14. Gerrard, J. M. et al. Am. J. Path. 96, 423-438 (1979)
- 15. Gerrard, J. M., Kindom, S. E., Peterson, D. A. & White, J. G. Am. J. Path. 97, 531-548
- 16. Bereziat, G., Chambaz, J., Trugman, G., Pepin, D. & Polonovski, J. J. Lipid Res. 19,
- Irvine, R. F. & Dawson, R. M. C. Biochem. biophys. Res. Commun. 91, 1399-1405 (1979).
- Irvine, K. F. & Dawson, K. M. C. Biochem. biophys. Res. Commun. 2, 1
 McKean, M. L., Smith, J. B. & Silver, M. J. J. biol. Chem. 256, 1522-1524 (1981).
 Mauco, G., Chap, H., Simon, M. F. & Douste-Blazy, L. Biochemie 60, 653-661 (1978).
 Agranoff, B. W., Gradley, R. M. & Grady, R. O. J. biol. Chem. 233, 1077-1083 (1958).
- 21. Bleasdale, J. E., Wallis, P., MacDonald, P. C. & Johnston, J. M. Biochim. biophys. Acta 575, 135-147 (1979).
- Malmsten, C., Granstrom, E. & Samuelsson, B. Biochem. biophys. Res. Commun. 68, 569-576 (1976).
- Lapetina, E. G., Schmitges, C. J., Chandrabose, K. & Cuatrecasas, P. Biochem. biophys. Res. Commun. 76, 828-835 (1977).
- Lapetina, E. G., Schmitges, C. J., Chandrabose, K. & Cuatrecasas, P. in Advances in Prostaglandin and Thromboxane Research Vol. 3 (eds Galli, C., Galli, G. & Porcellati, G.) 127-135 (Raven, New York, 1978). 25. Minkes, M. et al. J. clin. Invest. **59**, 449-454 (1977)
- Gerrard, J. M., Peller, J. D., Krick, T. P. & White, J. G. Prostaglandins 14, 39-50 (1977).
- 27. Feinstein, M. B., Becker, E. L. & Frazer, C. Prostaglandins 14, 1075-1093 (1977)

4',6-Dichloroflavan (BW683C), a new anti-rhinovirus compound

D. J. Bauer, J. W. T. Selway*, J. F. Batchelor, Margaret Tisdale, Ian C. Caldwell & D. A. B. Young

Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK

Derivatives of flavan have been synthesized as chemical intermediates, but the only reported biological action is the ability of certain alkyl and alkoxy derivatives to lower blood cholesterol concentrations1. It was therefore surprising to discover that flavan itself (Table 1) is a highly effective inhibitor of the replication of certain serotypes of rhinovirus, and that a simple derivative, BW683C (4',6-dichloroflavan), is the most potent antiviral compound yet reported. The present work examines the antiviral activity of flavan derivatives with a view to selecting the compound most suitable for trial in volunteers infected with a common cold virus.

4',6-Dichloroflavan, which is new to the chemical literature, has been prepared by methods used for the synthesis of substituted flavans²⁻⁵. It is a colourless crystalline solid, m.p. 101 °C, soluble in water only to the extent of 1 mg l⁻¹ at room tempera-

Antiviral activity was detected in vitro by means of plaque inhibition tests^{6,7} with monolayers of M-HeLa cells^{8,9} infected with rhinovirus 1B. Activity was measured by plaque reduction assays in which doubling concentrations of compound were incorporated into the overlay medium. Plaque counts, expressed as a percentage of the control value, were plotted against the logarithm of the compound concentration, to yield a doseresponse line from which the IC₅₀ value could be determined. The IC₅₀ values for BW683C and several analogues are shown in Table 1. Flavan ($R_6 = R_{4'} = H$), with an IC₅₀ of 0.046 μ M, is one of the least active of the compounds tested. The activity is generally increased by the presence of a single halogen substituent, and more so by the presence of two chlorine atoms, with the most active compound tested being BW683C which, with an IC_{50} of 0.007 μ M, is some six times more potent than the parent compound. The IC90 of BW683C was $0.02~\mu M$.

Structure-activity relationship of a selection of halogen-Table 1 substituted flavans against rhinovirus type 1B

$$R_6$$

R ₆	R ₄ '	IC ₅₀ (μM)	
Н	Н	0.046	
F	H	0.020	
ĈI	Ĥ	0.050	
Br	H	0.019	
H.	F	0.018	
Ĥ	C1	0.039	
Ĥ	Br	0.036	
F	F	0.068	
Cl	CI	0.007	
Br	Br	0.010	
ī	Ī	0.043	
•	-		

For any agent to be effective in the prophylaxis or treatment of the common cold, it must be active against a high proportion of rhinovirus serotypes. There are at least 89 serotypes, the most prevalent being 1A, 1B, 2, 4, 15, 29, 30 and 31 (ref. 10). IC₅₀ values were obtained for BW683C against 19 serotypes (Table 2). Seven of the eight most prevalent serotypes were inhibited, although they varied considerably in sensitivity. The sensitivity of the other 11 serotypes was also variable, but was sufficient to suggest that the compound may be clinically useful.

In tissue culture tests 4',6-dichloroflavan did not inhibit the replication of other RNA viruses, including bunyavirus, coronavirus, equine rhinovirus, influenza virus (NWS strain), measles virus, poliovirus (Sabin 1), Semliki Forest virus, Sindbis virus and respiratory syncytial virus. It also failed to inhibit the DNA viruses adenovirus type 5 and herpesvirus type 1.

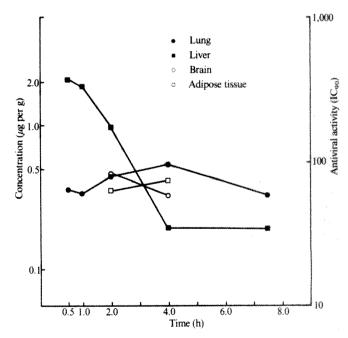


Fig. 1 Tissue concentrations of BW683C determined by gasliquid chromatography. Tissue homogenate (1 ml) was mixed with 0.5 ml ethylene glycol/water/2 M citric acid (2:2:1) and 5 ml hexane. The mixture was shaken for 30 min and centrifuged. The hexane layer was collected and mixed with 1 ml of a mixture of ethylene glycol/1 M Na₂CO₃ (1:9), shaken again and centrifuged. The upper layer was collected, dried in a stream of N₂ and the residue was dissolved in a small volume of toluene

^{*} To whom correspondence should be addressed.

Serotype	$IC_{50}(\mu M)$	Serotype	IC ₅₀ (μM)
1A*	0.013	13	0.66
1B*	0.007	14	Inactive
2*	0.04	15*	0.17
3	10.10	16	0.02
4*	Inactive	18	0.29
5	Inactive	19	0.81
8	8.00	21	1.70
9	0.011	29*	0.008
12	0.15	30*	52.00
		31*	0.013

^{*} Serotypes described by Roebuck¹⁰ as common.

Preliminary studies were carried out on the absorption and tissue distribution of 4',6-dichloroflavan in rats after its administration at 5 mg per kg by gavage as a suspension of finely ground particles in aqueous methylcellulose. Samples of plasma and selected tissues were collected at intervals up to 7.5 h after dosing, and unchanged compound was determined by analysis of tissue extracts by gas-liquid chromatography with electron capture detection or by specific ion monitoring using a mass spectrometer. Highest concentrations were found in liver (Fig. 1); peak concentrations of $\sim 7.3 \, \mu M$, some 350 times the IC₉₀, were observed at 0.5 h, and these declined rapidly. Concentrations of compound well in excess of the IC₉₀ value were also detected in plasma, lung, brain and adipose tissue, and the time of peak compound concentrations in lung samples seemed to occur between 2 and 4 h after dosing; compound concentrations in excess of the IC₉₀ level could still be found in lung 7.5 h after dosing. These results indicate that 4',6-dichloroflavan is adequately absorbed from the gastrointestinal tract, and that compound concentrations well above those required for maximum antiviral activity in vitro can be attained at doses as low as 5 mg per kg. The apparent persistence of this compound implies that infrequent administration may suffice to maintain effective antiviral concentrations in vivo. The above findings were supported by autoradiography of rats, which received 'H-BW683C as an aqueous suspension by gavage, and were killed 2 or 4 h later.

When BW683C was added to HeLa cells in culture no cytotoxic effects were observed at drug concentrations up to 3.6 µM, the limit of aqueous solubility and many times the IC₉₀ and IC₅₀ values determined with rhinovirus 1B. In the rat, the LD₅₀ after subcutaneous administration was ~300 mg per kg; animals showed no ill effects after oral administration at doses up to 1 g per kg or intraperitoneal administration at doses up to 700 mg per kg. BW683C showed no evidence of mutagenicity when tested in the presence or absence of a liver microsome preparation by the method of Ames¹¹

Preliminary studies showed that BW683C interacted directly with virus, reducing infectivity by about 0.5 log unit, but it was not a typical contact inactivator because inactivation did not increase with time. The possible binding of compound to virus particles was investigated by mixing tritiated compound with virus and centrifuging the mixture through a 15-45% sucrose gradient; radioactively labelled compound was found in the virus peak.

In a time-of-addition study, the compound was added at hourly intervals throughout a single growth cycle of rhinovirus 1B in M-HeLa cells. Maximum antiviral activity was obtained when the compound was added to the culture at the same time as the virus. Although some antiviral activity was observed when addition was delayed for 1 h or longer, it was no greater than that obtained by adding compound at the end of the cycle.

Further experiments with ³H-uridine-labelled virus indicated that the compound does not interfere with adsorption of the virus to the cell, nor with the uncoating or entry of the viral RNA into the cell. However, viral RNA synthesis was inhibited by the presence of the compound. BW683C therefore seems to bind to the virus and to inhibit some stage of viral replication immediately following entry of the viral RNA into the cell.

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- 1. US Patent No. 3,555,047 (1971).
- Robertson, A., Venkateswarlu, V. & Whalley, W. B. J. chem. Soc., 3137-3142 (1954).
 Clark-Lewis, J. W., Jemison, R. W., Skingle, D. C. & Williams, L. R. Chem. Ind., 1455-1456 (1967).
- 4. Jurd, L. Chem. Ind., 2175-2176 (1967)
- Jurd, L. Chem. Ind., 2175-2176 (1967).
 Hultzsch, K. J. prakt. Chem. 158, 275-294 (1941).
 Rada, B., B., Blaškovič, D., Šorm, F. & Škoda, J. Experientia 16, 487 (1960).
 Herrmann, E. C. Proc. Soc. exp. Biol. Med. 107, 142-145 (1961).
 Fiala, M. & Kenny, G. E. J. Bact. 92, 1710-1715 (1966).
 Scamans, E. M. thesis, Univ. London (1974).
 Roebuck, M. O. J. Hyg. Camb. 76, 137-146 (1976).
 Ames, B. N., McCann, J. & Yamasaki, E. Mutat. Res. 31, 347-364 (1975).

Mutation of gene encoding regulatory polypeptide of aspartate carbamoyltransferase

André Feller*†, André Piérard*†, Nicolas Glansdorff†‡, Daniel Charlier†‡ & Mariolène Crabeel†‡

* Laboratoire de Microbiologie, Faculté des Sciences, Université Libre de Bruxelles, 1, avenue E. Gryson, B-1070 Bruxelles, Belgium † Institut de Recherches du CERIA, 1, avenue E. Gryson, B-1070 Bruxelles, Belgium

‡ Erfelijkheidsleer en Microbiologie, Vrije Universiteit Brussel, E. Grysonlaan, 1-1070 Brussel, Belgium

In our studies on the regulation of the expression of the genes for the biosynthesis of arginine and pyrimidines in Escherichia coli, we discovered that an aspartate carbamoyltransferase (ATCase) synthesized in vitro from our λ transducing phage λ0TC3 lacked substrate cooperative interactions and was insensitive to feedback inhibition by CTP. We show here that these abnormal properties result from a mutation in the gene for the regulatory polypeptide chain of ATCase. We believe this to be the first report of a mutation in the gene for this regulatory

ATCase catalyses the first reaction specific to pyrimidine biosynthesis, the formation of N-carbamoyl-L-aspartate from L-aspartate and carbamoylphosphate. In E. coli, ATCase activity is modulated by feedback inhibition by CTP, the end product of the pyrimidine pathway, and activation by ATP, product of the parallel purine pathway¹. Because E. coli ATCase exhibits the various types of regulatory interactions characteristic of allosteric proteins, it has been considered a model system and has become the most extensively investigated regulatory enzyme^{2,3}. It differs, however, from most other allosteric proteins in that its catalytic and regulatory functions can be physically separated. Native ATCase, with a molecular weight (MW) of 300,000, consists of two trimeric subunits which possess catalytic activity but are insensitive to the allosteric effectors and three dimeric subunits which are catalytically inactive but bear the effector binding sites⁴⁻⁶. Thus, the completely different catalytic (C) and regulatory (R) chains (respective MWs 34,000 and 17,000) form a 2C₃: 3R₂ structure. Zn²⁺ ions, one per regulatory chain, are required to stabilize this quaternary structure7

The zinc-binding domains on the regulatory subunits participate in the R:C bonding domains essential for the allosteric interactions¹⁰. The gene pyrB coding for the catalytic chain of ATCase lies at 96 min on the $E.\ coli$ chromosome ^{11,12}, but the localization of the gene for the regulatory chain has been hampered by the failure to obtain mutations affecting this gene. However, a thorough investigation of their biosynthesis 13,14

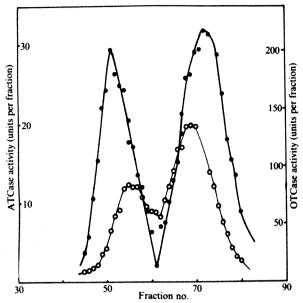


Fig. 1 Analysis of aspartate carbamovltransferase (ATCase) from strain PR7 by gel filtration on a column of Sephadex G-200. A 1-ml sample (containing 1,200 units and 10 mg protein) of crude extract of PR7 cells grown on minimal medium and extracted as described previously 21.22 was filtered on a 2.5 × 40-cm column of Sephadex G-200 equilibrated with 50 mM potassium phosphate buffer (pH 7.5). Elution was performed using the same buffer and 1.5-ml fractions were collected. E. coli and Streptococcus faecalis ornithine carbamoyltransferase (OTCase), exhibiting respective apparent MWs of 140,000 and 250,000 (ref. 23), were used as protein markers for calculating ATCase molecular weight. ATCase activity was determined by measuring the formation of ¹⁴C-carbamoylaspartate from ¹⁴C-carbamoylphosphate. The complete $(600 \,\mu 1)$ assay mixture containing 50 mM Tris-acetate (pH 8.0), 20 mM L-aspartate, 5 mM 14 C-carbamoylphosphate $((0.03 \,\mathrm{mCi \,mmol^{-1}})$ and enzyme sample was incubated at 37 °C for 15 min. The reaction was stopped by the addition of 400 $\mu l\,0.25~M$ trichloracetic acid. After 5 min boiling to destroy the non-reacted carbamoylphosphate, ¹⁴CO₂ formed was eliminated by bubbling air for 5 min. A 0.5-ml sample was counted in a model LS-100 Beckman liquid scintillation spectrometer in the presence of a scintillation cocktail containing 4 parts of Triton X-100 to 6 parts of a solution containing 0.5% 2,5-diphenyloxazole and 10%naphthalene in dioxane. A blank obtained by omitting the enzyme sample was subtracted from the results. OTCase was assayed as described previously²⁴. Units of enzyme activity are µmol of product formed per h. O, ATCase activity; O, OTCase activity.

suggested the existence of an operon encoding both types of ATCase chains in the order catalytic first and regulatory next. Moreover, in Salmonella typhimurium, which has a very similar ATCase¹⁵, pyrB deletions have been found to produce no detectable regulatory subunit¹⁶. The present study characterizes a λ transducing phage carrying the genetic determinants of an E. coli ATCase with modified regulatory subunits. This establishes the proximity of the genes encoding both types of chain. In agreement with this conclusion, the pyrB region of E. coli was

cloned recently as a 2,800-base pair DNA fragment encoding both subunits of ATCase (J. R. Wild & G. A. O'Donovan, personal communication). In agreement with Wild et al. (see accompanying report)¹⁷ in the present study we reserve the designation pyrB for the gene encoding the C chain of ATCase and use the symbol pyrI for the R chain of this enzyme.

 λ Bacteriophages transducing the contiguous *E. coli* genes argI (encoding ornithine carbamoyltransferase) and $pyrB^{18}$ have been used as a source of template DNA for in vitro synthesis of ATCase¹⁹. However, the enzyme synthesized using one of these DNA sources, λ 0TC3, was insensitive to CTP inhibition and exhibited hyperbolic aspartate saturation curves. Accordingly, bacteriophage λ 0TC3 was introduced into strain KMBL1510 G4, a thermoresistant derivative of strain KMBL1510 (F⁻ pyrB::Mu-1 cts62) that produces no detectable amount of either ATCase subunit (G. Hervé and B. Perbal, personal communication).

ATCase activity in the resulting strain PR7, was only slightly sensitive to CTP and lacked cooperative interactions with aspartate. When cell extracts of strain PR7 were submitted to gel filtration on a column of Sephadex G-200 (Fig. 1), two ATCases were observed with respective MWs of 300,000 and 100,000. Using the same experimental conditions, practically all the ATCase activity of a wild-type strain for this enzyme was present as a single activity peak with a MW of 300,000, and negligible amounts as the 100,000 MW species. Both ATCase forms of strain PR7 exhibited hyperbolic aspartate saturation curves (Fig. 2b, c), the half-saturation concentration of the larger form ($[S]_{0.5} = 6.5 \text{ mM}$) being significantly lower than that of the smaller form ($[S]_{0.5} = 23 \text{ mM}$). Whereas the larger ATCase of strain PR7 exhibited a reduced response to CTP and ATP, the smaller ATCase of this strain was totally insensitive to these effectors.

Comparison of the two ATCases of PR7 with the wild-type holoenzyme and its catalytic subunit (Table 1) suggested that the smaller ATCase (trimeric C₃) was identical with the wild-type catalytic subunit. The larger species corresponded to a 300,000-MW holoenzyme formed from this same catalytic subunit associated with a modified regulatory subunit. This interpretation is supported by the following experiments. (1) Treatment of the larger ATCase from PR7 with p-hydroxymercuribenzoate20 vielded a smaller ATCase (MW 100,000) having all the properties of the wild-type catalytic subunit. (2) A single ATCase species (MW 300,000) displaying sigmoidal aspartate saturation and effector responses similar to those of wild-type ATCase (Table 1 and Fig. 2a, d) could be reconstituted by combining the catalytic trimer of strain PR7 with purified wild-type regulatory subunit. (3) A similar reconstitution could be achieved in vivo by introducing the phage λ 0TC3 into strain 30S0U4 Hfr thi pyrB) harbouring a missense mutation in the pyrB gene but producing normal regulatory chain (Fig. 2e). These results indicate that phage λ 0TC3 encodes a wild-type catalytic ATCase chain and a modified regulatory chain.

The precise nature of the modification affecting the regulatory chains of ATCase remains to be determined. Nevertheless, heteroduplex analysis (not shown) of phage $\lambda 0 \text{TC}3$ indicates

Table 1 Comparison of aspartate carbamoyltransferases (ATCases) used in the present study

ATCase species	Wild-type (native)	Wild-type (catalytic subunit)	Modified (larger MW)	Modified (smaller MW)	Reconstituted holoenzyme (PR7 catalytic + wild-type regulatory subunits)
Molecular weight	300,000	100,000	300,000	100,000	300,000
Subunit structure	$2C_3:3R_2$	C_3	2C3:3R2	Ċ,	2C ₃ :3R ₂
Aspartate kinetics	Sigmoidal	Hyperbolic	Hyperbolic	Hyperbolic	Sigmoidal
$[S]_{0.5}$ value for aspartate	18 mM	25 mM	6.5 mM [†]	23 mM†	14 mM
CTP inhibition*	77%	No effect	11%	No effect	38%
ATC activation*	360%	No effect	38%	No effect	256%

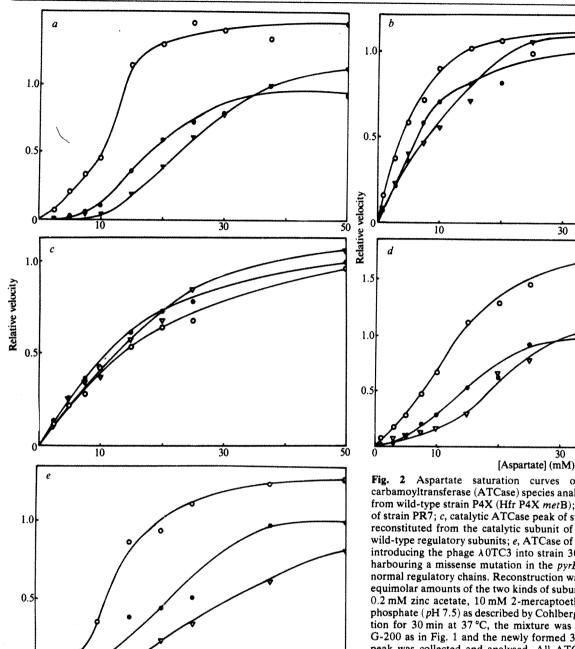
The $[S]_{0.5}$ value was determined at pH 8 as described in Fig. 1 legend.

† $[S]_{0.5}$ values calculated from Lineweaver-Burk plots of the data from Fig. 2b, c.

^{*} Inhibition by 2 mM CTP and activation by 5 mM ATP in the presence of 5 mM aspartate.

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that the modified gene lies at the extremity of a 7,300-base pair chromosomal segment inserted into the phage genome. It is therefore possible that a short 3'-terminal portion of the gene has been deleted during the formation of phage λ 0TC3. This hypothesis is being evaluated by cloning and sequencing the modified regulatory gene. Interestingly, the C-terminal end of the regulatory chain with its zinc-binding sites lies at the interface between the regulatory and catalytic chains 10. Even short 3'-terminal deletions affecting the regulatory chain may thus impair the R: C binding domain. This might be the case for the modified ATCase encoded by \(\lambda\) OTC3 which, as suggested by the presence of two ATCase forms in extracts of strain PR7, may be impaired in holoenzyme assembly.

[Aspartate] (mM)

Further work is being done on the purification and characterization of the heavy ATCase species encoded by λ 0TC3. A detailed analysis of this unusual enzyme and its comparison with the wild-type enzyme may be vital for determining the role of the R:C domain of bonding in the allosteric interactions of ATCase. For example, it will be of interest to investigate whether the modified regulatory subunits have altered proper-

Fig. 2 Aspartate saturation curves of various aspartate carbamoyltransferase (ATCase) species analysed here. a, ATCase from wild-type strain P4X (Hfr P4X metB); b, holo ATCase peak of strain PR7; c, catalytic ATCase peak of strain PR7; d, ATCase reconstituted from the catalytic subunit of ATCase of PR7 and wild-type regulatory subunits; e, ATCase of the strain obtained by introducing the phage $\lambda 0TC3$ into strain 30S0U4 (Hfr thi pyrB) harbouring a missense mutation in the pyrB gene but producing normal regulatory chains. Reconstruction was achieved by mixing equimolar amounts of the two kinds of subunits in the presence of 0.2 mM zinc acetate, 10 mM 2-mercaptoethanol and 50 mM K-phosphate (pH 7.5) as described by Cohlberg et al.9. After incubation for 30 min at 37 °C, the mixture was filtered on Sephadex G-200 as in Fig. 1 and the newly formed 300,000-MW ATCase peak was collected and analysed. All ATCase assay conditions were as for Fig. 1. Relative activities are normalized to the activity measured without effector in the presence of 50 mM aspartate. In the absence of allosteric effector; O, in the presence of 5 mM ATP; ∇ , in the presence of 2 mM CTP.

ties towards zinc. Although the ATCase distribution shown in Fig. 1 could not be modified following growth in the presence of high Zn²⁺ concentrations, preliminary experiments suggest that reaggregation of the two kinds of subunits of strain PR7 can be promoted in vitro provided that a high enough Zn2+ concentration is present.

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- Gerhart, J. C. & Pardee, A. B. J. biol. Chem. 237, 891-896 (1962). Gerhart, J. C. Curr, Topics cell. Regulation 2, 275-325 (1970).
- Kantrowitz, E. R., Pastra-Landis, S. C. & Lipscomb, W. N. Trends biochem. Sci. 5, 124-128, 150-153 (1980).
- Gerhart, J. C. & Schachman, H. K. Biochemistry 4, 1054-1062 (1965); 7, 538-552 (1968)
- Weber, K. Nature 218, 1116-1119 (1968). Wiley, D. C. & Lipscomb, W. N. Nature 218, 1119-1121 (1968).
- 7. Rosenbusch, J. P. & Weber, K. Proc. natn. Acad. Sci. U.S. 68, 1019-1023 (1971).

8. Nolbuch, M. B., Paget, V. P., Gorhardt, J. C. & Schachman, H. K. Biochemistry 11,

. .

- Cohlberg, J. A., Papet, V. P. & Schachman, H. K. Bischemerry 11, 3396-3411 (1972).
 Mosaco, H. L., Crawford, J. L. & Lipacomb, W. N. Proc. nam. Acad. Sci. U.S. A. 78, 5276-5280 (1978).
- 34/0-3480 (19/8).

 1. Beckwitz, J. R., Pardee, A. B., Anstran, R. & Jacob, F. J. molec. Biol. 8, 618-634 (1962).

 12. Beckmann, B. J. & Low, K. B. Microbiol. Rev. 44, 1-56 (1980).

 13. Perbal, B. & Hervé, G. J. molec. Biol. 70, 511-529 (1972).

 14. Parbal, B., Guagen, P. & Harvé, G. J. molec. Biol. 118, 319-340 (1977).

 15. O'Donoven, G. A., Holoubok, H. & Gerhart, J. C., Neture new Biol. 238, 264-266 (1972).

 16. Syvenen, J. M. & Roth, J. R. J. molec. Biol. 76, 363-378 (1973).

 17. Wild, J. R., Robremson, K. F., Roof, W. D. & O'Donoven, G. A., Mirror. 202, 272, 274.

- 17. Wlid, J. R., Foltermann, K. F., Roof, W. D. & O'Donovan, G. A. Nature 292, 373-375
- (1981), 18. Lagram, C., & Gianedorff, N. J. Bact. 128, 35–38 (1976).
 19. Fallor, A., Lassens, W., Gianedorff, N. & Pafrard, A. Arche Int. Physiol. Bischies. 86, 941–942 (1978).
- Gerhart, J. C. & Holoubeck, H. J. Mel. Chem. 242, 2886-2892 (1967) Chanadorff, N. Genetics St. 167-179 (1965)
- Margasy, M., Gusta, D., Bockmann, J., Glansdorff, N. & Piérard, A. Molec gen. Genet. 133, 299–316 (1974)
- Lagram, C. et al. Eur. J. Buchem. 20, 401–409 (1977).
 Wargmos, B., Lawrers, N. & Stalon, V. Bur. J. Buchem. 101, 143–152 (1979)

A mutation in the catalytic cistron of aspartate carbamovltransferase affecting catalysis, regulatory response and holoenzyme assembly

James R. Wild, Karen F. Foltermann*. William D. Roof* & Gerard A. O'Donovan*

Genetics Section and *Department of Biochemistry and Biophysics, Texas A & M University, College Station, Texas 77843, USA

We describe here a mutation in the gene encoding the catalytic subunit of aspartate carbamoyltransferase (ATCase, pyrB) which produces an enzyme retaining catalytic activity as holoenzyme (2C₃:3R₂) and catalytic trimer (C₃) but which shows neither cooperative substrate kinetics nor nucleotide effector response. Furthermore, the holoenzyme assembly seems quite fragile in that the enzymatic activity is recovered only partially as holoenzyme following growth of Escherichia coli in the presence of zinc. In contrast, the enzyme from wild-type E. coli strain E63 is recovered almost entirely in its dodecameric form in identical conditions. The gene encoding the mutant pyrB was isolated from a AdargI+pyrB+ transducing phage (obtained from N. Glansdorff). This mutation differs from other previously reported mutations affecting the catalytic subunit^{1,2} in that significant catalytic activity is retained but homotropic and heterotropic communication is lost³.

A 6.0-kilobase fragment was isolated from purified λDNA^4 , restricted with the endonuclease PstI (Bethesda Research Laboratory)⁵, and cloned on to plasmid pBR322 (ref. 6). The recombinant plasmid pPB-h204 (plasmid-pyrimidine-B cistron-holoenzyme-strain 204) was transformed into E. coli WR38, which contains a Mu insertion in the chromosomal pyrB and does not produce catalytic or regulatory polypeptides as determined by immunoassay with specific antisera (W.D.R., unpublished observations). This plasmid encodes both the catalytic (pyrB) and the regulatory (pyrI) polypeptides of ATCase. [A mutation in the cistron encoding the regulatory polypeptide is described in the accompanying report by Feller et al. $^{\circ}$. The cistron is designated pyrI in agreement with Bachmann (personal communication).] The synthesis of ATCase in the transformed strain, WR38-h204, is repressed by growth in uracil, so that the cloned fragment contains the operatorpromoter region as well as both ATCase cistrons. The catalytic activity recovered from this strain is distributed as ~40% holoenzyme with a molecular weight (M_r) of 300,000 and 60% as the catalytic trimer with a M_r of 100,000. Both forms of ATCase from this mutant lack cooperative homotropic kinetics for aspartate, do not respond to the allosteric effectors ATP and CTP, and seem to have altered affinities for aspartate.

ATCase is the archetype of allosteric enzymes because: (1) both its substrates, carbamoyl phosphate and aspartate, produce

Table 1 ATCase holoenzymes from wild-type E. coll strain E63 and mutant E. coli strain WR38-h204

	Wild-type	Mutant
[S] _{0.5} (mM aspartate) % Activity + CTP % Activity + ATP	5.5 mM 21% 166%	2.0–2.5 mM 97% 105%
ATCase activity at pH 7.0 ATCase activity at pH 8.4	3.1	0.51
Shape of velocity-substrate curve	Sigmoidal	Hyperbolic

ATCase assay mixtures of 2.0 ml volume contained 40 mM potassium phosphate, pH 7.0, 3.6 mM dilithium carbamoyl phosphate, pH 7.0, 5 mM potassium aspartate, pH 7.0, for the wild-type and 2.5 mM for the mutant (approximate $[S]_0$ values), and enzyme (holoenzyme fractions of G-200 cluate, Fig. 2). CTP or ATP was absent from control tubes and present at a concentration of 2 mM when inhibition (CTP) or activation (ATP) was measured. The control activity, that is, the activity in the absence of effector, is set at 100%. ATCase activity was assayed by measuring the amount of carbamoyl aspartate formed in 30 min at 30 °C as previously described23. Carbamoyl aspartate production was determined at 466 nm. Specific activity is expressed as nmol carbamoyl aspartate formed per min per mg protein. Specific activity of ATCase was determined in conditions in which carbamoyl aspartate formation was proportional to extract concentration and time. The value obtained for the ratio of ATCase specific activity at pH 7.0/ATCase specific activity at pH 8.4 may be used as an index for the presence or absence of cooperativity between catalytic sites for aspartate binding according to the methods of Kerbiriou and Hervé¹¹. A ratio of <1.0 signifies the absence of cooperativity; one of ≥2.0 reflects cooperative homotropic interactions. Practions from beneath the holoenzyme peak (Fig. 2) were used to estimate this ratio.

positive homotropic interactions between catalytic sites as evidenced by the sigmoidal dependence of activity on substrate concentrations^{9,10}, (2) substrate binding is subject to positive heterotropic interactions between catalytic and regulatory sites in the presence of ATP and negative heterotropic interactions with CTP¹¹⁻¹⁴, and (3) the binding of CTP is subject to negative homotropic interactions between the regulatory sites12 Moreover, ATCase is unusual among allosterically regulated enzymes, in that, as with yeast phosphofructokinase 16 regulatory protein is distinct and can be physically dissociated from the catalytic subunit. The assembly of the enzyme is a cytoplasmic event17 which produces a dodecamer with six regulatory and six catalytic polypeptides (r₆c₆) associated as two separable catalytic trimers and three regulatory dimers 18,19. The holoenzyme may be reversibly dissociated by mild treatment with mercurials such as p-chloromercuribenzoate20 or neohydrin²¹. The catalytic subunit (c₃) is insensitive to allosteric effectors, possesses a half-saturation concentration ([S]_{0.5}) higher for aspartate than the holoenzyme (8-10 mM compared with 5 mM) and produces a V_{max} that is two- to fourfold higher. The separate regulatory subunit (r2) has no catalytic activity although ATP and CTP may still be bound²⁰. On reassociation of the holoenzyme after removal of the mercurial by zinc replacement dialysis in the presence of dithiothreitol, the original catalytic and regulatory properties of the native enzyme are re-established²²

ATCase was prepared from E. coli wild-type strain E63 and the transformed mutant strain WR38-h204 by the methods of Wild et al.23. Several pertinent properties of the wild-type and mutant ATCases are compared in Table 1. We must emphasize three points regarding these data. (1) The mutant ATCase is not subject to allosteric regulation by either ATP or CTP at subsaturating concentrations of aspartate (1-5 mM). Minimal effector response was observed when velocity-substrate plots were examined over the range of 0.5 mM to 50 mM aspartate (<5% variation throughout the range). As noted by Gerhart² effects are due only to the direct competition for any phosphatecontaining compound. (2) The apparent [S]05 for aspartate of the mutant enzyme is significantly lower (2.0-2.5 mM) than the wild-type requirements (5.5 mM). (3) The homotropic kinetic responses of the mutant enzyme are dramatically reduced or abolished in the mutant enzyme as shown by the lack of sigmoidal dependence of activity on aspartate concentration (Fig. 1) even when plotted according to Eadie-Hofstee²⁵ (see insert Fig. 1). Thus, there is no apparent cooperativity from 0.5 to 10 mM (20% to fivefold [S]0.5, respectively). In addition, the

ratio of ATCase activity at pH 7.0 and 8.4 may be used as an index of the presence or absence of homotropic interactions between catalytic sites as first described by Kerbiriou and Hervé¹¹. A ratio of <1.0 signifies the absence of cooperativity and is always found for the catalytic subunits alone, whereas a ratio of \geq 2.0 reflects cooperative homotropic interactions¹¹. We have verified these observations in similar experiments (K.F.F. unpublished observations). By this criterion, the ATCase from strain WR38-h204 seems to lack cooperativity.

Figure 2 shows an analysis of the M_r values of the ATCases from mutant and wild-type enzymes in the presence of zinc. A single large molecular weight form ($\sim 300,000$) was observed from the wild-type extract. In contrast, more than half of the enzymatic activity from the mutant extract was observed as a smaller component ($M_r \sim 100,000$) which corresponds to the wild-type catalytic trimer. We have shown previously that all native ATCase from the enteric bacteria grown in sufficient zinc ($20 \,\mu\text{M}$) studied thus far are recovered entirely as holoenzyme²³. Only when zinc becomes limiting or there is an assembly defect can the catalytic trimer be recovered from cell-free extracts.

The following evidence suggests that these differences are consequences of an altered catalytic subunit. (1) The catalytic trimer from the mutant enzyme shows increased affinity for aspartate compared with the catalytic trimer ([S]_{0.5} of 5 mM aspartate compared with 8-10 mM) of the wild-type enzyme. (2) The M_r values of the catalytic trimers are indistinguishable, but they form clearly different holoenzymes when reconstituted with wild-type regulatory dimers (Table 2). The holoenzyme

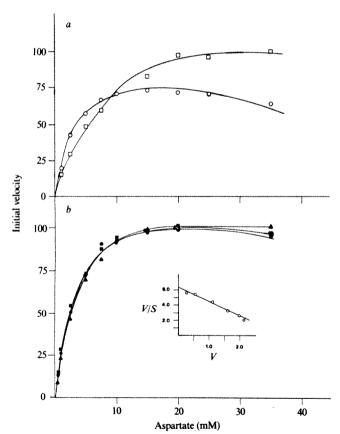


Fig. 1 Aspartate saturation curves for ATCase holoenzyme and its catalytic trimers from E. coli mutant strain WR38-h204. Enzyme samples were prepared as reported previously²³. Assay mixtures were as described in Table 1 legend except that the aspartate concentration was varied from 0.5 mM to 50 mM as indicated on the abscissa. CTP or ATP was absent from control tubes and present at 2 mM when inhibition (CTP) or activation (ATP) was measured. For the above comparisons initial rate values were normalized as described below. a, Velocity-substrate plots for aspartate of the ATCase holoenzyme (○) and its catalytic trimer (□) from the mutant. The maximum velocity for the catalytic trimers was set at 100 on the ordinate scale for convenience. b, Velocity-substrate plots for aspartate of the ATCase holoenzyme from the mutant in the absence of the effectors (●) and in their presence: CTP (△) or ATP (■).

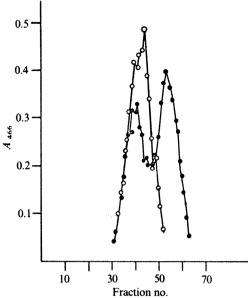


Fig. 2 Analysis of ATCase from E. coli wild-type E63 and mutant WR38-h204 by gel filtration on a G-200 Sephadex column; 1 ml, containing ~20 mg protein, of the appropriate cell-free extract was applied to the G-200 column, equilibrated previously with 40 mM potassium phosphate buffer pH 7.0, 0.1 mM dithiothreitol and 0.02 mM zinc acetate. The equilibration mixture was also used for elution. Individual fractions (abscissa) were assayed for ATCase by a spot assay. This differed from the usual assay²³ in that the final reaction volume was reduced from 2.0 ml to 0.2 ml and 30-50 mM aspartate was used. The ordinate gives absorbance at 466 nm, the wavelength for monitoring ATCase. A single wild-type peak (○) and two mutant peaks (●) are seen.

formed from wild-type regulatory dimers and mutant catalytic trimers has a M_r of $\sim 300,000$, but its regulatory and catalytic properties are those of the original mutant enzyme.

We have shown that a mutation in the pyrB gene, encoding the catalytic polypeptide of ATCase, can affect the homotropic and heterotropic interactions of the enzyme while maintaining catalytic activity. The reconstitution experiments with normal regulatory subunits purified from the native enzyme and catalytic subunits from both the mutant and wild-type strains prove that the catalytic polypeptide of the ATCase from strain WR38-h204 is altered. It is surprising that a mutation in the cistron encoding the catalytic polypeptide can affect such a wide range of enzymatic properties without completely disrupting the

Table 2 Catalytic trimers of ATCase from wild-type and mutant E. coli strains and their reconstituted holoenzyme derivatives

	Wild-type	Mutant
M_r for c_3	85,000-100,000	85,000-100,000
[S] _{0.5} aspartate for c ₃	8-10 mM	5 mM
M, for holoenzyme	280,000-300,000	280,000-300,000
[S] _{0.5} aspartate for		
holoenzyme	5.5 mM	2.0 mM
% Activity in 2 mM CTP	20	86-95
% Activity in 2 mM ATP	160	97-105

Reconstitution experiments were performed as follows. Catalytic subunits from the wild-type and mutant strains were mixed individually with excess of wild-type regulatory subunits and concentrated on an Amicon ultrafiltration system (PM-10 membrane) in the presence of 40 mM potassium phosphate buffer, pH 7.0, 0.2 mM zinc acetate, 2.0 mM dithiothreitol and 20 µM EDTA. The reconstitution of holoenzyme with both wild-type and mutant catalytic trimers was almost 100%. Effector response was measured exactly as described for Table 1. The effector response was determined at the appropriate [S]_{0.5} for each enzyme (that is 5 mM or 2.5 mM). Molecular weights were estimated using Sephadex G-200 ascending flow column chromatography. Standards used in column calibration were: RNase A, M_r 13,700; chymotrypsinogen A, 25,000; ovalbumin 45,000; aldolase 158,000; ATCase holoenzyme from E. coli 300,000; Blue Dextran 2000, 2×10^6 . These standard enzymes (5-20 mg of each, except ATCase) were prepared in a 2.0-ml volume of 40 mM potassium phosphate, pH 7.0, with 0.02 mM zinc acetate, 0.1 mM dithiothreitol, and applied to the column. The elution profiles of the various proteins were determined by monitoring absorbance at 280 nm. ATCase was used separately to verify the accuracy of the column at higher molecular weights. Catalytic and regulatory subunits from the wild type were prepared as described previously²¹. The catalytic subunit from the mutant was obtained from appropriate Sephadex G-200 column fractions (Fig. 2).

catalytic capabilities of the enzyme. This mutation should be compared with that affecting the regulatory polypeptide described by Feller et al. in the accompanying report. It is striking that the holoenzyme assembly is apparently deficient in both mutants, one affecting the catalytic sununit and the other the regulatory subunit.

. One final observation regarding the catalytic mutation seems pertinent. Examination of the kinetic characteristics (Fig. 1) of the mutant (with or without ATP added) revealed that they approximate the wild-type enzyme in the presence of ATP. Indeed, the high affinity exhibited by the mutant holoenzyme for aspartate suggests that the mutant ATCase may be frozen in its activated R state²⁶. The present data suggest that strikingly similar characteristics can exist for two independent mutations in either the catalytic or regulatory cistrons of ATCase.

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- Wall, K. A., Fintgeard, J. E., Gibbons, I & Schackman, H. K. J. biol Chem 254, 11910-11916 (1979)
- Beckwith, J. R., Pardes, A. B., Anstrian, R. & Jacob, R. J. molec. Biol. 5, 615-634 (1962). 3. Kentrowitz, H. R., Pestra-Landis, S. C. & Lipscomb, W. N. Trends blochess. Sci. 5, 124-128
- Deva, R. W., Botztein, D. & Roth, J. R. Manuel for Genetic Engineering, 80–82 (Cold Spring Harbor Laboratory, New York, 1960).
- Smith, D. E., Blatther, F. R. & Davies, J. Michic acid Res. 3, 343–353 (1976)
 Bollver, F. et al. Gene 2, 95–113 (1977).
 Taylor, A. L. Frec. natu. Acad. Sci. U.S.A. 89, 1043–1051 (1963).

- er, A., Fiferard, A., Glansdorff, N., Charber, D. & Crabesl, M. Namer 292, 370-373 (1981)
- Gerbert, J. C. & Scheck nn, H. K. Biechembury 7, 538-552 (1968).
- 10. Bethell, M. R., Smith, K. E., White, J. S. & Jones, M. E. Proc. nam. Acad. Sci. U.S.A. 60, 1442-1449 (1968).
- Karbiriou, D. & Harvi, G. J. molec. Biol. 64, 379-392 (1972).
 Karbiriou, D. & Hervi, G. J. molec. Biol. 78, 687-702 (1973).
- 13 Kantrowitz, E. R., Jacobsberg, L. B., Landfear, S. M. & Lipscomb, W. N. Proc. nem. Acad. Sci. U.S.A. 74, 111-114 (1977)
- Karbirion, D., Harvé, G. & Griffin, J. H. J. Isol. Chem. 252, 2881–2890 (1977)
 Wikitood, C. C. & Chamberlin, M. J. Brochem Maphys. Rev. Commun. 46, 43–49 (1970).
 Laurent, M., Chaffotts, A. F., Tenu, J. P. Roucous, C. & Seydoux, F. Bischem Mophys. Rev. Comprese. 80, 646-652 (1978).
- Thury, L. & Hervé, G. J. molec. Biol. 128, 515-534 (1978)
 Weber, K. Netters 218, 1116-1119 (1968).

- Weber, K. Nemer Alls, 1119-1119 (1998).
 Wiley, D. C. & Lipscomb, W. N. Nemer 218, 1119-1121 (1968).
 Gerhart, J. C. & Sobsoliman, H. K. Bacchestury 4, 1054-1062 (1965).
 Yang, Y. R., Kirschner, M. W. & Sobsoliman, H. K. Mesh. Enzyst. \$1, 35-41 (1978).
 Nelbach, M. E., Pigiet, V. P., Gerhart, J. C. & Schachman, H. K. Biochesticy 11, 315-327 (1978).
- (1972).
 Wild, J. R., Pottermann, K. F. & O'Donovan, G. A. Archa Binchem Biophys 201, 506-517
- Gerhart, J. C. Curr. Topics cell. Regulation 2, 275-325 (1970).
 Eache, G. S. J. biol. Chem. 146, 85-93 (1942).
- Monod, J., Wyman, J. & Changeux, J.-P. J. molec. Biol 12, 88-118 (1965).

Sequence dependence of the helical repeat of DNA in solution

Lawrence J. Peck & James C. Wang

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138, USA

Considerable progress has recently been made on the fine structure of DNA. X-ray diffraction studies of crystals of oligonucleotides of defined sequences have already provided several structures at atomic resolution 1-6. Whereas all the crystals studied so far show bihelical structures with antiparallel chains and Watson-Crick-type base pairing, there is a striking range of structural variability, from the right-handed B-type to the left-handed Z-type helices. In contrast to the well developed methology for crystal structure determination, few methods of high precision and low ambiguity are available for studies of DNA in solution. Here we report the application of the bandshift method previously developed in this laboratory to the determination of the dependence of the DNA helical repeat in solution on its nucleotide sequence.

Plearrad				Total areart length(top)
PBR322	:::ELECV	Pat I ata	ACSTC:::	0 .
PLP119	::: [[]		::::∰{A6:::	19
PLP140	···ETECATIA	ЖЖ	:::∰6646 :::	4 0
PLP144			:::: ;;;;; ;;;	44
M.P219	::: [Tecacco		::::::::::::::::::::::::::::::::::::::	19
PLP222	::: ETTECACCO		::::::::::::::::::::::::::::::::::::::	22
PLP225	··· (178866)			25
PLP234	::: [][34

Fig. 1 Sequences of homopolymer tract inserts. Clones were initially acreened for insert size by following the restriction endonuclease Hae III digest on a 6% polyacrylamide gel. For DNA sequencing, the insert containing the Alul-generated restriction fragment was isolated, end-labelled, and sequenced after strand separation according to the procedures of Maxam and Gilbert²¹ most cases both strands were sequenced. No changes in plasmid sequence were observed outside the PstI site.

The principle of the band-shift method has been reported elsewhere. Note that the helical repeat, ho, obtained from the band-shift method is the number of base pairs of the sequence inserted that will increase the average linking number of the DNA in its relaxed state by one. In cases where the insert does not have an intrinsic spatial writhe, h^0 is also identical to the number of base pairs that forms a complete helical turn.

In addition to the absolute magnitudes of the helical repeats of different sequences, the band-shift method gives the relative handedness of the helices. We shall make the generally accepted assumption that a double-stranded DNA of typical sequence is a right-handed helix in solution. The band-shift method then provides directly the handedness of the inserted helical segments as well.

To extend the band-shift method to the determination of the helical repeats of DNAs of defined sequences, families of covalent closed circular DNAs containing inserts of these sequences of known lengths are needed. The method used to insert homopolymer tracts of various lengths into the PstI site of pBR322, a plasmid with known nucleotide sequence 10, involved tailing with terminal transferase and one of the deoxynucleoside triphosphates11. On digestion with restriction endonuclease BamHI, preparative gel electrophoresis allowed isolation of the desired fragments. The appropriate pairs of DNA fragments thus obtained were ligated together with T4 polynucleotide ligase and competent Escherichia coli cells were transformed. The nucleotide sequences in the regions containing the inserts are given in Fig. 1. In several cases, at the junctions between the plasmid DNA and the homopolymer inserts, a few unexpected base changes occurred. These changes were probably due to a low level of $3' \rightarrow 5'$ exonuclease activity in the calf thymus terminal transferase used in the construction of these plasmids.

An example of the gel electrophoretic patterns of several DNA samples relaxed in identical conditions is shown in Fig. 2a; lane 9 illustrates the band pattern observed when a single DNA, that of plasmid pLP222, is present. In lene 8, a second DNA (pLP219) shorter than pLP222 by three GC base pairs (bp) is mixed in. It can be readily seen that the band pattern of the longer plasmids shows a relative upward shift of ~ 0.3 times the interband spacing between topoisomers.

The set of covalently closed DNA samples prepared are all positively supercoiled in electrophoresis conditions¹², thus the faster migrating topoisomers have higher linking numbers than the slower migrating ones. Therefore an upward shift (that is, a reduction in the distance migrated) by 0.3 times the interband

Table 1 Helical repeats of $(dA)_n \cdot (dT)_n$ and $(dG)_n \cdot (dC)_n$

	DNA pair	Length difference (bp)	Shift observed	Corrected shift	Linking difference deduced	Calculated h ⁰ (bp per turn)
$(dA)_n \cdot (dT)_n$ inserts	pLP140/pLP144	4	0.44	0.42	0.42	10
(a)	pBR322/pLP119	19	0.00	0.91	1.91	9.9
	pLP119/pLP140	21	0.15	0.05	2.05	10.2
	pLP119/pLP144	25	0.59	0.47	2.47	10.1
	pBR322/pLP140	40	0.15	0.95	3.95	10.1
	pBR322/pLP144	44	0.59	0.37	4.37	10.1
$(dG)_n \cdot (dC)_n$ inserts	pLP219/pLP222	. 3	0.28	0.27	0.27	11
	pLP219/pLP225	6	0.59	0.56	0.56	11
	pLP225/pLP234	9	0.88	0.84	0.84	11
	pLP222/pLP234	12	0.18	0.12	1.12	10.7
	pLP219/pLP234	15	0.47	0.40	1.40	10.7
	pBR322/pLP219	19	0.88	0.79	1.79	10.6
	pBR322/pLP222	22	0.17	0.06	2.06	10.7
	pBR322/pLP225	25	0.47	0.35	2.35	10.6
	pBR322/pLP234	34	0.35	0.18	3.18	10.7

Because of the particular construction of the homopolymer inserts and the base changes at some of the junctions, the homopolymer tracts do not make up the total length differences (second column) except for the pairs pLP119/pLP144, pLP219/pLP222 and pLP225/pLP234. For most of the other pairs sequences other than the homopolymers make up 5-10% of the total differences. Values of h^0 calculated are those of the total inserts. When the shift (third column) was < 0.2 or > 0.8 of the interband spacing, accurate measurements were obtained by the use of a common internal standard. For the pair pBR322/pLP119, for example, each member of the pair was measured against pLP144, and the same upward shift of 0.59 was observed. This shows that there is no shift between pBR322 and pLP119. For corrected shift an alternative way of making the length correction is to assume that the intrinsic length dependence of the electrophoretic mobility of a covalently closed topoisomer is the same as that for a nicked circular DNA. For DNAs of sizes similar to pBR322, the reduction in the distance migrated by a nicked circular DNA per bp increment in length was measured as 3.7×10^{-3} times the average spacing between two covalently closed topoisomers that differ by 1 in their linking numbers; this value was used to make the corrections in previous experiments. However, this procedure underestimated the correction, mainly because the topoisomers have migrated further than the nicked DNA, by a factor of ~ 1.2 on average. This results in a 20% underestimation. When this factor is taken into consideration, the two correction procedures give data which agree well. Note that although the absolute magnitudes of the helical repeats measured by the band-shift method are a function of the length correction factor, any differences between the helical repeats of particular sequences are not affected.

spacing agrees with a structure in which the 3-GC pair insert is in a right-handed helical form with a helical repeat of 3/0.3 or 10 base pairs per turn. The band-shift method cannot distinguish an upward fractional shift of 0.3 from a downward fractional shift of 0.7. Thus this single result by itself is equally consistent with the three GC pairs being in a left-handed helical form with a helical repeat of 3/0.7 or 4.3 base pairs per turn. Examination of several pairs of DNAs shows that consistent h^0 values are obtained only if the sense of the helical inserts is taken to be right handed. The same conclusion is arrived at with all other sequences examined.

Quantification of measurements for all pairs of DNAs were made on microdensitometer tracings of the negatives of gel photographs. A typical trace is shown in Fig. 2b to illustrate the resolution of the method. The observed shifts are given in column 3 of Table 1.

As described previously⁹, the band-shift method measures the displacement of one family of topisomers of a DNA with an x-bp insert relative to another family of topoisomers of the same DNA without the insert. Although for small inserts the electrophoretic mobilities of the topoisomers are affected by the inserts mainly by their effects on the deviations of the linking numbers of the topoisomers from the average linking number of the DNA in its relaxed state, the lengthening of a DNA per se does have a small but significant effect on its mobility. Correction for this intrinsic length effect is necessary to obtain data of high accuracy. Methods for making the length corrections have been discussed previously9. In one, the shift for each pair of DNAs is calculated from the average of the upward shift of the longer plasmid seen with both DNAs in the positively supercoiled form and the downward shift seen with both DNAs in the negatively supercoiled form. The intrinsic length effect enhances the upward shift but reduces the downward shift, thus the averaging cancels out this effect. An example is shown in Fig. 3

Using this method we have examined five pairs of DNAs with inserts 34-55 bp long and with sequences varying from

 $(dA)_n \cdot (dT)_n$ to $(dG)_n \cdot (dC)_n$. The average length correction factor calculated for these five pairs was 4.9×10^{-3} turns per bp with a s.d. of the mean of $\pm 0.2 \times 10^{-3}$. Deviation of the correction factor calculated for any one of the five pairs from that of the mean was found to be statistically insignificant, and the average was used for all the corrections.

The values for the calculated helical repeats after corrections for the length effect are given in the last column of Table 1. Clearly, when the lengths of the inserts are progressively increased the values for the helical repeat of GC inserts rapidly approach 10.7 ± 0.1 bp per turn. This is not significantly different from the helical repeat of 10.6 ± 0.1 bp per turn found for a 58-bp segment in the cro gene region of phage λ when the refined length correction reported here is applied to the published results. In contrast, the homopolymer $(dA)_n \cdot (dT)_n$ shows a helical repeat that is significantly different from those of the other sequences examined. The value 10.1 ± 0.1 bp per turn obtained for this sequence is the only one measured so far that agrees, within experimental error, with the classical 10-fold B-type helix of DNA.

The values measured are for the DNAs in electrophoresis buffer at room temperature. Effects of counterions and temperature on h^0 are well known but are <1% within the ranges generally considered to be physiological^{13,14}. The handedness of the helices is identical for all the sequences examined, and is presumably dexiotropic.

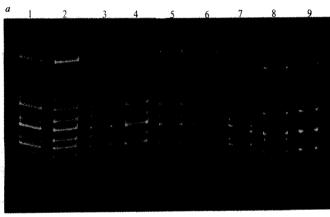
For sequences that are more or less random, the average helical repeat measured by the band-shift method for DNA in solution agrees remarkably well with that measured from the nuclease digestion pattern of DNA adsorbed on solid surfaces 15. The two very different methods give the same value of 10.6 ± 0.1 bp per turn. The theoretical calculation of Levitt 16 also gives a similar value for the helical repeat of DNA in solution.

There is a significant discrepancy between the solution measurements and X-ray diffraction studies of DNA fibres. Although DNA fibres show polymorphism when the counter-

ions and humidities are varied, the high-humidity form for DNAs of typical sequences is the B-type structure with 10 bp per turn. Recent measurements with highly solvated fibres gave the same results¹⁷.

There are various possible reasons why the fibre results do not agree with solution measurements. One potential complication brought into focus by a crystal structure of a dodecamer is the spatial writhe of DNA^{7,8}, and the plausible effect of lateral association of DNA molecules in a fibre on this writhe. Another explanation (suggested by D. M. Crothers) is that electric dichroism measurements of DNA in solution indicate that the structure is sensitive to aggregation induced by heavy metal ions. The magnitude of the dichroism in the absence of heavy metal ions can be interpreted by the structure calculated by Levitt¹⁶ with the base pairs assuming propeller twists and a 10.6-bp periodicity. In the aggregated state, the magnitude of the transient dichroism changes to that of a B-type structure with the base pairs almost perpendicular to the helical axis. A third possibility is that packing forces in fibres or crystals tend to favour structures with integral helical repeats¹⁸. Even in highly wetted fibres, the packing forces might be significant relative to the free energy differences among different polymorphic forms.

In the case of the homopolymer sequence $(dA)_n \cdot (dT)_n$, the solution measurements agree with fibre diffraction studies. The agreement between the helical repeat of poly(dA) · poly(dT) from fibre diffraction measurements and our solution measurements provides a clue to the reason for the disagreements between other sequences. The sequence $(dA)_n \cdot (dT)_n$ seems to be unique in its inertness to environmental effects ¹⁹⁻²². Both IR and fibre diffraction studies indicate that the B-type to A-type transition which can be induced for the



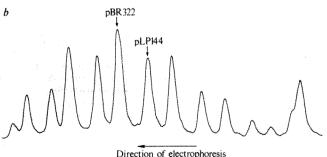


Fig. 2 a, Electrophoretic patterns of DNAs. DNA samples were relaxed overnight at 0 °C with calf thymus topoisomerase I in 10 mM Tris-HCl pH 8, 0.2M NaCl, 0.1 mM EDTA. The reaction was stopped and the enzyme removed by extraction with neutralized phenol. Electrophoresis in 0.7% agarose was carried out at room temperature after dialysis of the DNA samples against the electrophoresis buffer (90 mM Tris-borate pH 8.3, 2.5 mM EDTA). Plasmids present in the gel lanes are: 1, pLP225/pLP140; 2, pLP140/pLP144; 3, pBR322/pLP144; 4, pBR322/pLP225; 5, pLP219/pLP234; 6, pLP219/pLP225; 7, pLP222/pLP225; 8, pLP219/pLP222; 9, pLP222. b, Microdensitometer tracing of the negative for the electrophoretic pattern of the pair of DNAs pBR322/pLP144 (lane 3 of a).

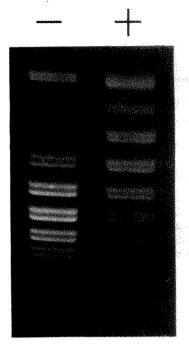


Fig. 3 Electrophorectic gel pattern of a mixture of pTR161 and pTR182, two DNAs that differ by an insert of 53 base pairs of a sequence in the cro gene region of phage λ^9 . Relaxation of the DNAs was the same as described in Fig. 2 legend except that during the topoisomerase I reaction the sample on the left contained ethidium. The covalently closed DNAs in the left lane are negatively supercoiled whereas those in the right lane are positively supercoiled. The DNAs were identified by comparing the gel patterns with those of positively and negatively supercoiled marker

other sequences does not occur for poly(dA) · poly(dT). The sensitivity of other sequences to environmental effects and packing forces may underlie their polymorphism in fibres and crystals, and cause structural differences between the condensed and soluble states.

The possible biological significance of different helical geometries associated with DNAs of different sequences has been widely discussed. The solution measurements have the advantage that the DNA is in a more physiological state and is free from packing forces. Our results clearly demonstrate that the sequence (dA), (dT), has a distinct helical geometry in solution. We hope that the method described here will find increasing application in cases where the molecular geometries of particular sequences are thought to have significant biological

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- Viswamitra, M. A. et al. Nature 273, 687-688 (1978).
- Wang, A. H.-J. et al. Nature 282, 680–686 (1979). Crawford, J. L. et al. Proc. natn. Acad. Sci. U.S.A. 77, 4016–4020 (1980).
- Drew, H., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. Nature 286, 567-573
- Wing, R. et al. Nature 287, 755-758 (1980).

- Wang, A. H.-J. et al. Science 211, 171-176 (1981).
 Drew, H. R. et al. Proc. natn. Acad. Sci. U.S.A. (in the press).
 Dickerson, R. E. et al. Proc. natn. Acad. Sci. U.S.A. (in the press).
- Wang, J. C. Proc. natn. Acad. Sci. U.S.A. 76, 200-203 (1979); Trends biochem. Sci. 5, 219-221 (1980).
- 10. Sutcliffe, J. G. Cold Spring Harb, Symp. quant. Biol. 43, 77-90 (1979)
- Lobban, P. E. & Kaiser, A. D. J. molec. Biol. 78, 453-471 (1973). Depew, R. E. & Wang, J. C. Proc. natn. Acad. Sci. U.S.A. 72, 4275-4279 (1975).

- Wang, J. C. J. molec. Biol. 43, 25–39 (1969).
 Bauer, W. R. A. Rev. Biophys. Bioengag 7, 287–313 (1978).
 Rhodes, D. & Klug, A. Nature 286, 573–578 (1980).
 Levitt, M. Proc. natn. Acad. Sci. U.S.A. 75, 640–644 (1978).
- 17. Zimmerman, S. B. & Pheiffer, B. H. Proc. nam. Acad. Sci. U.S.A. 76, 2703-2707 (1979).

- 18. Dover, S. D. J. molec. Biol. 110, 699-700 (1977).
- 19. Wells, R. D., Larson, J. E., Grant, R. C., Shortle, B. E. & Cantor, C. R. J. molec. Biol. 54,
- 20. Gray, D. M. & Gall, J. G. J. molec. Biol. 85, 665-679 (1974).
- Arnott, S. Nucleic Acids Res. 2, 1493-2502 (1975).
 Pilet, J., Blicharski, J. & Brahms, J. Biechemistry 14, 1869-1876 (1975).
- 23. Maxam, A. M. & Gilbert, W. Proc. natu. Acad. Sci. U.S.A. 74, 560-564 (1977).

Sequence-dependent helical periodicity of DNA

D. Rhodes & A. Klug

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

We have recently described a new approach to measuring the helical periodicity of random sequence DNA in solution1. It consists of binding short, stiff pieces of DNA to various flat surfaces and using DNase I to probe the most accessible phosphodiester bonds. The differences in length of the fragments produced are measured with high precision to give the helical repeat directly. This approach has now been extended to study the periodicity of defined-sequence DNAs using another enzyme, micrococcal nuclease, as well as DNase I. We find the helical repeat of poly(dA-dT) to be $10.5 \pm < 0.1$ base pairs (bp), a value very close to that found earlier by us for randomsequence DNA, 10.6±0.1 bp, and confirmed here. Poly(dA).poly(dT) exhibits a distinctly different helical repeat, 10.0 ± 0.1 bp.

In our earlier work we used two different experimental approaches. The first used short DNA fragments of defined length, nominally 140 bp extracted from nucleosome cores, and the ³²P label incorporated beforehand at the 5' termini. This method, besides giving accurate distances between DNase I cutting sites, also enables the probability of cutting at each site along the length of the DNA fragment to be determined. The second used DNA fragments with a wide distribution of lengths, an average of ~200 bp, and the ³²P label was introduced after DNase I digestion. As a less laborious alternative, the 32P label has now been incorporated by nick translating each DNA sample. As fragments of poly(dA-dT) and poly(dA) poly(dT) of defined length are not readily available, double-stranded fragments a few hundred base pairs long were produced by shearing samples of calf thymus DNA (Sigma, 'highly polymerized'), long poly(dA-dT) and poly(dA).poly(dT) (both BCL Boehringer) in a sonicator2, which gives a broad fragment-length distribution of

Figure 1a, b and c show the results from experiments where DNA. poly(dA-dT) and poly(dA).poly(dT) were immobilized on calcium phosphate microcrystals and mica powder, then lightly digested with micrococcal nuclease or DNase I and the products fractionated by polyacrylamide gel electrophoresis3,4 The autoradiographs show patterns of fine bands, each band representing single-stranded fragments differing in size by one nucleotide. The intensities of the fine bands vary, showing band maxima at regular intervals ~10 bases apart. A set of intense fine bands represents a set of fragments produced by cutting with the nuclease at two different groups of accessible phosphodiester bonds ('sites') in the same DNA molecule. The 'ladder' formed by successive groups of fine intense bands is a direct visualization by the nuclease of the helical repeat.

The average distance between nuclease cutting sites can be obtained by counting the number of fine bands between maxima and then dividing by the number of maxima traversed. The calculation can easily be carried out from the densitometer tracings of the autoradiographs shown in Figs 2 and 3. For example in Fig. 3, in the densitometer tracing of poly(dA).poly(dT) digested with DNase I, the number of fine peaks between F3 and F10 is 70, giving a value of 70/7 = 10.0bases

For random-sequence DNA (Fig. 1a) the distance between DNase I cutting sites is 10.5-10.6 bases as found previously¹. The same value was obtained using micrococcal nuclease (not shown), but here the lengths of a proportion of the fragments produced is gradually reduced as the digestion proceeds. This is in contrast to the case of the two synthetic (A, T) polymers, where all fragments are progressively reduced in length (see below). We interpret the difference as due to the relative resistance to micrococcal nuclease⁵ of (G,C) stretches, compared with (A.T) stretches, present in random sequence DNA. The mechanism of trimming of DNA fragments has consequences for the production of nucleosome cores from

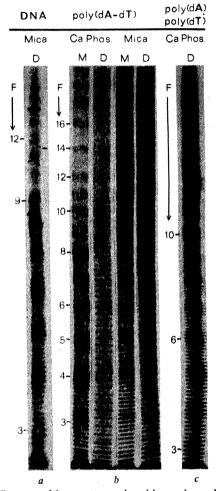
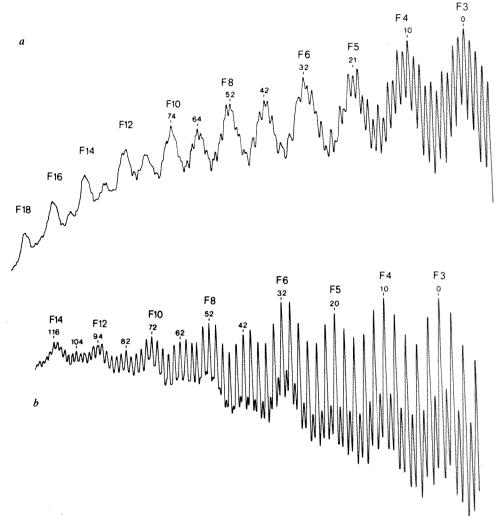


Fig. 1 Patterns of fragments produced by nuclease digestion of three DNAs immobilized on flat surfaces. After nick translation in the presence of $[\alpha^{-32}P]$ dATP (ref. 23), each DNA sample was extracted, precipitated and the DNA and poly(dA-dT) precipitates 20 mM Tris pH 8.0 and 60 mM Poly(dA),poly(dT) was dissolved in 20 mM Tris pH 8.0 only, because even low concentrations of salt tend to favour the formation of triple strand helices²⁴. Finally, the two synthetic polymers were annealed at 40 °C for 10 min and then stored at 5 °C. The purity of the synthetic DNAs was checked by nearest neighbour analysis. The calcium phosphate surface and the mica powder were prepared as described earlier1, except that the concentration of the buffer in which the mica is resuspended has been halved, to reduce the destabilizing effect that Mg²⁺ ions have on the double helices of (A. T) polymers²⁵ a Autoradiography of readon services. a, Autoradiographs of random sequence DNA fragments from electrophoresis in a high-resolution denaturing gel made with 8% acrylamide and 1.35% methylenebisacrylamide. This system can resolve random sequence fragments differing in size by one nucleotide3. b, Poly(dA-dT); c, poly(dA).poly(dT) fragments from electrophoresis in denaturing gels made with 6% acrylamide and 0.3% methylenebisacrylamide, as used in DNA sequencing⁴. All nuclease digestions were carried out at 20 °C. D signifies DNase I and M signifies micrococcal nuclease. F stands for a set, or group, of fragments and the number indicates the size class, for example, F3 represents fragments of ~ 30 bases in length. The gel tracks shown in b have been assembled from the same gel, containing additional experiments not shown here.

Fig. 2 Densitometer tracings of the autoradiograph shown in Fig. 1b, (first two lanes); poly(dA-dT) digested with a, micrococcal nuclease; and b, DNase I. The fine peaks which represent poly(dA-dT) fragments differing in size by one nucleotide are numbered from the centre of band F3. In a, because of the base specificity of micrococcal nuclease, there is an alternation of intensity in the fine peaks and in b, because of the base specificity of DNase I (see text), fine peaks every two nucleotides dominate the pattern.



chromatin and will be discussed elsewhere (M. Cockell, D.R. and A.K., in preparation).

The results from a typical experiment on poly(dA-dT) immobilized on two different surfaces and digested with micrococcal nuclease or DNase I are shown in Fig. 1b. The intensity variations between successive fine bands is caused by the particular base specificities of the two nucleases used. Micrococcal nuclease cleaves poly(dA-dT) in solution at Ts with only a slight preference over As⁶ whereas DNase I cleaves almost exclusively at Ts, producing a large proportion of fragments differing in size by two nucleotides⁷ (Fig. 2a,b). The sets of fragments produced by micrococcal nuclease (lanes M) lie one or two nucleotides below the sets of fragments produced by DNase I (lanes D). This reduction in length depends on the extent of digestion, but all fragments seem to be reduced in length to an equal extent. Consequently, the distance between micrococcal nuclease cutting sites remains essentially constant.

The helical repeat of poly(dA-dT) bound to calcium phosphate, obtained directly from the densitometer tracings (Fig. 2a,b), is 10.5 bp with both nucleases. The intensity distribution in Fig. 1b (first two lanes) and Fig. 2a,b indicates that the helical periodicity is very close to 10.5 bp. There is an alternation in intensity between successive sets of intense fine bands, especially visible in the longer fragments, for example, F10 and F18. This shows that the repeat length seen by the enzyme is two helical turns corresponding to an exact integral number of base pairs $(10.5 \times 2 = 21)$. Poly(dA-dT) bound to mica showed a slightly lower helical periodicity (Fig. 1b, last two lanes). The alternation of the intensities of the longer fragments is not so evident and the densitometer tracings (not shown) give a helical repeat of 10.4 bases.

Figure 1c shows the result of an experiment with the third polynucleotide, poly(dA), poly(dT), bound to calcium phosphate

and digested with DNase I. The densitometer tracing in Fig. 3 shows that the helical periodicity of poly(dA).poly(dT) is distinctly different from that of DNA or poly(dA-dT). The repeat length of the fragments produced is 10.0 bases. The periodicity observed using micrococcal nuclease is the same as that found with DNase I, but because the fragments are reduced in size very rapidly the measurements vary from site to site and are therefore less accurate (not shown). We were unable to obtain a ladder reflecting the double-helical screw from poly(dA).poly(dT) immobilized on mica. Presumably, when the polymer comes into contact with Mg²⁺ present in the mica suspension buffer, its double-helical nature is lost because of the propensity of poly(dA).poly(dT) to form triple-stranded helical structures?

The enzyme digestion method for determining the helical periodicity of DNA is simple, direct and requires no corrections. The accuracy of the measurements of the distance between

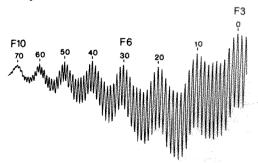


Fig. 3 Densitometer tracing of the autoradiograph shown in Fig. 1c. The fine peaks which represent poly(dA) poly(dT) fragments differing in size by one nucleotide are numbered from the centre of F3 to F10.

nuclease cutting sites depends mainly on the length of the largest DNA fragments that can be resolved to a resolution of a single nucleotide in the gel electrophoresis system, that is, on the number of helical repeats counted. The resolution of the homopolymer fragments is very good because the gel electrophoresis system used for sequencing DNA can be used⁴, but the random-sequence DNA fragments cannot be fractionated to the same resolution³. In our earlier paper on random-sequence DNA we mentioned that the changes caused by cation type and concentration and temperature on the screw of DNA^{10,11} would not be >0.1 bp per turn. In the present study with poly(dA-dT), where the precision of the results is higher, we find just such a change. The decrease in screw in the presence of millimolar Mg^{2+} is about 1% = 0.1 bp and is consistent both in sign and magnitude with previous work by others¹¹

The results we have obtained demonstrate that the helical periodicity of DNA depends on the nucleotide sequence. Thus, the number of 10.0 bp per turn of the poly(dA).poly(dT) double helix in solution is distinctly different from the value of 10.4-10.6 we found for poly(dA-dT) and random-sequence DNA. These values are in good agreement with measurements by Wang who, using a completely different experimental approach from ours, has reported a value of 10.5 bp for DNA and 9.9 for poly(dA).poly(dT)^{12,13}.

It is not surprising that the same helical screw is found for random-sequence DNA and poly(dA-dT) because the former must contain, on average, equal numbers of purine and pyrimidines on each strand. The different helical periodicity observed for poly(dA).poly(dT) can perhaps be correlated with physicochemical studies which suggest that this polymer is different in some way from all other DNAs studied14. Poly(dA).poly(dT) fibres give essentially the same B-form X-ray diffraction pattern as other DNAs, but, unlike all other DNAs studied, no conditions have been found for its transition to a different form¹⁵. Furthermore, poly(dA).poly(dT) is the only DNA with a UV circular dichroism spectrum which does not fit a simple additivity rule accommodating many different DNA duplexes16

The distinctly different helical screw we have found for poly(dA).poly(dT) could be of some biological significance. The previous finding that nucleosomes cannot be reconstituted from poly(dA).poly(dT), but are easily obtained from reconstitution with poly(dA-dT) or DNA^{17-19} , was previously explained by the high salt in the reconstitution system used, disproportionating poly(dA).poly(dT) into triple helical structures. However, the present results suggest an alternative explanation. There are also examples where the A+T-rich spacer regions between genes, such as the 5S genes of yeast^{20,21} and the histone genes of sea urchin²², contain long runs of As. Although these findings might not be coincidental, it is premature to conclude that such stretches of poly(dA).poly(dT) would exclude nucleosomes directly.

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- Rhodes, D. & Klug, A. Nature 286, 573-578 (1980).
- Record, M. T., Woodbury, C. P. & Inman, R. B. Biopolymers 14, 393-408 (1975).
- Lutter, L. C. Nucleic Acids Res. 6, 41-56 (1979). Sanger, F. & Coulson, A. R. FEBS Lett. 87, 107-110 (1978).
- Anfinsen, F. B., Cuatrecasas, P. & Taniuchi, H. in *The Enzymes Vol.* 4 (ed. Boyer, P. D.) 177-204 (Academic, London, 1971).
- 177-204 (Academic, London, 1971).
 Lomonossoff, G. P. thesis, Univ. Cambridge (1980).
 Scheffler, I. E., Elson, E. L. & Baldwin, R. L. J. molec. Biol. 36, 291-304 (1968).
 Sulkowski, E. & Laskowski, Sr. M. J. biol. Chem. 14, 3818-3822 (1969).
 Miles, H. T. & Frazier, J. Biochem. biophys. Res. Commun. 14, 21-28 (1964).
 Wang, J. C. J. molec. Biol. 43, 25-39 (1969).
 Anderson, P. & Bauer, W. Biochemistry 17, 594-601 (1978).
 Wang, J. C. Proc. natn. Acad. Sci. U.S.A. 76, 200-203 (1979).
 Wang, J. Trends biochem. Sci. 5, 219-221 (1980).
 Leslie, A. G. W. Arnott, S., Chandrasekaran, R. & Ratliff, R. L. J. molec Biol. 143

- 14. Leslie, A. G. W., Arnott, S., Chandrasekaran, R. & Ratliff, R. L. J. molec Biol. 143, 49-72 (1980).
- Arnott, S. & Selsing, E. J. molec. Biol. 88, 509-521 (1974).
- Arnott, S. Nucleic Acids Res. 2, 1493-1502 (1975)
- Simpson, R. T. & Künzler, P. Nucleic Acids Res. 6, 1387-1415 (1979). Rhodes, D. Nucleic Acids Res. 6, 1805-1816 (1979).
- McGhee, J. D. & Felsenfeld, G. A. Rev. Biochem. 9, 1115-1156 (1980). Valenzuela, P., Bell, G. I., Mesiarz, F. R., De Gennaro, J. L. & Rutter, W. J. Nature 267, 641-643 (1977)

- 21. Maxam, A. M., Tizard, R., Skryabin, K. G. & Gilbert, W. Nature 267, 643-645 (1977).
- Grosschedl, R. & Birnstiel, M. L. Proc. natn. Acad. Sci. U.S.A. 77, 7102-7106 (1980).
 Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. J. molec. Biol. 113, 237-251 (1977).
- Krakauer, H. & Sturtevant, J. M. Biopolymers 6, 491-512 (1968)
- Spatz, H. Ch. & Baldwin, R. L. J. molec, Biol. 11, 213-222 (1965)

In vivo consequences of plasmid topology

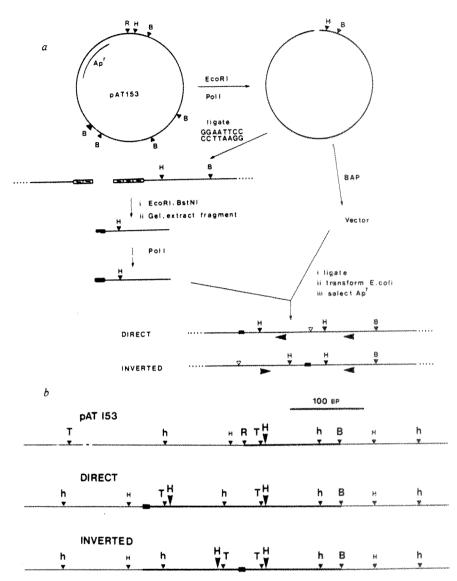
David M. J. Lilley

Molecular Genetics Department, Searle Research Laboratories, Lane End Road, High Wycombe HP12 4HL, UK

The topology and physical chemistry of closed circular DNA molecules are well understood, but the significance for events within living cells is less well appreciated. It has been demonstrated recently 1-3 that the torsional constraint which arises from negative supercoiling (that is, reduction of linkage) can induce localized novel secondary structure in isolated plasmid and phage DNA. Inverted repeats adopt hairpin-loop structures not found in relaxed DNA. This structural perturbation might be expected to have functional significance within the living cell. but clearly this requires that the torsional free energy be available for unhindered partition between alterations of twist and writhe. Microheterogeneity in DNA structure has recently attracted considerable interest, especially with regard to left-handed sections of duplex⁴⁻⁶. The inverted repeats identified as sites of hairpin formation are relatively small, with stems of 13 base pairs (bp) or less. Whilst these hairpins could result in a relaxation of $\sim 10\%$ of the plasmid supercoiling energy, it was of considerable interest to try to construct stem-loop features about 10 times larger so as to study the topological consequences. In the cloning experiment described here, designed to produce direct or inverted 130-bp repeats depending on insertional orientation, no inverse species could be discovered, and deletion events were frequent. It is concluded that the inverted repeat deprives Escherichia coli of its antibiotic resistance. Cruciform adoption by the inverted species can totally relax the torsional constraint in the plasmid. These experiments highlight the importance of topological considerations in the genetics of closed circular DNA, and confirm the availability of torsional constraint in vivo.

Figure 1a shows the experimental basis of the investigation. A 130-bp EcoRI, BstNI fragment of pAT153 (ref. 7) was inserted back into the parent plasmid to generate either a direct repeat, or an inverted repeat with a 6-bp central loop, depending on orientation. Resulting colonies were screened by restriction enzyme analysis of plasmid DNA, using the predicted restriction maps shown in Fig. 1b. The most straightforward screening procedure involved HindIII digests of plasmid mini-preparations⁸ from each colony. As pAT153 has a single HindIII site at 29 bp (relative to EcoRI at 0 bp), the direct repeat recombinant should give a 130-bp fragment, and the corresponding fragment size from the indirect repeat molecule should be 70 bp. Figure 2a shows a typical restriction cleavage analysis of some recombinant plasmids. Analysis of 67 ampicillin-resistant colonies revealed that these comprised 21 recyclized parent plasmids, 25 direct repeat species, no inverted repeat species and 21 deletants. The most important comparison is that between direct and inverted repeats of 25/0. As the probability of insertion in either orientation is 50%, it is quite clear that selection at another level is operating in this experiment. A rather high level of deletants was observed (31%), several of which seem to be mutations which disrupt the symmetry of what would otherwise be inverted repeats. This again implies that it is the symmetry of the inverted repeat which is responsible for its non-viability. The high level of recyclized vector (31%) arises from the inherent low efficiency of blunt-end ligation reactions, coupled with the removal of inverse orientation events from the statistics. The statistics show clearly that an inverted repeat of unit length 136 bp confers lethal properties on pAT153. This could arise by interference with the mechanism of plasmid replication or the expression of the ampicillin-resistance gene, such that resistance is no longer conferred on the host bacterium. The act of insertion per se is clearly not responsible for the effect, because the direct

a, Schematic outline of the construction of recombinant molecules derived by reinsertion of the 130-bp EcoRI, BsfNI fragment of pAT153 (ref 7) into the flush-filled EcoRI site of pAT153, with hexanucleotide asymmetric joining section. pAT153 was cleaved by EcoRI and the resulting 5' extensions 'filled in' using 2 units of the Klenow fragment of E. coli DNA polymerase I (Boehringer) in 50 mM Tris pH 7.5, 10 mM MgCl₂, 1 mM β -mercaptoethanol with 0.25 mM of each of the four deoxynucleotides for 30 min at 15 °C. One-quarter of this was dephosphorylated using 20 µg bacterial alkaline phosphatase (F grade, Worthington) in 20 mM Tris pH 7.5, 0.1% SDS for 30 min at 37 °C, for subsequent use as cloning vector. EcoRI linker (Collaborative Research) was 5 phosphorylated in two stages. 2.5 µg of the doublestranded octanucleotide were incubated in 50 mM Tris pH 7.5, 10mM MgCl₂, 5 mM dithiothreitol, 0.1 mM spermidine using 100 μCi of 5,000 Ci mmol⁻¹[γ-³²P]ATP (Amersham) and 3 units of T4 kinase (New England Biolabs) at 37 °C for 60 min, followed by addition of unlabelled ATP to 1 mM and further kinase and incubation for an additional 60 min. The phosphorylated octanucleotide was ligated to the remainder of the flush-ended EcoRIcleaved pAT153 by the blunt-end ligation procedure19, using 0.2 units of T4 DNA ligase (Bethesda Research) in 50 mM Tris pH 7.5, 10 mM MgCl₂, 20 mM dithiothreitol, 1 mM ATP for 2 h at 15 °C. After EcoRI, BstNI cleavage, the fragments were resolved on 5% polyacrylamide gel electrophoresis²⁰ and the 136-bp fragment removed by homogenization and extraction in 0.5 M NH₄-acetate, 10 mM Mg-acetate, 0.1% SDS, 0.1 mM EDTA²¹ at 37 °C, followed by binding to DEAE cellulose (Whatman), washing, elution and ethanol precipitation. After polymerase filling to give flush ends, this fragment was blunt-end ligated into the flush-ended EcoRI -cleaved and dephosphorylated pAT153. After ligation, DNA samples were diluted to 100 µl in 10 mM Tris pH 7.5, 10 mM CaCl₂ and 10 mM MgCl₂ and used to transform 200-µl aliquots of CaCl₂-shocked *E. coli* K12 HB101 (ref. 22) as described previously²³. After 20 min at 37 °C in L broth containing 100 µg mlcarbenicillin (Pyopen), 200-µl aliquots were plated on to agar containing 100 µg ml⁻¹ carbenicillin and carbenicillin and incubated at 37 °C for 16 h. (All restriction enzymes used in this study were from New England Biolabs or Bethesda Research Laboratories, and used as directed.) Key: R. EcoRI site; H. HindIII sites; B. BsfNI sites; BAP, dephosphorylation using bacterial alkaline phosphatase; PolI, flush-filling using DNA polymerase I (Klenow fragment); Apr, resistance to ampicillin. The rectangular boxes represent the repeats do not possess these properties, and thus we are left with the symmetry created by inverse insertion. However, the symmetry cannot be solely responsible for the lethal properties because smaller inverted repeats occur in ColE1 and pBR322^{1,2} and in recombinants derived from these plasmids³. The major



synthetic EcoRI linker fragment, and the filled box represents the double-stranded AATTCC unit. Filled triangles denote restriction enzyme sites, and open triangles denote restriction sites deleted during construction. b, Restriction maps of the EcoRI region of pAT153, and calculated maps for the direct and inverse recombinants, all derived from the sequence of pBR322 (ref. 24). Key: R, EcoRI; H, HindIII; B, BstNI; T, TaqI; H (smaller), HaeIII; h, HhaI. The thicker line denotes sequences between the EcoRI and BstNI sites of pAT153 which become duplicated in the recombinants. The filled boxes represent the hexanucleotide joining section.

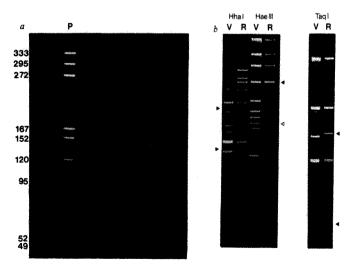


Fig. 2 Analysis of recombinants by restriction enzyme cleavage. a, HindllI cleavage of plasmid mini-preparations grown from individual colonies. The marker fragments (P) are HaeIII-digested phage PM2 DNA. Colonies were picked into 1 ml L broth solutions containing 100 µg/ml⁻¹ carbenicillin and grown at 37 °C for 16 h in the absence of chloramphenicol. DNA was prepared using a modification of the alkaline preparation method^a where all standing times were reduced to 5 min. After two ethanol precipitations and an optional phenol extraction, the DNA was sufficiently pure to be digested by restriction enzymes. This method gives a low chromosomal DNA background but to visualize small DNA fragments it was necessary to remove contaminating RNA by inclusion of 50 µg ml⁻¹ RNase A (Sigma, preincubated at 95 °C for 10 min to remove potential DNase activity) in the restriction enzyme digestion. b, Detailed analysis of one clone containing a full-length 136-bp insertion. A 100-ml culture was grown from a 1-ml overnight incubation of one full-length recombinant, for 16 h at 37 $^{\circ}$ C in L broth containing 100 μg ml $^{-1}$ carbenicillin but in the absence of chloramphenicol amplification. Plasmid DNA was purified from cell lysates by banding in caesium chloride²⁵. Key: V, pAT153 digests; R, recombinant digests. Solid triangles denote new (or double intensity) fragment bands; open triangles denote bands missing from the recombinant digests. Note that these digests unambiguously confirm the orientation of the inserted DNA, and the increase in length of the 206-bp Hhal and 368-bp Tagl fragments confirms the presence of the joining hexanucleotide

difference between the inverted repeat created in this experiment, and those found in ColE1 and pBR322, is one of size. The ColE1 repeat has a unit size of 13 bp, whereas that in the potential pAT153 recombinant is 10-fold larger-130 bp.

I have observed previously that small inverted repeats present in supercoiled circular DNA are centrally hypersensitive to S₁ nuclease, implying the adoption of hairpin-loop structure. On forming a cruciform type of structure an alteration of twist occurs for the molecule, given by

$$\Delta T w = n/10.4 \tag{1}$$

where n is the total number of base pairs involved in the formation of the hairpin. (The number of 10.4 is derived from an estimate of the twist of solution DNA being 10.4 bp per helical turn⁹.) The parameters which describe the topological properties of supercoiled DNA are related by the expression

$$\Delta Lk = \Delta Tw + Wr \tag{2}$$

where ΔLk is the alteration in linkage from the most relaxed topoisomeric conformation, and Wr is the writhing number. The linking number for pAT153 has been measured as 18±1 (Fig. 3), which equation (2) shows must be partitioned between alteration of twist or writhe, either of which is energetically unfavourable. However, the twist alteration produced by cruciform formation allows the rest of the molecule to 'relax' by the extent

$$R = \frac{-n}{10.4 \ \Delta Lk} \times 100\% \tag{3}$$

thus, for ColE1 $(n = 30 \text{ and } \Delta Lk \simeq -27)^{12}$ a relaxation of twist and writhe of 11% should occur. For the inverse recombinant pAT153 species relaxation becomes 142% (n = 266), that is, the molecule can be totally relaxed. This, then, is the likely basis of the lethal properties of these large inverted repeats.

Further examples of selection against inverted symmetric units can be found in the literature 13.14, and another example has recently been noted in this laboratory (W. Tacon, unpublished results), when it was found that only tandem repeats of a 111-bp fragment could be obtained in a multiple-copy cloning experiment. One explanation previously proposed¹⁴ was that the symmetry might facilitate strand switching during replication. However, there is no a priori reason that such a process should occur only in longer inverted repeats. It seems more likely,

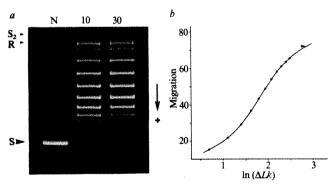


Fig. 3 Measurement of the relative linking number (ΔLk) of pAT153 by gel electrophoretic resolution of partial topoisomerizations^{26,27}. a, 1.2% agarose gel electrophoresis (room temperature) of native supercoiled and partially relaxed pAT153. 0.8 µg of pAT153 was incubated with 2 units of Agrobacterium tumefaciens topoisomerase (Bethesda Research) at 37 °C in 20 mM Tris pH 7.8, 2 mM MgCl₂, 7 mM β-mercaptoethanol for the times indicated (min), followed by electrophoresis at 50 V for 16 h in 40 mM Tris pH 8.0, 5 mM Na-acetate, 1 mM EDTA²⁸. Key: N, fully supercoiled pAT153; S, R and S₂, positions of migration of fully supercoiled monomer, nicked monomer and supercoiled dimer species. b, Extrapolation of migration of topoisomers to that of the native supercoiled pAT153 (shown by the arrow) indicates that $\Delta Lk = -18$. Migration of individual topoisomers measured by microdensitometer scanning of photographic negatives. The were assigned by comparison of identical topoisomerizations on agarose gels run at room temperature and 5 °C (ref. 16). Thus, the most slowly resolved topoisomer in a was assigned to $\Delta Lk = -2$. A small error (estimated to be ± 1) may result from the extrapolation procedure.

therefore, that the topological effects are the basis of these observations. Another instance of such properties was noted by Gellert et al. 15, who were unable to propagate a circular dimer of the longer EcoRI, BamHI fragment of pBR322. Another example may be found in a study of post-transformational rearrangement in modified pSM19035 (ref. 16), where manipulations designed to generate a large perfect inverted repeat resulted in spontaneous deletion events which removed the centre of symmetry.

Several consequences follow from these observations. First, the strong selection against inverted repeats demonstrates that topological effects are operative on DNA within the living bacterium, that is, that the torsional constraint arising from linkage reduction by gyrase is 'available', rather than being locked up in writhing around histone-like proteins. This energy is available to drive processes requiring strand separation, such as transcription^{17,18}, and possibly the alteration of DNA secondary structure 1-3. It is therefore perhaps not surprising that the (possibly transient) release of this free energy into cruciform structures has deleterious consequences. Second, the present study demonstrates that inverted repeats can strongly influence the functional properties of supercoiled molecules. The difference between the stable direct species and the unstable inverted species constructed in these experiments is quite subtle—the reversal of polarity of 130 bp in a 3.7-kbp molecule at a region distinct from both ampicillin-resistance and replication functions. Yet this modification in some way deprives the cell of its resistance to the antibiotic. Finally, these studies have practical significance, for they imply that the topological consequences of certain cloning operations should be considered. Thus, 'head to head' dimers of fragments above a limiting size are likely to be impossible to clone. Furthermore, plasmid 'libraries' may be incomplete if inverted repeats are present in the original nucleic acid cloned.

The size limitations on cloning ability are imprecise at present, but some limits may be set. The stability of cruciform structures will be proportional to stem length, but inversely proportional to loop size^{1,3}, whereas the topological consequences depend on the size of the whole feature. Stem sizes of 9-13 bp do not result in plasmid instability¹, whilst we have noted in this laboratory that 30-bp duplications (R = 32%) cannot be cloned inverted in pAT153. However, longer inverted repeats have been propagated where the loop size is 12 nucleotides. As I have noted previously³, the symmetry requirements for cruciform structure formation are stringent.

I thank many of my colleagues at Searle, especially Drs W. Tacon and M. Houghton, and also Professor D. Sherratt for useful discussion.

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- Lilley, D. M. J. Proc. natn. Acad. Sci. U.S.A. 77, 6468-6472 (1980). Panayotatos, N. & Weils, R. D. Nature 289, 466-470 (1981). Lilley, D. M. J. Nucleic Acids Res. 9, 1271-1289 (1981).
- Wang, H-K, et al. Nature 282, 680-686 (1979)
- Drew, H., Takano, T., Tanaka, S., Itakura, K. & Dickerson, R. E. Nature 286, 567-573 (1980)
- Arnott, S., Chandrasekaran, R., Birdsall, D. L., Leslie, A. G. W. & Ratliff, R. L. Nature 283, 743-745 (1980).
- Twigg, A. J. & Sherratt, D. Nature 283, 216-218 (1980)
- Nugg, A. J. & Sherrau, D. Nature 283, 210-218 (1980).
 Birnboim, H. C. & Doly, J. Nucleic Acids Res. 7, 1513-1523 (1979).
 Wang, J. C. Proc. natn. Acad. Sci. U.S.A. 76, 200-203 (1979).
 Vinograd, J. & Lebowitz, J. J. gen. Physiol. 49, 103-125 (1966).
 Fuller, F. B. Proc. natn. Acad. Sci. U.S.A. 68, 815-819 (1971).
 Shishido, K. FEBS Lett. 111, 333-336 (1980).

- snisnido, K. PEBS Lett. 111, 353-350 (1980).
 Bolivar, F. et al. Proc. natn. Acad. Sci. U.S.A. 74, 5265-5269 (1977).
 Sadler, J. R. et al. Gene 3, 211-232 (1978).
 Gellert, M., Mizuuchi, K., O'Dea, M. H., Ohmori, H. & Tomizawa, J. Cold Spring Harb. Symp. quant. Biol. 43, 35-40 (1978).

- Symp. quant. Biol. 43, 35-40 (1978).

 16. Behnke, K., Malke, H., Hartmann, M. & Walter, F. Plasmid 2, 605-616 (1979).

 17. Wang, J. C. J. molec. Biol. 87, 797-816 (1974).

 18. Richardson, J. P. Biochemistry 13, 3164-3169 (1974).

 19. Ullrich, A. et al. Science 196, 1313-1319 (1977).

 20. Maniatis, T., Jeffrey, A. & Van de Sande, H. Biochemistry 14, 3787-3794 (1975).

 21. Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560-564 (1977).

 22. Boyer, H. W. & Roullard-Dussoix, D. J. molec. Biol 41, 459-472 (1969).

 23. Cohen, S. N., Chang, A. C. Y. & Hsu, L. Proc. natn. Acad. Sci. U.S.A. 69, 2110-2114 (1972).
- Sutcliffe, J. G. Cold Spring Harb. Symp. quant. Biol. 43, 77-90 (1979).
 Katz, L., Kingsbury, D. K. & Helinski, D. R. J. Bact. 114, 557-591 (1973).
 Keller, W. Proc. natn. Acad. Sci. U.S.A. 72, 4876-4880 (1975).
 Shure, M. & Vinograd, J. Cell 8, 215-226 (1976).
- Sharp, P. A., Sugden, B. & Sambrook, J. Biochemistry 12, 3055-3063 (1973).

Sequence homologies between tonin, nerve growth factor γ -subunit, epidermal growth factorbinding protein and serine proteases

Claude Lazure, Nabil G. Seidah, Gaétan Thibault, Roger Boucher*, Jacques Genest & Michel Chrétien

Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7

Tonin¹, a protein isolated from rat submaxillary gland, has interesting proteolytic activities. It is able to liberate angiotensin II from angiotensinogen (or a shorter synthetic tetradecapeptide corresponding to the first 14 residues) and from angiotensin I; it is also able to release peptides from β -lipotropin (β -LPH), corticotropin (ACTH) and their common precursor proopiomelanocortin (POMC) leaving intact the β -endorphin and the Met-enkephalin entities^{2,3}. Here, we describe the sequencing of 95 of the 272 amino acids of tonin. Our results reveal extensive sequence homology of tonin with the γ -subunit of nerve growth factor (NGF) and epidermal growth factor (EGF)-binding protein (both of which also have proteolytic activities), and also with serine proteases.

Tonin is a single polypeptide chain of 272 amino acids having a molecular weight of 28,700; sequence analysis has revealed isoleucine and proline at the NH₂ and COOH termini respectively⁴. We have now extended the NH₂-terminal sequence up to 65 cycles with excellent repetitive yield while identifying blanks and uncertain residues in the previously reported sequence, as shown in Fig. 1a. In addition, we identified 30 other residues in a cyanogen bromide fragment, giving the definitive sequence of 35% of the molecule (Fig. 1b). While this work was being carried out, the sequence of the γ -subunit of mouse NGF became available⁵, making it possible to compare the amino acid sequences.

As indicated in Fig. 2a, alignment of the first 65 residues of the γ -subunit and tonin reveals only 15 differences—77% homology. Furthermore, the sequence Val-Leu-Thr-Ala-Ala-His-Cys, which is found in all serine proteases except haptoglobin, a recently proposed serine protease homologue⁶, is present in rat tonin; it does contain a conservative substitution, namely the isoleucine at position 35 in place of the common leucine residue. This segment is very important because it contains the histidine residue typical of the active site of all serine proteases. The cysteine residue at position 24 is part of the disulphide loop maintaining the proper orientation of the histidine residue. The relationship between rat tonin and serine proteases can also be seen by comparison of the first 65 residues with known sequences: the homology with rat tonin is highest for pancreatic enzyme kallikrein⁷ (55%) and trypsin⁷ (48%), with other serine proteases slightly less homologous⁶⁻¹³.

Comparison of the NH₂-terminal sequence of a cyanogen bromide fragment of tonin with positions 95–124 of the γ -subunit (Figs 1b and 2b) also reveals 21 identical positions out of 30, thus showing a striking similarity (70%) between rat tonin, γ -subunit and serine proteases.

Although the present results reveal only a partial (35%), but definitive, sequence of rat tonin, it is nevertheless clear that tonin isolated from rat submaxillary gland is very closely related to the γ -subunit of mouse NGF and also probably to the EGF-binding protein. Our data do not show conclusively that tonin represents a variant of the γ -subunit of the rat NGF because NGF could not be detected in the submaxillary gland of the rat ^{14,15}; however, this possibility cannot be ruled out. Also interesting is the fact that the isolated tonin is a single poly-

Fig. 1 a, Automatic NH2-terminal degradation of reduced and alkylated rat tonin. Sequencing was carried out on 2.6 mg (87 nmol) of protein using 3 mg of polybrene precycled seven times and a 0.3M Quadrol programme on an updated Beckman 890B sequenator fitted with a cold trap. Phenylthiohydantoin (PTH) derivatives were obtained after conversion in methanol/HCl using in-line sequemat Po autoconverter. Separation and quantification were carried out by HPLC using an ultrasphere-ODS column and a tetrahydrofuran acetonitrile gradient. Quantitative yields of PTH-amino acids corrected for background and normalized to PTH-norleucine internal standard are illustrated as a function of residue number. The slope and intercept were obtained by a linear regression analysis on selected stable PTH-amino acids. Repetitive yield thus obtained was 95.2% while the carry-over never exceeded 1% except for Glu and Asp residues which are retained by the polybrene. b, Automatic NH2-terminal degradation of a reduced and alkylated (using ³H-iodoacetic acid) fragment of rat tonin obtained by cyanogen bromide cleavage. Sequencing was carried out on ~10-15 nmol using the conditions described above; the repetitive yield for that sequence was 89%.

No. cycles

peptide chain comprising 272 amino acids, whereas the γ -subunit, as reported, is composed of 233 amino acids distributed in what seems to be two or three chains linked by disulphide bridges. This observation supports Thomas et al.'s proposal's that a limited, perhaps autocatalytic, proteolysis of a single-chain form of γ -subunit generates a two- or three-chain form. Rat tonin is clearly related to the serine proteases family, especially to trypsinogen, kallikrein and plasminogen. On the other hand, the substrate specificity of this enzyme seems to be

^{*} Deceased

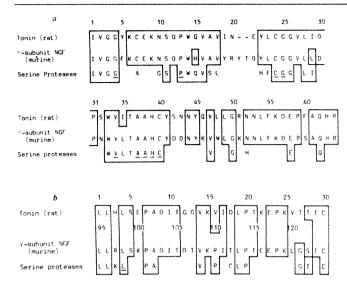


Fig. 2 a, Comparison of the amino acid sequence (in the singleletter code, ref. 16) of the NH₂-terminal portion of rat tonin with corresponding segment of y-subunit of mouse NGF as determined by Thomas et al.⁵ and with conserved segments of serine proteases according to Dayhoff⁷. The underlined residues correspond to invariant position in the known sequence of all serine proteases. b, Comparison of the amino acid sequence of a cyanogen bromide fragment of rat tonin with residues 95-124 of y-subunit of mouse NGF and conserved segments of serine proteases.

quite different from the arginine-specific activity of NGF γ subunit and EGF-binding protein because it is able to cleave after specific arginine residues and after suitably located lysine, phenylalanine, tyrosine and tryptophan residues depending on the pH used for the digest^{2,3}. For example, at pH 5.0 it can selectively cleave the pro-opiomelanocortin precursor to yield intact ACTH involving an arginine and phenylalanine cleavage at the NH₂ and COOH termini respectively. Thus, this enzyme expresses a selective chymotryptic-tryptic-type activity. The digestion studies clearly indicate the importance of the conformation of the substrate in directing the site of cleavage^{2,3}. Originally, it was proposed that tonin might be involved in the local generation of angiotensin II in tissue; its newly found similarity with NGF γ -subunit and EGF-binding protein suggests other possible roles for this enzyme and its substrate specificity.

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- Demassieux, S., Boucher, R., Grisé, C. & Genest, J. Can. J. Biochem. 54, 788-795 (1976).
- Seidah, N. G. et al. Biochem. biophys. Res. Commun. 86, 1002–1013 (1979).
 Seidah, N. G. et al. in Proc. 6th Am. Peptide Symp. (eds Gross, E. & Meienhofer, J.) 921–924 (Pierce Chemical Company, 1979).
- Seidah, N. G. et al. Can. J. Biochem. 56, 920-925 (1978).

- Seidah, N. G. et al. Can. J. Biochem. 50, 920-925 (1978).
 Thomas, K. A., Baglan, N. C. & Bradshaw, R. A. J. biol. Chem. (in the press).
 Kurosky, A. et al. Proc. natn. Acad. Sci. U.S.A. 77, 3388-3392 (1980).
 Dayhoff, M. D. (ed.) Atlas of Protein Sequence and Structure Vol. 5, suppl. 3 (1978).
 Mole, J. E. & Niemann, M. A. J. biol. Chem. 255, 8472-8476 (1980).
- Christie, D. L., Gagnon, J. & Porter, R. R. Proc. natn. Acad. Sci. U.S.A. 77, 4923-4927
- Walz, D. A., Hewett-Emmett, D. & Siegers, W. H. Proc. natn. Acad. Sci. U.S.A. 74, 1969-1972 (1977).
- 11. Butkowski, R. J., Elion, J., Downing, M. R. & Mann, K. G. J. biol. Chem. 252, 4942-4957 Woodbury, R. G., Katunuma, N., Kobayashi, K., Titani, K. & Neurath, H. Biochemistry 17,
- 811-819 (1978). Volanakis, J. E., Bhown, A. S., Bennett, J. C. & Mole, J. E. Proc. natn. Acad. Sci. U.S.A. 77,
- 1116-1119 (1980).
- Bradshaw, R. A. A. Rev. Biochem. 47, 191-216 (1978).
 Thoenen, H. & Barde, Y.-A. Physiol. Rev. 60, 1284-1335 (1980).
 Eur. J. Biochem. 5, 151-153 (1968).

Methionyl-tRNA synthetase shows the nucleotide binding fold observed in dehydrogenases

J. L. Risler, C. Zelwer & S. Brunie

Centre de Génétique Moléculaire, 91190 Gif sur Yvette, France

A striking common structural feature has emerged from the comparison of the X-ray crystallographic studies of several dehydrogenases. In lactate dehydrogenase¹, soluble malate dehydrogenase², alcohol dehydrogenase³ and glyceraldehyde-3-phosphate dehydrogenase⁴ similar foldings have been described in the region which binds the coenzyme NAD, whereas no significant similarities were observed in the chemical sequences. The occurrence of a characteristic 'nucleotide binding fold' (the so-called Rossmann fold) has also been observed in horse muscle phosphoglycerate kinase⁵, in phosphorylase^{6,7} and, with some topological deviations, in other kinases 8-11 as well as in the flavin-binding domain of flavodoxin12. If one assumes that these structural homologies are the result of a divergent evolutionary process, it is then tempting to predict a similar pattern of structure-function relationship in other nucleotide-binding proteins, in particular in aminoacyl-tRNA synthetases. We show here that methionyl-tRNA synthetase does show the same nucleotide binding fold.

Native Escherichia coli methionyl-tRNA synthetase, a dimer of molecular weight (MW) 172,000, has never been obtained in a crystalline form. However, a fully active monomeric fragment which can be prepared by a controlled proteolysis¹³, crystallizes in space-group P2, with a = 78.1 Å, b = 46.2 Å, c = 87.9 Å, $\beta = 108.8^{\circ}$. The crystals contain one molecule of MW 64,000 per asymmetric unit14. The structure of this active fragment has been determined at 2.5 Å resolution by the multiple isomorphous replacement (MIR) method using five heavy-atom derivatives. The diffracted intensities were recorded photographically with an oscillation camera and measured using a rotating drum densitometer (Centre de Microdensitométrie, Orsay). Some statistical data on the quality of the measurements and the heavy-atom derivatives are given in Table 1. The MIR phases were improved by a solvent modification technique similar to that used by Schevitz et al. 15 in the study of yeast tRNA, Met. The resulting electron density map was easily interpreted for most of the molecule, except in one small region situated in the bottom right part of Fig. 1. A detailed account of the experimental procedures and a complete description of the



Fig. 1 Stereoscopic drawing of the α -carbon backbone of methionyl-tRNA synthetase based on unrefined coordinates measured from the model. The central part (thick line) corresponds to the nucleotide binding domain. The molecule is viewed down the crystallographic b axis.

structure will be given elsewhere. An atomic model (scale 1.25 cm per Å) was built, which accounts for 480 amino acid residues.

There is no complete chemical sequence available for methionyl-tRNA synthetase but its molecular backbone is

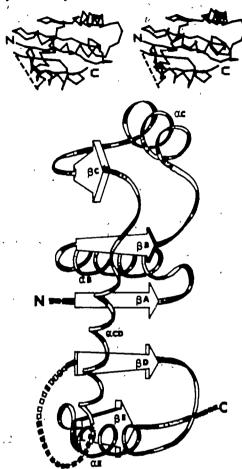


Fig. 2 A stereo view of the nucleotide binding domain in methionyl-tRNA synthetase, together with a schematic representation of its structural elements. The nomenciature of the helices and β strands are those proposed by Hill et al.². The β strands are represented by arrows pointing towards their C-terminus. The dotted chain between helix α CD and strand β D represents that part of the molecule which is depicted at the bottom of Fig. 1 (thin line). The view is approximately down the a^{α} axis. N and C represent the amino and carboxyl ends of the chain, respectively.

shown in Fig. 1. The elongated molecule $(90 \times 52 \times 44 \text{ Å})$ consists of three distinct domains: (1) a central core (thick line in Fig. 1) formed by five parallel strands separated by helices and containing the N-terminus; (2) a less regular structure (thin line at the bottom of Fig. 1) containing ~ 135 amino acid residues, which can be visualized as an intrusion between the third and fourth strands of the central core; and (3) a third domain (thin line, top of Fig. 1) rich in helices, which pears the C-terminus of the polypeptide chain.

The most salient feature of the structure is, by far, the central core, which shows the topological characteristics of a 'nucleotide binding domain': (1) it is composed of a five-stranded parallel pleated sheet with helices connecting the strands. (2) The sequential order of the strands, as shown in Fig. 2 is, from top to bottom, $\beta C - \beta B - \beta A - \beta D - \beta E$. (3) Helices αB and αC are 'below' the pleated sheet (Fig. 2) while helices α CD and α E are 'above' it. (4) The nucleotide 8-bromo-ATP, which was used as a heavy-atom marker, binds near the C-terminal end of the pleated sheet (in our interpretation this binding involves contacts with the loop between strand βA and helix αB and with side-chains of helix aB). These features clearly indicate a remarkable structural homology with the nucleotide binding domain of dehydrogenases. Note that although the structure of tyrosyl-tRNA synthetase from Bacillus stearothermophilus seemed at first to bear no structural relationship to other proteins¹⁶, an improved model revealed the existence of a Rossmann fold around the ATP binding site 17.

Methionyl-tRNA synthetase is thus a new member of the family of proteins which possess the characteristic Rossmann fold. This supports the hypothesis that many nucleotide binding proteins may derive from a common ancestral gene 18,19. By gene fusion and duplication, this gene would have evolved in a divergent evolutionary process to give rise to the modern dehydrogenases and aminoacyl-tRNA synthetases (the situation is less clear for kinases which show a rather wide variety of topologies in their ATP-binding domain). The nucleotide binding domain of methionyi-tRNA synthetase, however, has a unique feature, that is, the helix α CD and the strand β D are far apart in the sequence (they are separated by ~ 135 residues). These residues build a domain (bottom of Fig. 1) whose structure bears little resemblance to a Rossmann fold. This extra domain could be the result of the insertion of a segment of DNA into the ancestral gene²⁰. Conversely, aminoacyl-tRNA synthetases could mirror the existence of a longer ancestral gene which, after deletions, would now code for the nucleotide binding domains in the dehydrogeneses. Interestingly, the presence of an extra domain has also been reported for pyruvate kinase (PK)¹¹ as compared with triose phosphate isomerase (TIM)²¹. The two enzymes show a characteristic barrel of eight

•	∞–7.3	7.3-5.5	5.5-4.4	4.4-3.7	3.7-3.2	3.2-2.8	2.8-2.5	Overall
N .	713	942	1,544	2,209	2,961	3,621	4,020	16,010
R _{eyes} (native)	0.015	0.022	0.025	0.027	0.037	0.060	0.078	0.040
R _{eyes} (Pt)	0.020	. 0,025	0.026	0.030	0.040	0.060	0.073	0.041
R _{epon} (U)	0.018	0.022	0.024	0.026	0.034	0.051	0.063	0.035
R _{eyen} (Sm)	0.021	0.026	0.027	0.030	0.038	0.054	0.062	0.039
R _{spee} (Pt+U)	0.034	0.038	0.037	0.040	0.052	0.073	0.10	0.053
R (8-Br-ATP)	0.029	0.033	0.037	0.040	0.049	0.071	0.089	0.052
P (Pt)	2.26	2.02	1.72	1.38	1.39	1.30	1.17	1.44
P (U)	2.30	2.12	1.65	1.54	1.44	1.34	1.17	1.46
P (Sm)	2.07	2.32	1.69	1.59	1.56	1.68	1.50	1.66
P(Pt+U)	2.13	2.55	1.69	1.58	1.56	. 1.49	1,28	1,59
P(8-Br-ATP)	1.10	1.08	0.84		,			0.97
m	0.75	0.73	0.70	0.68	0.66	0.64	0.57	0.65

Statistical data on the quality of intensity measurements and heavy-atom derivatives, given as a function of resolution. N is the number of phased reflections. Due to the geometry of the oscillation method, about half of the reflections are measured more than once; the R_{sym} factor $(R_{\text{sym}} = \sum |< F| > -F|/\sum F|)$ gives a measure of the agreement between symmetry-related reflections. P is the phasing power of the heavy-atom derivatives, that is, the ratio of the r.m.s. heavy-atom scattering to the r.m.s. lack of closure: $P = [\sum f_b^2/n]^{1/2}/[\sum (F_{\text{ph}}^{\text{obs}} - F_{\text{ph}}^{\text{obs}})^2/n]^{1/2}$. The derivatives used were obtained with $\mathbb{K}_2\text{Pt}(\text{CN})_4$, $\mathbb{K}_3\text{UO}_2F_5$, $\text{Sm}(\text{NO}_3)_3$ and 8-bromo-ATP (8-Br-ATP). The last derivative was used only at 4 Å resolution. m, Figure of merit. For each derivative (including the parent) $\sim 20\%$ of the measured reflections were rejected (too weak or poor R_{sym}).

parallel strands connected by helices which is continuous in TIM, and in PK contains ~100 additional residues, inserted between the third strand and the third helix.

Knowledge of the complete amino acid sequence for methionyl-tRNA synthetase may hopefully provide further insight into its taxonomic relationships with other nucleotidebinding proteins.

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- Rossmann, M. G. et al. Cold Spring Hasb. Symp. quant. Biol. 36, 179-191 (1971).
 Hill, E., Tsernoglou, D., Webb, L. & Banaszak, L. J. J. molec. Biol. 72, 577-589 (1972).
 Eklund, H. et al. J. molec. Biol. 102, 27-59 (1976).
- Buehner, M., Ford, G. C., Moras, D., Oisen, K. W. & Rossmann, M. G. J. molec. Biol. 90,
- Blake, C. C. F. & Evans, P. R. J. molec. Biol. 84, 585-601 (1974).
 Fletterick, R. J., Sygusch, J., Semple, H. & Madsen, N. B. J. biol. Chem. 251, 6142-6146
- Weber, I. T. et al. Nature 274, 433-437 (1978)

- R. Evans, P. R. & Hudson, P. J. Nature 279, 500-504 (1979).
 Schulz, G. E. & Schirmer, R. H. Nature 250, 142-144 (1974).
 Steitz, T. A., Fletterick, R. J., Anderson, W. F. & Anderson, C. M. J. molec. Biol. 104, 197-222 (1976).
- Levine, M., Muirhead, H., Stammers, D. K. & Stuart, D. I. Nature 271, 626-630 (1978).
 Burnett, R. M. et al. J. biol. Chem. 249, 4383-4392 (1974).

- Cassio, D. & Waller, J. P. Eur. J. Biochem. 20, 283-300 (1971).
 Waller, J. P., Risler, J. L., Monteilhet, C. & Zelwer, C. FEBS Lett. 16, 186-188 (1971).
- 15. Schevitz, R. W., Podjarny, A. D., Zwick, M., Hughes, J. J. & Sigler, P. B. Acta Crystallogr.
- (in the press).

 16. Irwin, M. J., Nyborg, J., Reid, B. R. & Blow, D. M. J. molec. Biol. 105, 577-586 (1976).

 17. Bhat, I. N., Blow, D. M., Brick, P., Monteilhet, C. & Nyborg, J. Abst. EMBO-FEBS (RNA)
- Workshop, Strasbourg, 1980.

 18. Rossmann, M. G., Moras, D. & Olsen, K. W. Nature 250, 194-199 (1974)
- Ohlsson, I., Nordström, B. & Bränden, C. I. J. molec. Biol. 89, 339-354 (1974).
 Calos, M. P. & Miller, J. H. Cell 20, 579-595 (1980).
- 21. Banner, D. W. et al. Nature 255, 609-614 (1975)

Cationic control of O₂ affinity in lugworm erythrocruorin

Roy E. Weber

Institute of Biology, University of Odense, DK 5230 Odense M. Denmark

The erythrocruorins (extracellular haemoglobins from annelids¹) have molecular weights of 3-4×10⁶, contain 60-192 O2-binding haem moieties per molecule and are much more complex than the tetrameric vertebrate haemoglobins1-However, they perform the same function, carrying O2 from the respiratory surfaces to the tissues, and exhibit similar cooperativity in O2 binding and inhibitory heterotropic interactions between O2- and proton-binding sites (Bohr effects), although these functions show greater adaptive variation than in the vertebrate pigments^{2,3}. Whereas erythrocruorin- O_2 affinity is insensitive to the anionic organic phosphate cofactors like glycerate-2,3-bisphosphate and ATP1-3, which depress the O2 affinity of vertebrate haemoglobin inside the red blood cells, it is increased by inorganic salts⁶⁻⁸. This effect is important physiologically because annelids lack significant capacity for osmotic regulation and experience large fluctuations in blood electrolyte levels8,9. I show here that, in contrast to the situation in man, where anionic cofactors and protons decrease haemoglobin-O2 affinity by specifically depressing the O2 association equilibrium constant of the pigment in the deoxygenated state 10-13 inorganic cations govern the O_2 affinity of erythrocruorin from the burrowing, intertidal lugworm, Arenicola marina, by preferentially modifying the association constant of the (almost fully) oxygenated form. In contrast to the vertebrate mechanism, which optimizes O2 unloading in the tissues, this alternative control mechanism in erythrocruorin seems to be adaptive to O2 loading at the low O_2 tensions generally characteristic of the microenvironments of erythrocruorin-bearing annelids¹⁻³.

Figure 1 shows that NaCl increases O2 affinity and cooperativity (measured as n_{max} , the maximum slopes of the Hill plots) of Arenicola erythrocruorin by increasing the association constant for binding the last O2 molecules, without significantly affecting that for binding the first molecules to the deoxygenated pigment. These constants, which are respectively referred to as K_R and $K_{\rm T}$, by analogy with the R and T states of vertebrate haemoglobin, without implying the existence of only two discrete conformational states, are estimated by lines drawn asymptotic to the experimental curves at extreme high and low O₂ saturations (see Fig. 1). Divalent cations exert greater effect than monovalent ones at the same anion concentration and also slightly increase K_T (Fig. 1). These results imply preferential cation binding to erythrocruorin in the oxygenated form and provide a rare example of an heterotropic effector that increases O2 affinity in haem pigments.

The differential effects of salts on K_T and K_R raise the free energy of haem-haem interaction, ΔG (which equals RT In (K_R/K_T) where R is the gas constant and T the absolute temperature¹⁴). At pH 7.37, which approximates the in vivo value at 15 °C (refs 1, 2), the K_R/K_T ratio indicates a 33-fold higher affinity for the last compared with the first O2 molecules bound, and a ΔG value near 8.4 kJ mol⁻¹ in the absence of salts. The presence of 0.6 M Na⁺ or 0.12 M Mg²⁺ (which approximate the blood levels in lugworms from normal seawater¹²) raises K_R/K_T to ~68 and ΔG to ~10.0 kJ mol⁻¹ (Fig. 1, right The mechanism of ionic interaction in erythrocruorin is radically different from that in human haemoglobin, where glycerate-2,3-bisphosphate lowers $K_{\rm T}$ (reducing the association constants for the first three but not the last O2 molecule) and where neutral salt acts in a qualitatively similar manner except that it also slightly increases K_R (refs 10-12). Both control mechanisms, however, have the same net effects, increasing ΔG

Proton concentration also acts mainly on K_R of erythrocruorin but in the opposite direction to salt concentrations, shifting the oxy-asymptote to lower affinities and reducing ΔG and n_{max} (Fig. 2). This also contrasts with human haemoglobin, where lower pH reduces K_T and only slightly affects K_R , raising ΔG and cooperativity¹³. The present data allow estimation of the Bohr effects for binding of the first and last O_2 molecules (Δ $\log K_T/\Delta pH$ and $\Delta \log K_R/\Delta pH$, respectively) as -0.20 and -0.74, showing that most protons are released on binding of the last O2 molecules.

The shifts in the oxy-asymptotes of Arenicola erythrocruorin imply the existence of different R states whose O2 affinities depend on cation and proton binding. Ions thus affect not only the apparent equilibrium constant for the transition between the deoxy- and oxy-forms but also the non-exclusive binding coefficient $c(=K_T/K_R)$, in contradiction of the two-state $model^{15}$ as defined for human haemoglobin, where c is invariant of the concentration of the allosteric effector.

The fact that the ion sensitivity of erythrocruorin resides with $K_{\rm R}$, rather than with $K_{\rm T}$ as in human haemoglobin, seems to be adaptive to O2 transport in hypoxic (O2-poor) conditions in the natural habitat of annelid worms. In contrast to air-breathing animals where loading O2 tensions generally are sufficient to saturate the pigment fully, so that O2 transport can only be improved by increasing O2 unloading, O2 transport in hypoxic microenvironments depends on O_2 loading and thus K_R . In animals like Arenicola, a salt-induced increase in K_R and thus in n_{max} and O_2 affinity will favour oxygenation of the erthrocruorin in the gills during tidal emersion, when severe burrow hypoxia 16 coincides with high blood salt levels as a result of saline, dense, high-tide water remaining in the burrows8. Again, the proton sensitivity of KR, resulting in a greater Bohr effect at high degrees of O₂ saturation, will augment the O₂ affinity difference between blood at relatively high O2 tension and pH (as in the gills) and blood at lower O₂ tension and pH (as in tissues)¹⁷. As the published data show that in nature tissue O2 tensions in Arenicola marina fall sufficiently to unload the pigment at low tide16 and that gill-tissue pH differences do occur18, the salt and

Fig. 1 Effects of inorganic salts on O₂ equilibria of Arenicola erythrocruorin, measured in 0.05 M Tris-HCl buffer at 15 °C and pH 7.37; haem concentration s 0.17 mM; \tilde{Y} is the fractional O_2 saturation. Left: Is 0.17 mM; Y is the tractional O₂ saturation. Left: Hill plots in the absence of cation (♠) and in the presence of 0.12 M NaCl (○), 0.25 M NaCl (●), 0.62 M NaCl (□) and 0.12 M MgCl₂ (♠). Right: effects of NaCl (♠) and MgCl₂ (♠) on the association equilibrium constant for the first and last O₂ molecules bound (K_T and K_R, respectively) and on the free energy of hearm-harm interaction. AG. The cert energy of haem-haem interaction, ΔG . The erythrocruorin, collected as described previously, was stripped of ions by passage through mixed ion-exchange resin (Amberlite MB-3) and dialysis against distilled water. Cation concentrations were varied by the addition of solutions of analytical grade cationchloride salts and controlled by chloride measurement using a Radiometer CMT-10 titrator. The O₂ equilibria were measured with a diffusion chamber technique 19,20 except that the stepwise increases in O₂ tensions were generated by cascaded gas mixing pumps (Wösthoff) while absorbance changes between zero and full saturation (when equilibrated with pure N2 and O2, respectively) were monitored on recorders with high sensitivity (Radiometer REA 112) units. Extremely low and high saturations were measured by amplifying absorbance increments near zero and full O₂ saturation in separate runs, observing previously described precautions¹¹. The asymptotes were fitted by eye. With the upper asymptotes the saturation points were additionally estimated from extrapolations in plots of $\Delta \hat{V}$ against 1/p. (cel. 12) The tions in plots of ΔY against $1/p_{\rm O_2}$ (ref. 13). The application of small amounts of erythrocruorin solutions (layer thickness $\sim 10~\mu \rm m$) minimized equilibration time and meterythrocruorin formation, which was always less than 1% and corrected for graphically²⁰.

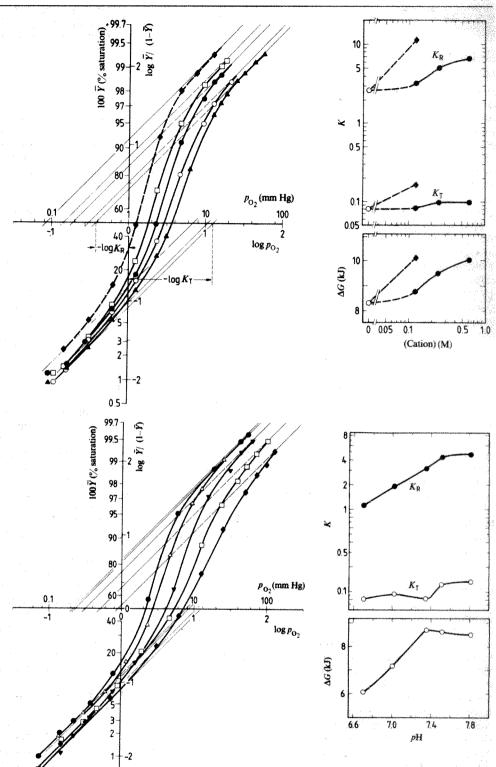


Fig. 2 Effects of pH on O₂ equilibria of Arenicola erythrocruorin, measured in 0.05 M HEPES-NaOH buffer at 15 °C. pH values (left panel): 7.81 (♠), 7.51 (♠), 7.35 (♥), 7.02 (□) and 6.70 (♠). The cor-Tris buffers (compare ▼ with ▲ in Fig. 1) indicates the absence of significant buffer effects. Other details are as for Fig. 1.

proton interactions will increase the capacity of the blood for transporting O₂.

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- 1. Weber, R. E. in Physiology of Annelids (ed. Mill, P. J.) 393-446 (Academic, New York,
- 2. Magnum, C. F. & Adaptation to Environment (ed. Newell, R. C.) 191-278 (Butterworths. Magnuin, C. F. 89 Maaptation to Environment (ed. Newell, R. C.) 191-218 (Dutters London, 1976).

 Weber, R. E. Am. Zool. 20, 79-101 (1980).

 Chung, M. C. M. & Ellerton, H. D. Prog. Biophys. molec. Biol. 35, 53-102 (1979).

 Wood, E. J. Essays Biochem. 16, 1-47 (1980).

- Everaarts, J. M. & Weber, R. E. Comp. Biochem. Physiol. A48, 507-520 (1974).
 Weber, R. E., Bonaventura, J., Sullivan, B. & Bonaventura, C. J. comp. Physiol. 123, 177-184 (1978).
- 8. Krogh-Rasmussen, K. & Weber, R. E. Ophelia 18, 151-170 (1979).

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- 9. Oglesby, L. in Physiology of Annelids (ed. Mill, P. J.) 555-658 (Academic, New York,
- 10. Tyuma, I., Katsuhiko, K. & Imai, K. Biochem. biophys. Res. Commun. 43, 423-428 (1971).

- Iyuma, I., Katsuhiko, K. & Imai, K. Biochem. biophys. Res. Commun. 43, 423-428 (1971).
 Imai, K. Biochemistry 12, 798-808 (1973); J. biol. Chem. 23, 7606-7612 (1974).
 Tyuma, I., Imai, K. & Shimizu, K. Biochemistry 12, 1491-1498 (1973).
 Imai, K. & Yonetani, T. J. biol. Chem. 250, 2227-2231 (1975).
 Wyman, J. Adv. Protein Chem. 19, 223-286 (1964).
 Monod, J., Wyman, J. & Changeux, I. -P. J. molec. Biol. 12, 88-118 (1965).
 Toulmond, A. Resp. Physiol. 19, 130-144 (1973).
 Warren, L. M., Wells, R. M. G. & Weber, R. E. J. exp. mar. Biol. Ecol. (in the press).
 Mangum, C. P. Physiol. Zool. 49, 85-99 (1976).
 Weber, R. F. Comm. Biochem. Physiol. 35, 170, 180 (1970).
- Weber, R. E. Comp. Biochem. Physiol. 35, 179-189 (1970).
 Sick, H. & Gersonde, K. Analyt. Biochem. 32, 362-376 (1969).

MATTERS ARISING

Rebuttal of criticisms of remote viewing experiments

MARKS¹ has argued that our first two remote viewing (RV) studies (with subjects Price and Hammid)² should be discounted as evidence for the RV hypothesis. He claims that sensory cues in the subject-generated RV transcripts provide sufficient information to permit judges to blind-match transcripts to target sites on an artefactual basis. The evidence, however, does not support this claim.

First, with regard to the Hammid series. When one examines the details of Marks' criticism (see ref. 3) one finds that the criticism about cueing is based on an error-in-fact on the part of Marks; he erroneously assumed that the target list given to judges was in the order of target usage, that is, unrandomized. That is incorrect. That list, which Marks used to formulate his criticism, was randomized by random number generator to prevent the very possibilities he raises. When this fact is taken into account, one finds that his criticism is voided at its foundation. Specifically, his criticism is derived from the fact that the transcripts were dated; however, the dates do not provide the useful cue information posited by Marks-they would have done so only under the (incorrect) assumptions about target order made by Marks. As a result. Marks' attempt to associate the transcripts to the target sites on the basis of the alleged cues failed.

With regard to the Price series. While not questioning the data collection procedures or commenting on the near-photographic accuracy of some of the individual descriptions, Marks and Kammann hypothesized in their first critique that the successful judging of the Price series might be due to sensory cues in the transcripts that provide information as to, for example, whether a given trial was early or late in the series4. In response to this we arranged to have the series independently rejudged, using transcripts from which the suggested cues had been removed. The rejudging resulted in the same seven out of nine target/transcript matchings as in the original study, indicating that it was the quality of the transcripts themselves, rather than the presence of cues, that was responsible for the successful outcome². This rejudging is rejected by Marks because of the potential confounding factor of the publication of the original studv.

This leads us to a second re-analysis of the Price series for which, in order to give the Marks' hypothesis its best chance, we assume all potential cues remain and are used to maximum advantage; we then rigorously assess the consequences. In the strongest case for cues the probability of a correct match can at most be increased from one-ninth to one-third; in another

case from one-ninth to one-seventh; and in two other cases from one-ninth to oneeighth. The remaining five cases gain no direct advantage from cues, just indirect advantage from not using as possibilities the ones already used in the more advantageous cases. When one takes into account the resulting constraints due to cues, the number of possible target/transcript matchings is reduced from 9!= 362,880 to 68,760 combinations, by exact count. Assuming that the cues are used to maximum advantage, we find that the significance level associated with obtaining at least seven matches in nine trials (as was done) in a forced-choice, nonindependent assignment of transcripts to target sites (the most conservative statistic) is only reduced from $P = 10^{-4}$ to P = 3.9×10^{-4} , still a quite significant result. (A paper containing the details of this analysis has been published elsewhere⁵.) Thus, the Marks hypothesis that the presence of extraneous cues accounts for the significance of the Price series result receives no support.

We would summarize by first indicating that we recognize that our original RV studies are not without flaw, and the potential effect of possible cues deserves critical examination. However, in our continuing re-examination of this work we find that the suggested cueing artefacts, although providing some potential for confounding, are yet well below the magnitude necessary to account for the strength of the results. As a result, this re-examination of the data has provided yet additional support for the hypothesis indicating the existence of a human remote sensing capability. This conclusion is supported by the continuing replication of RV experiments in our own and other laboratories^{6,7} for which the Marks concerns no longer apply due to the increased sophistication of the procedures in use.

H. PUTHOFF R. TARG

Radio Physics Laboratory, Menlo Park, California 94025, USA

- 1. Marks, D. Nature 292, 177 (1981).
- Tart, C. T., Puthoff, H. E. & Targ, R. Nature 284, 191 (1980).
- Marks, D. & Kammann, R. The Psychology of the Psychic (Prometheus, New York, 1980).
 Marks, D. & Kammann, R. Nature 274, 680-681 (1978).
- Marks, D. & Kammann, R. Nature 274, 681-681 (1978).
 Tart, C. T., Puthoff, H. E. & Targ, R. Research in Parapsychology (Scarecrow Press, Metuchen, New Jersey, in the press).
- Dunne, B. J. & Bisaha, J. J. Parapsychol. 43, 17-30 (1979).
 Schlitz, M. & Gruber, E. J. Parapsychol. 44, 305-317 (1980).

Expression of herpesvirusinduced antigens in human cervical cancer

DREESMAN ET AL. have described the expression of two herpes simplex virus type 2 (HSV-2) antigens, designated ICSP

11/12 and ICSP 34/35, in 38% of cervical tissues with pathological findings of severe dysplasia or carcinoma. Their undocumented statement that previous studies used antisera against HSV virions and gave rise to equivocal results is inaccurate and generates controversy where none exists. Indeed, the expression of HSV-2 antigens in cervical anaplastic tissue has been consistently and unequivocally demonstrated using antisera prepared against extracts of HSV-2-infected cells that contain both structural and nonstructural viral proteins. To our knowledge, there is no report describing the failure to identify HSV-2 antigens in cervical anaplastic tissue when such have been studied. Had the authors' comment on the equivocal nature of previous results been valid, their data demonstrating the presence of two antigens, the viral identity of which may be questionable to some critics, in only 38% of patients with anaplastic findings could hardly support their conclusion that HSV-2 is a factor in human cervical cancer1.

Using antisera against total viral antigens, HSV-2 antigens were demonstrated by immunodiffusion and crossed immunoelectrophoresis in extracts of cervical tumour cells²⁻⁴. Similarly prepared antisera were used by Athanasiu et al.5 in immunofluorescent staining of frozen sections of cervical tumour biopsies. Expression of HSV-2 antigens was described in 8 of 24 (33.3%) specimens from cervical carcinoma, a percentage remarkably similar to that described by Dreesman et al. , using similar specimens. The observation that viral antigens are preferentially expressed in cells on the peripheral part of the carcinoma mass towards the vagina and/or cervical canal^{6,7} has led some investigators to conclude that the proportion of reactive patients could be greatly increased by studying the cervical cells that have naturally exfoliated or have been induced to exfoliate artificially. Immunoflorescent staining of such specimens with antisera against total viral antigens in six independent studies⁶⁻¹¹ has demonstrated the expression of HSV-2 antigens in cells of 61-81.2% of patients with dysplasia and 92-100% of those with invasive cancer or carcinoma in situ (CIS). This compares with a reactivity of 0-9.4% in normal women (Table 1).

We showed previously that antisera against an antigenic fraction designated AG-e, that consists of two viral proteins (ICP 12, ICP 14)¹², stains cervical anaplastic cells from patients with dysplasia, CIS or invasive cancer, but not from normal women¹⁰. Consistent with this finding, antisera against ICP 12 or ICP 14 also stain the cells from patients with these pathological findings¹¹. It is significant that ICP 12 shares a number of

Table 1 % Of patients exfoliated cells staining with antisera to viral antigens*

Diagnosis	Total†	AG-e‡	ICP 12	ICP 14
Normal (N)	0-9.4	0	0	0
Dysplasia (D)	61-81.2	69-72.6	57	51
CIS and invasive (Inv)	91.6-100	91.4	100	75

* Athanasiu et al. 5: 33.3% of frozen sections of cervical carcinoma stained with antiserum to total viral antigens. Normal tissue was negative.

† Pacsa et al.⁸: N = 9.4%, D = 61%, CIS/Inv = 94%. Adelusi et al.⁹: N = 0, Inv = 100%. Minhui et al. 6 : N = 9%, Inv = 100%. Aurelian et al. $^{7.10}$: N = 0, D = 81.2%, CIS/Inv = 91.6%. 4 Smith et al. 11 : N = 0, D = 72.6%, CIS/Inv = 91.4%. Aurelian et al. 10 : N = 0, D = 67.6%, CIS/Inv = 86.4%.

properties with Dreesman's ICSP 11/12—the two proteins have the same molecular weight and both bind DNA^{1,13} Antisera against them stain the nuclei of HSV-2-infected cells, but staining of cervical anaplastic cells is cytoplasmic and perinuclear f.11

We suggest that the findings of Dreesman and his colleagues cannot be considered in isolation of previous evidence in this field. They neither offer conceptually new information, nor do they help elucidate a hitherto equivocal or controversial issue. The significance of their findings lies specifically in their confirmatory nature. Only in this context can the authors' conclusion be accepted, that HSV-2 is a factor in cervical carcinoma.

> C. C. SMITH P. K. GUPTA L. AURELIAN

Departments of Comparative Medicine. Pathology and Biophysics, The Johns Hopkins Medical Institution, Baltimore, Maryland 21205, USA

- 1. Dreesman, G. et al. Nature 283, 591-593 (1980).
- 2. Gall, S. A. & Haines, H. G. Gynecol. Oncol. 2, 451-459 (1974).
- Ibrahim, A. N., Ray, M., Megaw, J., Brown, R. & Nahmias, A. J. Proc. Soc. exp. Biol. Med. 152, 343-347 (1976).
 Ibrahim, A. N., Robinson, R. A., Marr, L., Abdelal, A. T. H. & Nahmias, A. J. J. natn. Cancer Inst. 63, 319-323 (1979)
- 5. Athanasiu, P., Nastas, E., Stoian, M., Predesiu, M. & Hozoe, M. Revue roum. Med. Virol. 29, 251-253 (1978).
 6. Minhui, C., Yuexin, P., Xuejun, Y. & Wenxian, Z. Chin. J.
- Oncol. 1, 255-259 (1979).
- 7. Aurelian, L., Strandberg, J. D. & Davis, H. J. Proc. Soc.
- Aurelian, L., Strandoerg, J. D. & Davis, H. J. Proc. Soc. exp. Biol. Med. 140, 404-408 (1972).
 Pacsa, A. S., Kummerlander, L., Pejtsiky, B., Kromer, K. & Pali, K. Cancer Res. 36, 2130-2132 (1976).
 Adelusi, B. B., Osunkoya, B. O. & Fabiyi, A. Obstet. Gynecol. N. Y. 47, 545-548 (1976).
 Aurelian, L. et al. Analyt. quant. Cytol. 1, 80-102 (1979).

- Smith, C. C. et al. Analyt. quant. Cytol. 2, 131-143 (1980).
 Smith, C. C. & Aurelian, L. Virology 98, 255-260 (1979).
 Smith, C. C., Aurelian, L., Cohen, G. H., Eisenberg, R. & Schaffer, P. A. (submitted).

POWELL ET AL. REPLY-We have no intention of generating controversy as Smith, Gupta and Aurelian have suggested. Our aim was to examine sections of cervical tissue biopsy specimens for the presence of two specific HSV antigens. We detected both proteins in about onethird of the samples of abnormal tissues¹. As we detected the HSV antigens in the unstressed biopsy specimens, this result clearly differs from that of Aurelian et al.2, in their paper "HSV-2 antigens absent from biopsied cervical tumor cells: a model consistent with latency" wherein

they were unable to demonstrate the presence of HSV-2 antigens in cervical tumour biopsies from 29 patients. We would agree that all published reports have found at times expression of HSV antigens in exfoliated cervical cells.

Previous results are equivocal. Thus whilst Athanasiu et al.3 reported the presence of virus antigens in cervical tumour biopsy specimens using antisera to crude virus antigens and the indirect immunofluorescence test, this result was not obtained by Nahmias et al.4, Aurelian et al.2 or by ourselves1. Even in studies of exfoliated cells, reports differ as to the presence of virus antigens in cells from normal patients or from patients with bland disorders and in the proportion of patients with overt disease showing positive reactions with antisera to crude virus protein mixtures^{2,5,6}. It is important not to gloss over such differences as they may be highly significant.

Our approach was quite different from that of previous reports. We selected two proteins ICSP 11, 12 and ICSP 34, 35 because they are both DNA-binding proteins. Both are known to be virus specific and have been mapped using intertypic recombinants to specific regions of the viral genome. They were purified to homogeneity and only then used to prepare antisera. The sera were tested to ensure that they only reacted with the polypeptide used for immunization. These reagents obviously differ considerably from the crude or partially purified antigen preparations used by other groups to prepare antisera for previous studies^{2,4-6}. With these reagents two independent studies have detected expression of HSV antigens in about onethird of cervical tumour biopsies^{1,7}. We were totally unable to detect HSV antigens in the same specimens using potent general antisera to total virus proteins, in contrast to results reported by Aurelian's group with exfoliated cervical cells (60-90% of dysplasic CIS/invasive cancer patients samples positive even using antisera to crude infected cell extracts).

Finally, we are amazed at the casual way in which Smith, Gupta and Aurelian associate their ICP 12 and ICP 14 (which they have clearly shown to be structural proteins capable of inducing neutralizing antibody⁸) with ICSP 11, 12 which is a non-structural protein totally absent from even the worst preparations of purified virus. Our reagents and our results are clearly different from those of Aurelian and her co-workers.

K. L. POWELL D. J. M. PURIFOY

Department of Microbiology, University of Leeds, Leeds LS2 9JT, UK

G. R. DREESMAN J. BUREK E. ADAM

R. H. KAUFMAN J. L. MELNICK

Departments of Virology and Epidemiology and of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas 77030, USA

- 1. Dreesman, G. R. et al. Nature 283, 591-593 (1980).
- Aurelian, L., Strandberg, J. D. & Davis, H. J. Proc. Soc. exp. Biol. Med. 140, 404-408 (1972).
- Athanasiu, P., Nastas, E., Stoian, M., Predesiu, M. & Hozoe, M. Revue roum. Med. Virol. 29, 251-253 (1978). 4. Nahmias, A. J., Del Buono, I. & Ibrahim, I. 2nd int. Symp Oncogenesis and Memesviruses II (eds De The, G
- Epstein, M. A. & Zur Hausen, H.) 309-313 (IARC Scientific Publs, Geneva, 1975).
- Scientific Publs, Geaeva, 1973.
 Pasca, A. S., Kummelander, L., Pejtsik, B., Krommer, K. & Pali, K. Cancer Res. 36, 2130–2132 (1976).
 Smith, C. C. et al. Analyt. quant. Cytol 2, 131–143 (1980).
 McDougal, J. K. et al. Cold Spring Harb. Conf. cell. Proli-
- feration 7, 101-116 (1980).

 8. Smith, C. C. & Aurelian, L. Virology 98, 255-260 (1979).

Recent thrusting in the Appalachians

STUDIES of incremental strain have been used in an attempt to understand recent tectonism in regions, such as eastern North America, which are generally considered to be tectonically inactive. Yet one of the best pieces of evidence of recent tectonism and incremental strain is seismicity, and eastern North America has historically been seismically quite active1.

Schäfer² has studied apparent recent tectonism in the Appalachians using offset drill holes to indicate incremental strain. Incremental strain is observed in drill holes following construction of presplit highway cuts in the Cumberland-Allegheny Plateau, and in the western Valley and Ridge of Tennessee, West Virginia and Pennsylvania.

We have examined three of the four localities described by Schäfer, and other localities nearby. Our additional data suggest that tectonic stresses resulting in faulting are responsible for the observed offsets but that there is no evidence of post-palaeozoic tectonism.

Examination of exposures along Interstate Highways 40 and 75 in Tennessee and at Oak Flat, West Virginia, revealed numerous offset drill holes in Pennsylvanian and Devonian sandstone (and sandstone/shale). All observations were confined to near-vertical presplit roadcuts in which the rocks are minimally weathered providing less chance of error caused by measurements on loose blocks.

In all offset holes containing multiple offsets, or in any succession of offsets from

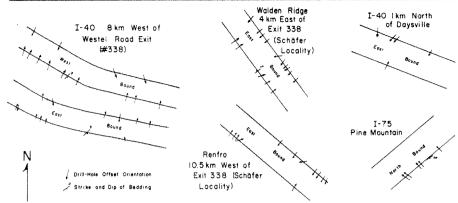


Fig. 1 Orientations of displacement for drill holes at the localities in Tennessee.

the base to the top of a cut in the Tennessee localities, the amount of offset increases upwards. Also, in most drill holes in which there are multiple offsets, the offset vectors are nearly parallel. Where offset vectors are not parallel, differences in motion may generally be explained by relative rotation of one of the upper blocks during blasting, later slumping, readjustment across major joints, or some other local process. At the West Virginia locality south-west of Oak Flat, the sense of movement is reversed from the base to the top of the cut on the north side of the highway. On a lower-to intermediate-level fracture the drill holes are offset away from the highway, whereas on an upper-level fracture the offset vectors have moved towards the highway.

A consistent relationship between offset vectors and highway cut orientation was noted. Orientation vectors are consistently oriented approximately normal to highway cuts. Most vectors symmetrically converge towards the centre of the highway (Fig. 1). At one locality in Tennessee, measurements were made in two parallel cuts of westbound and eastbound lanes separated by 30-50 m of rock. Offset vectors converge symmetrically towards the centre of the westbound lane, but vectors on both the north and south sides of the eastbound lane point north (Fig. 1). Using an elastic rebound mechanism, this pattern may be explained by excavation of the westbound lane first, initiating rebound and release of elastic strain energy throughout the mass. The differential in total or average offset of drill holes from the westbound to the eastbound lane (consistently greater effects in the westbound lane) favours a difference in time of excavation for the two lanes.

Offsets may cease to exist from one side of prominent joints (properly oriented) to another. One side of a joint may be offset as much as 30 cm whereas drill holes on the other side of the joint may be offset only 0 to <2 cm.

Stresses which produce observed strains, such as offset drill holes, may be simply interpreted as having a tectonic origin². However, relatively high near-surface in situ stresses may also originate from non-tectonic processes³.

Static loads derived from the weight of rock material composing a topographic high may become resolved into shearing stresses directed along properly oriented existing planar discontinuities, such as subhorizontal bedding. As long as these stresses are less than the shearing strengths of the rocks involved or less than the stresses necessary to reactivate existing planar discontinuities, no permanent strain would result. There would also be some dependence of storage of elastic strain energy on the elastic moduli of the rock mass. The most favourable orientations for static load-derived shearing stresses to be relieved would obviously be those which would permit movement outwards from the source of stress. A downward-directed static load may be relieved effectively by horizontal motion, provided this otherwise normal stress may be transformed into shearing stresses (Fig. 2). Existing subhorizontal planar discontinuities (bedding or fractures) may provide shear surfaces along which the stresses may be relieved. Motion along these surfaces may be accelerated during blasting where rapidly expanding gases are forced along bedding or fracture surfaces. The gases separate the rock mass and effectively lubricate it to overcome the coefficient of internal friction along the surfaces, so that accumulated elastic strain energy is released. Further strain may be caused by subsequent creep along discontinuities.

Blocks which are surrounded by properly oriented joints may contain no stored energy. Joints generally dip 70° or more. Those which are sufficiently open (not locked) and are oriented at low to moderate angles to the outward-directed shearing stresses may be relieved without offsetting drill holes.

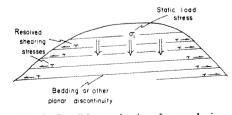


Fig. 2 Possible mechanism for producing subhorizontal shearing stresses from a nontectonic source.

We conclude that offset drill holes are a common phenomenon in the Cumberland-Allegheny Plateau of Tennessee and West Virginia where bedding is nearly horizontal. Drill holes are offset by release of stored elastic strain energy produced by static load. Offset drill holes in the Cumberland-Allegheny Plateau and in the Valley and Ridge are not related to recent tectonism. All tectonic structures present here predate highway construction and are probably Palaeozoic structures.

ROBERT D. HATCHER JR Department of Geology, University of South Carolina, Columbia, South Carolina 29208, USA

FRED WEBB JR
Department of Geology,
Appalachian State University,
Boone, North Carolina 28608, USA

- 1. Sbar, M. L. & Sykes, L. R. Bull. geol. Soc. Am. 84, 1861
- 2. Schäfer, K. Nature 280, 223 (1979)
- Schaeffer, M. F., Steffens, R. E. & Hatcher, R. D. Jr. Southeastern Geol. 20, 129 (1979).

SCHÄFER REPLIES—Hatcher and Webb have observed additional offsets of drill-holes with vectors oriented normal or oblique to the highway cuts. I agree that many of these displacements result from the release of static load-derived strain which is a common feature in the Appalachians and in other mountainous areas.

hatcher and Webb stated that I simply interpreted those offsets as having a tectonic origin. However, I did not consider any offset vector which was directed towards or away from a highway, although a tectonic component could also be involved. Offset vectors oriented parallel to the highway cuts were not included, either, in myanalysis when the excavation of the raod was directed radially to a topographic high. As of sites 1 and 2 at Interstate highway 40, Tennessee. which is NW-SE-oriented, a topographic high is located north-east of the highway. Also at the West Virginia site the highway cut runs tangentially to a topogkraphic high in the north-west. Thus, it is not surprising that Hatcher and Webb observed numerous drillholes with offset vectors normal or oblique to the road cuts. Other non-tectonic offset vectors may even run in the direction of the road-cut when static load-derived tangential tensile stresses induce dip-slip movements.

The borehole offsets which I considered, however, are all parallel (in one case sub-parallel) to the highway cuts revealing considerable rock masses to be upthrusted by tectonic forces and not by blasting. The tectonic origin of these faults evidently is not doubted by Hatcher and Webb as they did not mention any of these borehold offsets in their discussion.

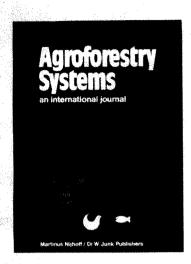
KARLHEINZ SCHÄFER Institut für Geowissenschaften der Universität Bayreuth, D-8580 Bayreuth, FRG

1. Schäfer, K. Nature 280, 223 (1979).



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BOOK REVIEWS

Time to change

T. W. Marshall

THIS, book deserves to be read, and discussed, at several different levels, and not all levels can be covered in a single short review. The chemist or physicist who wishes to understand Prigogine's concept of "dissipative structure", and its application to systems far from thermodynamic equilibrium, for which he received the Nobel prize for chemistry in 1977, will find in Part II — "The Physics of Becoming" - a clear explanation of where both equilibrium and linear non-equilibrium thermodynamics become inadequate, and of how an externally imposed inhomogeneity, such as a temperature gradient, produces a new kind of order. Examples are given from physics (convection in the atmosphere), chemistry (periodic chemical reactions) and biology (cell accretion).

As its title implies, the book is more than that. Prigogine's Nobel award citation referred to his "bridging the gap between biological and social fields of enquiry", and he sets out here to share his insights with a wider reading public. His central character is Time, and he states the problem in his preface: to relate three different concepts of time occurring in dynamics (time as a simple parameter with no preferred direction), thermodynamics (time has direction, but leads inescapably towards "heat death") and biology (time as evolution and, ultimately, history).

In the preface, Prigogine acknowledges a debt to past giants in the field, Ludwig Boltzmann and Jacques Monod. In this work and its companion volume (La Nouvelle Alliance, written jointly with Isabelle Stengers and published by Gallimard in 1979), he makes a strong bid to join them.

But he is no philosophical soulmate of these two. Both describe themselves unhesitatingly as materialist in outlook, and Prigogine recounts how Monod rebuffed his attempt to establish common ground, classifying his programme for unifying the physical and social worlds as a variety of animism. Possibly this somewhat intemperate rejection led Prigogine, in his turn, to misunderstand Monod. For he quotes the passage from Chance and Necessity about man being "a stranger in the world from which he evolved by accident" as though Monod was referring to life as the strange feature. A more careful reading reveals that the strange feature, peculiar to our species, is consciousness - that which leads us

From Being to Becoming: Time and Complexity in the Physical Sciences. By Ilya Prigogine. Pp.272. ISBN hbk 0-7167-1107-9; ISBN pbk 0-7167-1108-7. (W. H. Freeman: 1980.) Hbk \$24.95, £16.70; pbk \$12.95, £7.95.

ultimately to scientific and philosophical enquiry about our environment. So if, as Prigogine proposes, we need a "second time" to describe irreversible processes, including life, how can he be sure that we do not need a "third time" to describe consciousness?

It is to Boltzmann, however, that Prigogine mainly addresses this work. He begins by noting, like Boltzmann and Gibbs before him, that in a purely dynamical system, based on a hamiltonian evolution function, it is not possible to construct a state function having the time-directional property of entropy. The "Poincare-Misra theorem" (Chapter 7) simply puts this long-known result into the modern terminology of Lyapunov functions. Yet every thermodynamic system has an entropy. Ask a physical chemist and he will measure it for you. Hence the problem.

Gibbs's solution, which Prigogine rejects, was to say that the irreversibility of thermodynamic processes is an illusion, a creation of the physical chemist's imagination. Prigogine quotes some fragments of the Einstein-Besso correspondence which suggest that Einstein also held this view. But a closer examination of those letters, especially the passages dealing with Brownian motion, shows that, in fact, Einstein supported Boltzmann.

Boltzmann's solution was to propose that, in addition to the dynamical laws of evolution of a large system, we should require the initial state to be one of "molecular chaos". He anticipated Prigogine's diagram 7.3 and asserted that, since a state with all the molecular velocities reversed does not have this property, it will almost never occur in any actual physical system.

Prigogine, applying some new results obtained by mathematicians working in ergodic theory, thinks he has *derived* Boltzmann's molecular chaos from the dynamics. But has he? I believe not

That is not to deny that he and his coworkers have achieved a great deal. They have shown, more clearly than most, that Boltzmann's definition of chaos has to be extended to include higher order correlations in the motions of neighbouring molecules. And, for certain simple timeevolving systems, of which the most discussed is the non-dynamical Baker's transformation, they show how to construct an entropy operator which is compatible with the evolution operator. But the very fact that they end up with an entropy operator shows that they, like Boltzmann, are considering not a single microstate, but a set of states on which only certain averages are specified. To specify a probability measure on this set is to impose a more sophisticated molecular chaos hypothesis. So Boltzmann, rather than Prigogine, invented the concept of "second time"

In his remarks on Einstein's refusal to accept quantum mechanics, Prigogine is really out of his depth. It is the case that some quantum theorists are now proposing to abandon the 400-year-old notion of space going from Galileo through Einstein. on which all science since the Renaissance is based, preferring a "biological" space in which a change in one place produces instantaneous changes everywhere else. Such a feature, called "non-locality" seems to be an unavoidable consequence of quantum theory, and Einstein drew our attention to it nearly 50 years ago. But, in his enthusiasm for biological models, based on such phenomena as the cooperative motion of the parts of a chicken embryo, Prigogine thinks he sees common ground with the elementary particle 'zoologists' in their high-energy physics laboratories. This is unwise, because the non-locality some physicists speak of has never been observed, and quite possibly it never will be. On the other hand, the nonlocality exhibited by a chicken embryo certainly does not require superluminal signals to sustain it. It is easily subsumed within a materialist world-picture of the type supported by Boltzmann and Einstein. This is especially the case once full account is taken of the field concept. something which Prigogine almost totally ignores — Faraday does not rate a mention, and Maxwell enters only through his contribution to kinetic theory.

This is not a great book, and Prigogine has not yet achieved what Boltzmann did in *Populäre Schriften* or Einstein in *The World As I See It*. But it is a very good book, and the ideas contained within it promise better things to come.

T. W. Marshall is a Lecturer in Mathematics at the University of Manchester.

What's new in the mechanisms of enzyme regulation?

Edwin G. Krebs

Protein Phosphorylation in Regulation: Recently Discovered Systems of Enzyme Regulation by Reversible Phosphorylation. Vol. 1 of Molecular Aspects of Cellular Regulation. Edited by P. Cohen. Pp.273. ISBN 0-444-80226-6. (Elsevier/ North-Holland Biomedical: 1980.) \$63.50, Dfl. 130.

As the title of this first volume in a series on cellular regulation suggests, the emphasis is on new systems that for the most part have not been reviewed extensively heretofore. The editor of the series, Philip Cohen, who is one of the world's foremost authorities on protein phosphorylation, provides an introductory chapter that gives an excellent summary of the well-established role of this process in the regulation of glycogen metabolism. His succinct account will provide readers with a broad outline of the history and evolution of the general principles of protein phosphorylation, but at the same time will not burden them with an enormous load of information that is available elsewhere. A more exhaustive treatment of glycogen metabolism in this book would have detracted from the reviews that follow. These include chapters by different authors on pyruvate kinase, acetyl CoA carboxylase, hydroxymethylglutaryl CoA reductase, steroidogenesis, triacylglycerol synthesis, muscle contraction, protein synthesis, the phosphorylation of ribosomal proteins and the phosphorylation of histone H1. Parenthetically, it should be noted that the word "enzymes" is used in a very loose sense in this volume, in that the topics covered include the phosphorylation of proteins ordinarily not thought of as enzymes.

The individual contributions, which, with one exception, are clear and well written, follow a general theme developed in the introductory chapter. Brief descriptions of the more classical enzymology of a given system, including non-covalent regulation, are followed by the more recent work concerned with phosphorylation-dephosphorylation. The latter, which constitutes the principal thrust of each article, is generally handled by progressing from in vitro phosphorylation to the more difficult in vivo situations. Most of the authors make an effort to apply criteria of physiological relevance to the phosphorylation phenomena that have been observed. This aspect is especially well handled in sections on the regulation of pyruvate kinase, the control of protein synthesis and the modification of histone H1. A final chapter in the book, again written by Cohen, highlights common features of the various phosphorylation systems and forecasts future directions that will be taken by workers in this field; in addition,

he introduces an interesting hypothesis concerning the mechanism of action of insulin in the regulation of protein phosphorylation.

The book can be recommended for readers who desire timely, concise reviews of work on various aspects of metabolic regulation controlled by protein kinases and phosphoprotein phosphatases. It is

designed more for the non-specialist than for the specialist, but anyone reading the volume will profit from the experience, particularly if he reads it in its entirety.

Edwin G. Krebs is a Senior Investigator at the Howard Hughes Medical Institute and Chairman of the Department of Pharmacology, University of Washington, Seattle.

Newtonian style and scientific revolution

Richard S. Westfall

The Newtonian Revolution, with Illustrations of the Transformation of Scientific Ideas. By I.B. Cohen. Pp.414. ISBN 0-521-22964-2. (Cambridge University Press: 1981.) £18, \$37.50.

TWENTY-five years ago, Professor Cohen's influential Franklin and Newton (American Philosophical Society, 1956) established him as the foremost student of Newtonian natural philosophy. Over the intervening years a constant stream of publications has at once expanded our understanding of Newton and the scientific revolution and maintained Cohen's position. To that stream he now adds another major volume, The Newtonian Revolution, the revised text of the Wiles Lectures delivered at the Queen's University of Belfast. As the two-part title suggests, the book divides into two major sections, one on the Newtonian revolution in science, the other on the doctrine of transformations as an interpretive explanation of the development of scientific ideas. Though the book contains several lengthy asides which are connected only tenuously to the central argument, the two sections maintain a fundamental unity by focusing on the same developments in Newtonian science. In Cohen's view — a view that most historians of science would enthusiastically endorse — the elaboration of the Principia was the single most important step forward in the course of modern science. The appearance of Professor Cohen's detailed analysis of it constitutes a major event in the historiography of science.

While the title of Part I recalls Thomas Kuhn's Structure of Scientific Revolutions, it is Part II, Transformations of Scientific Ideas, which speaks directly to the issues Kuhn raised. Where Kuhn argued for discontinuities in the growth of science, revolutions characterized by sudden switches in the perception of nature, Cohen stresses continuity in which every revolution is achieved by the transformation of received concepts. He is presently at work on a general exposition of

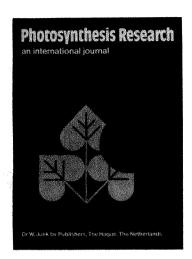
his theory of scientific growth. In the present volume he illustrates it by examining the same events that are central to his understanding of the Newtonian revolution. For the latter he develops the important concept of the Newtonian style by which he seeks both to identify the essence of Newton's contribution and to characterize the nature of modern science. Central to the Newtonian style is the use of mathematical constructs which successive modifications make ever more adequate to the full complexity of physical reality. Equally important is the postponement of enquiry into causes until maximum agreement between the construct and the world of experience has been attained. The idea of the Newtonian style enables Cohen to distinguish Newton's approach from that of other quantifiers and geometrizers in seventeenth century science. No one insisted more on the role of geometry in natural philosophy than Kepler, but, as Cohen shows, Kepler started with causal hypotheses and arrived at mathematical constructs, whereas the Newtonian style proceeded in the opposite order. The primacy of causal hypotheses likewise separated Newton from his continental critics, who were not inconsiderable mathematicians. As I indicated, the concept of a Newtonian style in science, which Cohen argues persuasively was a more important ingredient in his enduring influence than the specific content of the Principia, strikes me as a major contribution to our growing understanding of the scientific revolution.

I feel bound to add that, in my opinion, there are problems attached to this exposition of the concept. Professor Cohen has confounded what philosophers call the context of discovery with the context of justification and has insisted, almost perversely, in using a concept adapted to the context of justification to expound Newton's process of discovery. Few scientists have been less autobiographical in their publications than Newton. He was on the one hand an extremely introverted man who was always concerned to keep his



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(vol. 3/4 issues, 1982)

Editor-in-chief: Dr. R. Marcelle, Laboratory of Plant Physiology, Research Station of Gorsem, Brede Akker 3, B-3800 Sint Truiden, Belgium.

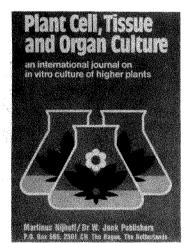
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(vol. 2/4 issues, 1982)

Editor-in-chief: Dr. D.K. Dougall, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946, USA.

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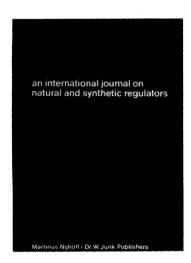
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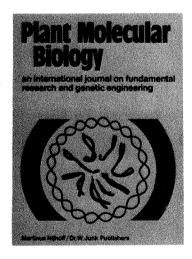
Plant Growth Regulation

(vol. 2/4 issues, 1982)

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Plant Molecular Biology

(vol. 2/4 issues, 1982)

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distance from others. On the other hand, he held advanced standards of rigour and elegance. More than any other scholar, Professor Cohen himself has taught us to appreciate Newton's immense care in his presentation as he continually revised everything he wrote to express his exact meaning. In the present book, he now asks us to receive these same writings as autobiographical accounts of the process by which Newton arrived at his conclusions. Paradoxically, this leads Cohen wholly to reject the validity of genuine autobiographical statements which appear in some of Newton's manuscripts. No doubt the statements were usually self-serving. At best they must be handled with caution, and perhaps some or even all of them need in the end to be dismissed entirely. Surely, however, it is not the published form of non-autobiographical works that can determine what to accept and what to reject.

Cohen's approach leads him to insist that, because Newton expressed reservations, he never seriously entertained the existence of particulate forces in matter and that, because he stated in the Principia that gravitational attraction may be performed by impulse, he always believed that the final explanation of gravity would involve a material aether. Cohen's own theory of the Newtonian style applied to the context of justification puts this material in quite a different light. With a remarkably cold eye, Newton distinguished demonstration from speculation, even when the speculation contained some of his most cherished convictions. He chose not to mix mere belief, no matter how deeply held, with what he took to be demonstrations. When we approach his work from this angle, the very concept of a Newtonian style leads us to an account of the development of Newtonian science that is quite different from the one Professor Cohen presents. In my opinion, it is a more convincing account. We see a Newton who was the boldest speculator of the century, a man who was able to break out of the confines of received natural philosophy and to put questions no one else could formulate. The Newtonian style, the separation of speculative explanatory hypotheses from mathematically demonstrated regularities in nature, controlled the speculation by proposing what he could not demonstrate under titles such as "Oueries".

Thus I like the notion of a Newtonian style; it gives definite form to important characteristics of Newton that have hitherto not been clearly perceived and articulated. At the same time, I remain to be convinced that the Newtonian style is a useful instrument in the elucidation of Newton's own scientific development.

Richard S. Westfall is Professor of History and Philosophy of Science at Indiana University, Bloomington, and author of Never at Rest: A Biography of Isaac Newton (Cambridge University Press, 1981).

Minerals through the microscope

R.A. Howie

Optical Mineralogy: The Nonopaque Minerals. By Wm Revell Phillips and Dana T. Griffen. Pp.677. ISBN 0-7167-1129-X. (W.H. Freeman: 1981.) £24.60, \$39.95.

ALTHOUGH this book was originally intended to be the companion volume to Mineral Optics: Principles and Techniques (1972), to supply the necessary tables of properties and mineral descriptions, it developed into a more comprehensive work. There are two quite distinct parts: Part I contains detailed descriptions of most varieties of the common rockforming mineral groups, while Part II presents an "abbreviated summary" (pp.399-665) of the optical properties of "all" non-opaque mineral species (including some first described as recently as 1979). The optical descriptions are illustrated with orientation sketches and determinative graphs of optical properties against composition, though unfortunately refractive indices are given as n_a , n_{ε} etc. rather than as α , ε as recommended by the International Mineralogical Association. For a small proportion of the minerals there are diagrams of the crystal structure, but although the compositional ranges are discussed there are no phase diagrams at all (even for the Al, SiO, system of the plagioclase series). Notes on zoning, alteration, distinguishing features and occurrence are included, together with a few selected references.

In addition to summaries of the optical and physical constants of the non-opaque minerals, Part II also gives the typical habit, colour (in thin-section and in hand specimen), alteration and occurrence, together with remarks (solubility, group, polymorph etc.) and one reference. The authors state in their preface that it is hoped that this volume will be accepted as the logical successor to A.N. Winchell's

Science and the President. Pergamon Press have recently issued in paperback Science Advice to the President, a volume tackling many of the same questions raised by Harvey Brooks in his review of Science at the White House by E.J. Burger (Nature 290, 634). The book is a collection of 23 essays by all but one of the past Science Advisers and by other well-qualified individuals, edited by William T. Golden. Prices are hbk £25, \$50; pbk £5, \$9.95.

British Anthozoa. In his review of British Anthozoa (Nature 291, 520) Sir Maurice Yonge regretted the absence of colour illustrations from the book. R.L. Manuel has compiled another guide to anthozoans which contains colour photographs of all but three of the British species. The book was published last year by the Underwater Conservation Society and is available from UCS Sales, 148 College St. Long Eaton, Nottingham NG10 4GX, price £16.

Elements of Optical Mineralogy, II: Descriptions of Minerals (Wiley, 1951) and Larsen and Berman's The Microscopic Determination of the Nonopaque Minerals (US Geological Survey, 1934), both long out of date and out of print. Comparison might also be made with Kostov's Mineralogy (author's translation edited by P.G. Embrey and J. Phemister), published by Oliver & Boyd in 1968. Certainly, Part II of the present work will prove invaluable. With the rapid discovery of new minerals (currently at the rate of around 80 each year) no single work can ever contain all critical data on all non-opaque minerals. but mineralogists will be in debt to Phillips and Griffen for some years.

In Part I, the detailed descriptions of the commoner rock-forming minerals are wellpresented, yet surely are not quite detailed enough. Comparison is inevitably to be made with Battey's Mineralogy for Students (Oliver & Boyd, 1972) or Deer, Howie and Zussman's Introduction to the Rock-Forming Minerals (Longmans, Green, 1966). Admittedly the present work is concerned with optical mineralogy, but it does include sections on the occurrence of the various minerals and these are not always sufficient for a petrologically inclined mineralogist, e.g. for the garnet group. Nor are the references particularly up-to-date - the most recent augite reference is to a 1970 paper, and for biotite, 1966, though perhaps this is merely a reflection of the increasing use of X-ray and electron microprobe techniques in preference to those of optical mineralogy. The amphibole sections take no account of the IMA rulings (1978) for the nomenclature of this group. Nevertheless this descriptive part of the book is well-produced and attractively presented with numerous clear diagrams and quite a few microphotographed pairs (taken with plain-polarized light and with crossed polars). It must be said, however, that after the colour photographs in MacKenzie and Guilford's recent Atlas of Rock-forming Minerals in Thin Section (Longman, 1980), these black-andwhite photographs seem uninspiring.

Perhaps the biggest surprise about this book is the juxtaposition of the two parts which would seem to cater for rather different levels of readership. The listing of properties of all known non-opaque minerals in Part II will appeal to final-year honours students and particularly to research workers at all levels, whereas the mineral descriptions in Part I would, in my opinion, be insufficient for a final year honours course in geology. It is, however, remarkably good value at under £20; all libraries catering for the earth sciences should have it.

R.A. Howie is Professor of Mineralogy at King's College, University of London.

Hennig in English: hypotheses for entomology's systematists

R. A. Crowson

Insect Phylogeny, By W. Hennig. Translated and adapted by A. C. Pont, with revisionary notes by D. Schlee. Pp.514. ISBN 0-471-27848-3. (Wiley: 1981.) £28, \$84.

WILLI Hennig's 1969 volume, Die Phylogenie der Insekten (published by Waldemar Kramer), while undoubtedly a work of scholarly importance, received rather less than due attention in the English-speaking world. At the time of his death in 1976, Hennig was accumulating notes for a revised English-language version of the work. In its lieu, the present volume provides a translation of the 1969 text into good and readable English, interspersed with Hennig's surviving notes for a revision, and supplementary notes by younger, mainly German entomologists designed to bring into consideration work

published since 1968. The bibliography now includes some references as recent as

The "methodological introduction" summarizes Hennig's phylogenetic methods, more fully set out in his Phylogenetic Systematics (University of Illinois Press, 1966). It is followed by a review of Palaeozoic and Mesozoic fossil data on insects, a systematic account of the phylogenetic relations of modern insects down to about order or suborder level, and a more detailed review of the phylogenetic relations of Mesozoic insects. Illustrations are mainly dendrograms or pictures of wings; the bibliography is extensive though far from exhaustive.

The main phylogenetic conclusions, not significantly changed by the later annotations, agree largely with those of Boudreaux's Arthropod Phylogeny with Special Reference to Insecta (Wiley, 1979) who also applied Hennigian methods, though Hennig goes into more detail. A notable feature is the use of a numerical system denoting taxa, based on Hennig's belief that phyletic splittings are almost always binary, these numbers being directly convertible into a topologically correct dendrogram. One effect of the binary system is the use of a large number of classificatory levels — thus it takes eight splittings to get from Insecta (= Hexapoda) to an order such as Diptera, though only two to Diplura.

Perhaps the most important of the additions are due to Dieter Schlee, who brings into consideration a good deal of recently described fossil evidence, not known to Hennig in 1969. Otherwise, the later annotators seem mainly concerned with promulgating their own views about particular orders, not always in a very scholarly manner. Some of these writers, and even Hennig himself, may at times be accused of unduly dogmatic assertion thus Hennig states that the theory of a blood-sucking ancestor for Diptera "is certainly not correct"; Mickoleit writes "There is no longer any doubt that the Mecoptera are a monophyletic group"; and Kinzelbach states flatly that "Strepsiptera have no evident gula". Hennig himself, and M. Baehr, are both guilty of misunderstanding and misrepresenting my own views on the Coleoptera at some points.

For the non-entomological systematist, the work should be of interest as the example of Hennig's own application of his method to higher-level classification of a major section of the animal kingdom. The data used in this work are drawn almost entirely from the fields of classical comparative anatomy and palaeontology; its conclusions should be taken as scientific hypotheses, which could be tested against evidence from fields such "macromolecular systematics" not taken into account by Hennig and his co-

It is to be regretted that neither Hennig nor his annotators have tried seriously to establish ancestral modes of life for the taxa with which they are concerned. The result is that the book has little of interest to the new, rising school of palaeoecologists, or even to palaeogeographers, despite Hennig's own interest in geographical distribution as evidence for phylogeny. The main users of the book will undoubtedly be entomologists and palaeoentomologists, for whom this well produced volume should be a desirable acquisition.

Forms of magnetic behaviour

B. R. Coles

Introduction to the Magnetic Properties of Solids. By A.S. Chakravarty. Pp.696. ISBN 0-471-07737-2. (Wiley: 1981.) £35,

MAGNETISM continues to present challenges to our physical insight and abilities to produce firmly based formal theories. With its wide range of phenomena and richness in relevant concepts (spin, exchange, spin-waves, domains, ferromagnetism, spin density waves, crystal fields and so on), it is a much more difficult subject to present to the student in an orderly fashion than, on the one hand, classical electricity and magnetism or, on the other, quantum mechanics. Faced with this dilemma authors either accept the complexity and diversity of the real world of the magnetic properties of matter or turn their back on it until they have laid the formal theoretical foundations which they are convinced will ultimately be the basis of explanations of the behaviour of all magnetic matter. As this book (which is firmly in the second category) shows, the difficulty of the latter approach is that the materials and properties described tend less to be those of intrinsic interest than those that illustrate the bits of theory that can be tackled fairly rigorously. It contains very few figures showing the behaviour of real materials (the first is on p.150 and the second on p.265) and those are chosen solely to compare with rather specific calculations rather than because of their power to illustrate important effects.

For the theoretical physicist who seeks

interesting problems that give promise of possible solutions with existing techniques. this will be a helpful introduction that moulds his thinking along the lines that have been thoroughly explored and found useful. It does not, however, give the introduction promised in the title to the magnetic properties of real solids in all their diversity or even to some modern theoretical developments. Some omissions are understandable, given the tenor of the book, but it is strange to find a transition from an account of the band theory of solids to theories of itinerant ferromagnetism - which oddly fails to refer the student to Herring's classic text (Magnetism edited by G. Rado and H. Suhl, Vol IV; Academic Press, 1972) with no reference to the susceptibilities of metals that do not become magnetically ordered. The treatment of spin-wave spectra is surprisingly brief and there is no discussion of the powers of neutron scattering in their investigation. There is also no discussion of the phase transition aspects of magnetism which are currently a focus of much theoretical effort -"critical phenomena" and "renormalization group" do not appear in the index. It is a pity that space that might have introduced even these theoretical aspects was taken up with extended treatments of standard background theoretical formalism which are easily found elsewhere.

B. R. Coles is Professor of Solid State Physics at Imperial College, University of London.

R. A. Crowson is a Senior Lecturer in the Zoology Department at the University of Glasgow.

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RESEARCH FELLOWSHIP

Applications are invited for a Research Fellow to work with Dr S J Ferguson on an SERC project entitled "Structural and Mechanistic Studies on Proton Translocating ATPases". The main emphasis will be on identifying and sequencing specific peptides from functionally important parts of the mitochondrial and bacterial enzymes, but some enzymological work is also anticipated. Experience in protein chemistry advantageous although not essential.

The appointment is for three years commencing 1st October 1981 (or as soon as possible thereafter) in the range £6,070 to £10,575. Starting salary not expected to exceed £6,880.

Applications (2 copies), including a curriculum vitae and the names of three academic referees, should be sent by 20th August 1981 to Jane Nelson, Senate Registry, University of Birmingham, PO Box 363, Birmingham B15 2TT, from whom further details may be obtained.

(9158)E

Molecular Biologist/Biochemist

Sittingbourne, Kent

Shell Research Limited has a vacancy for a post-graduate molecular biologist/biochemist to join a multidisciplinary group investigating the nature of chemical carcinogenesis and developing novel techniques for predicting carcinogenicity. The major challenge of the job will be to apply modern techniques of molecular biology, particularly recombinant DNA methodology, to solve toxicological problems.

The candidate we are looking for will be a first class experimentalist, aged up to 30 years, who possesses an excellent grasp of modern concepts of the structure, function and regulation of DNA.

The Research Centre has modern, well-equipped laboratories situated on a 450 acre farmland site near Sittingbourne. We offer a competitive salary and assistance will be given with relocation expenses where appropriate. You will be able to join our pension fund and other benefits include a subsidised staff restaurant and excellent sports and social facilities. Please write or telephone for an application form to:

Shell Research Ltd., Recruitment Division, PNEL/25 (N2), Shell Centre, London, SE1 7NA. Telephone:- 01-934 2948.



UNIVERSITY OF NEWCASTLE UPON TYNE

FACULTY OF AGRICULTURE

RESEARCH ASSOCIATE

Applications are invited for the temporary post of Research Associate in the Department of Agriculture for a period of 2 years to work on a project sponsored by the Opencast Executive of the NCB. The work will be to evaluate and improve the agricultural potential of land restored after opencast coal mining and will involve detailed investigation of grass and cereal root growth in both undisturbed and restored sites. Candidates must have recent research experience in a related area of study.

The appointment will be made at the appropriate point on the scale for research and analogous staff: Range 1A £6,070 — £10,575 according to age, qualifications and experience.

Further particulars may be obtained from the Senior Assistant Registrar (FP), University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU, with whom applications (3 copies) together with the names and addresses of 3 referees should be lodged not later than 1st September 1981. Please quote reference N.

(9122)A

THE UNIVERSITY OF MELBOURNE CHAIR OF MATHEMATICS

The University of Melbourne invites applications for a Chair of Mathematics, which is one of four chairs in the Department of Mathematics. The Chair recently became vacant following the resignation of Professor Leon Simon to take up a chair in the Institute of Advanced Studies, Australian National University. The appointee will be expected to develop teaching and research in a branch of Mathematics. Applicants in analysis, differential geometry, and topology are particularly sought. It is not intended to appoint a person whose main interests are in the applications of mathematics.

Salary: \$A41,509 per annum.

Further information about the position, application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment are available from the Registrar or from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H OPF. All correspondence (marked "Confidential") should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia

Applications close on 31 October 1981. (9149)A

CONNECTIVE TISSUE BIOCHEMIST

Exceptional opportunity to join the Merck Sharp & Dohme Research Laboratories highly regarded professional staff. We are presently seeking a PhD Biochemist to join a team of Cell Biologists, Immunologists and Biochemists who are studying various aspects of the effector mechanisms of immune-based inflammation with particular emphasis on connective tissue degradation. The successful candidate will be expected to develop and utilize state-of-the-art technology for measurement of connective tissue breakdown and turnover with the aim of developing new animal models of chronic inflammation. A good basic training in Biochemistry with emphasis on connective tissue biology and immunological methods is highly desirable.

Salary and benefits highly competitive. Send résumé to: Susan R. Jenkins, Dept. N., Personnel Manager, Merck & Co., Inc., P.O. Box 2000, Rahway, New Jersey 07065



(NW759)A

THE UNIVERSITY OF GARYOUNIS, THE SOCIALIST PEOPLE'S LIBYAN ARAB JAMAHIRIYA, INVITES APPLICANTS TO TEACH FROM THE BEGINNING OF SEPTEMBER 1981 IN THE FOLLOWING FACULTIES:

Faculty of Science (Benghazi) in the following fields.

Geology Department:

- 1. Geophysics.
- 2. Crystallography.
- 3. Petroleum and Subsurface Geology.
- 4. Field methods and Geological Mapping.

General requirements:

- 1. Applicants should hold a PhD degree or its equivalent from a recognised university.
- 2. Experience in university teaching preferable.
- 3. Teaching is in Arabic or English language.

General information: Basic annual salary:

 Professor
 8640 to 9720 LD

 Associate Professor
 7560 to 9640 LD

 Assistant Professor
 6840 to 7560 LD

 Lecturer
 6240 to 6843 LD

Assistant Lecturer

5265 to 6237 LD

One Libyan Dinar (LD) is equivalent to approximately £1.50 or US 3.33 Dollars. Housing is provided by the University: additionally a furniture allowance of 2 months basic salary is provided for married accompanied staff: single staff will be

accommodated in a furnished flat within the campus.

Research and consultation opportunities are available. Funding individual research projects will be considered. Details furnished upon request. The academic year begins on 1 September 1981. Ranks and salaries commensurate with experience. On termination the staff member will receive a gratuity of two months salary for each completed year of service payable at the end of contract. Round trip air tickets from the place of recruitment to Benghazi are given to the staff member and family (up to four children under 18 years) at the beginning and end of contract. In addition a baggage allowance of up to 25% of the price of the air ticket. Leave travel tickets to the place of recruitment for the staff member and his family are given every year. The University provides free medical treatment.

Applications including resumés should be sent to:

The Secretary of the Popular Committee, Faculty of Science, University of Garyounis, Benghazi, The Socialist People's Libyan Arab Jamahiriya.

(9138)A

Wessex Regional Cytogenetics Unit, General Hospital, Salisbury, Wiltshire

A vacancy exists in the above Unit for a Clinical Cytogeneticist (Senior Scientific Officer).

Applications are invited from candidates with experience in human cytogenetics research and with special interest in developing methods for early diagnosis of neoplasia from serious effusions and solid tumours.

He/she should be able to plan and maintain the research programme of the Unit.

Application forms can be obtained from the District Personnel Officer, Odstock Hospital, Salisbury, Wilts SP2 8BJ.

Further details from Dr M Seabright, Tel: (0722) 6212 ext 669. Closing date for receipt of completed applications 17th August 1981.



SALISBURY Health District_

GLASSHOUSE CROPS' RESEARCH INSTITUTE

requires a
BOTANIST OR
HORTICULTURIST

to join the Hardy Ornamental Nursery Stock Section of the Crop Science Division to work on container production of hardy ornamentals with emphasis on the physical aspects of container growing and/or problems associated with field establishment of plants from containers.

The post is designated Scientific Officer Band II/Higher Scientific Officer Band II and is suitable for person with interest in agrometeorology and environmental physics.

Qualifications: Degree in relevant subject or HNC with appropriate experience for appointment at SO level, and at least 3 years postqualifying experience required for higher grade.

Salary within scale £4,809 to £6,480 (SO), £6,075 to £7,999 (HSO) according to qualifications and experience. Non-contributory superannuation scheme. Annual leave 20 days (SO) or 22 days (HSO).

Application forms and further details from Administrative Assistant, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, West Sussex BN16 3PU. Closing date for applications 14 August, 1981.

(9164)A

Applications are invited for two vacancies of Research Demonstrators in the Department of Genetics. Candidates should have or expect shortly to have a PhD and be active in research. For one post, experience in evolutionary genetics, and for the other, experience in microbial genetics

active in research. For one post, experience in evolutionary genetics, and for the other, experience in microbial genetics (especially bacterial plasmids) is desirable. The successful applicants will be involved in laboratory and tutorial teaching.

Research Demonstrators

The appointments which will date from October 1 1981 or as soon as possible thereafter, will be for 1 year in the first instance and will be on a scale up to £6,880 pa together with USS/USDPS benefits.

benefits.
Informal enquiries may be made by telephone to Professor J A Beardmore or Dr J M Parry (0792 205678) but application forms must be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea SA2 8PP to which office they should be returned by August 20, 1981. (9169)A

ENZYME MECHANISM Ph.D's

THE INTERNATIONAL PLANT RE-SEARCH INSTITUTE (IPRI) invites applicants for its rapidly expanding programs in advanced molecular genetics. Applicants with significant records of frontier achievements are invited to join a strong team of innovative scientific leaders in the pleasant San Francisco area.

Send résumé with recent publications

DIVISION OF **MOLECULAR** GENETICS, IPRI, 853 Industrial Road, San Carlos, Calif. 94070.

Equal Opportunity Employer, M/F (NW763)A

UNIVERSITY OF YORK CANCER RESEARCH UNIT 1 POST-DOCTORAL RESEARCH FELLOW 2 RESEARCH ASSISTANT

Applications are invited from suitably qualified persons for the posts of postdoctoral research fellow and research assistant in the above Unit, which specialises in chemical carcinogenesis.

The above staff are required to work on a project funded by the Health and Safety Executive on investigating the biological activity of nitrated polycyclic aromatic hydrocarbons (PAHs) and their possible presence in air-particulates in industrial locations.

The project is funded for three years from 1 October 1981. Salary scales are as follows:

Post-Doctoral Research Fellow -Research Scale 1A (£6,070 — £10,575) Research Assistant Research Scale 1B (£5,285 — £7,700)

Starting salary according to age, qualifications and experience.

Six copies of applications, including full curriculum vitae and naming three referees, should be sent by 7 August 1981 to the Registrar, University of York, Heslington, York YO1 5DD, from whom further particulars are available. Please quote reference number 6125 for Post-doctoral Research Fellow applications and 7095 for Research Assistant applications. (9120)A

AGRICULTURAL RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

SCIENTIFIC SERVICES & DEVELOPMENT DIVISION PHYSICIST/PHYSICAL **CHEMIST** (SCIENTIFIC

OFFICER/HIGHER SCIENTIFIC OFFICER) Scientific Officer/Higher

Scientific Officer is required to join a team of three in the Mass Spectrometry Group to be responsible for the development of techniques in mass spectrometry and related chromatography and microscopy to meet the needs of the Institute's research programme and to provide a central Mass Spectrometry Service to other ARS Institutes and Units.

Qualifications: A 1st or upper second class Honours degree in Physics or Physical Chemistry together with experience of mass spectrometry and HPLC. minimum of 2 years post-qualifying experience will be required for HSO grading and a PhD will be preferred.

Salary: On a scale £4,809 — £6,480 (SO), £6,075 — £7,999 (HSO), Under review. Non-contributory superannuation scheme.

Further particulars application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting ref. 80/38. Closing date: 6th August

CSIRO AUSTRALIA

Research Scientist/ **Senior Research Scientist** (Research Fellow)

Division of Forest Research Canberra Act

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,400 employees - 2,500 of whom are research and professional scientists located in Divisions and Sections throughout Australia.

Field: Forest Fire Suppression (Project Aquarius).

General: The Division of Forest Research is concerned with the long term use of Australian forests - both conifer plantations and the native eucalypt forests - for wood production, water supply, wildlife conservation and recreation. The Division will establish a project team to investigate the effectiveness of alternative techniques for suppressing forest fires in Australia. The project will investigage:

- The effectiveness of large air-tankers and fire retardants for suppressing fires of varying intensity.
- The effectiveness of conventional fire fighting techniques under similar conditions.
- A cost/benefit analysis of forest and bush fire suppression in Australia.

The multidisciplinary project team will be expected to carry out extensive field work. It will work with officers of a State Forest Service and scientists from other organizations engaged in contract research on this project.

Duties: As part of a team, design, undertake and evaluate experiments to measure the suppression effectiveness of a large air tankers dropping water and fire retardant chemicals on fires of varying intensities.

Note: Duties will involve prolonged field work of up to three months duration in summer.

Qualifications: A PhD or equivalent research experience in an appropriate area, e.g. forest fire suppression, air tanker operations. Operational experience on fire suppression desirable.

Salary: Research Scientist/Senior Research Scientist (Research Fellow) \$A19,662 - \$A28,564 pa. Outstanding applicants may be considered for appointment at a higher level

Tenure: Three years. Superannuation benefits available.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number A3665 should reach: The Personnel Officer, Australian Scientific Liaison Office. Australia House, Strand, London WC2B 4LA by 28 August 1981. Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036.

Current vacancies in CSIRO appear on PRESTEL page 252903.

CHAIRMAN

DEPARTMENT OF GENETICS

The University of Utah, School of Medicine, Salt Lake City, Utah, invites nominations and applications for the position of Professor and Chairman of the new Department of Genetics. This position requires a person with an outstanding research program in genetics, as well as, administrative and leadership qualities, who will interact with both clinical and basic science faculty. This new department will provide the opportunity and requirement to recruit strong faculty.

The university is an equal opportunity/affirmative action employer, thus applications from women and minorities are encouraged.

Inquiries including a curriculum vitae, should be sent to Dr John A Dixon, Chairman Genetics Search Committee, Department of Surgery, Dixon, Chairman Genetics Search Committee, Department School of Medicine, Salt Lake City, Utah 84132. Closing date (NWX764)A

Hereford Area Public Health Laboratory

Director

Leptospira Reference Unit Principal Grade Microbiologist Salary scale £9,720 — £13,635

The Leptospira Reference Unit, currently located at Colindale London, but planned to transfer to become an integral part of the Hereford Area Public Health Laboratory requires a successor to the present Director who will be retiring in November.

The person appointed will work initially as the Deputy Director at Colindale, and thereafter at Hereford, as Head of the Reference Unit, under the overall direction of the Director of the Hereford Public Health Laboratory Service Laboratory.

The Unit provides a reference and a research centre for work on pathogenic leptospires with emphasis on the diagnosis of leptospiral infection in man and on support for epidemiological investigations. The Microbiologist appointed will be responsible for the development of the Unit's scientific programme and for the day to day management of the diagnostic and reference services.

Candidates for this post should have a relevant higher degree or other qualifications supported by experience of both research and medical microbiology, experience in the field of leptospires whilst desirable is not essential.

Interested candidates who would like to discuss this appointment may do so by arrangement with: The Director of the Service, Dr. J. E. M. Whitehead. Telephone 01-200 1295 extension 35.

Further particulars are obtainable from the Secretary to the Public Health Laboratory Service Board, 61 Colindale Avenue, London NE95EQ to whom applications should be sent stating date of birth, qualifications, experience and published work, and naming three referees. Closing date 14th August 1981.

PHLS Centre for Applied Microbiology and Research Bacterial Metabolism Research Laboratory

Senior Microbiologist

Starting salary £7,674 rising by annual increments to £9,921

Applications are invited from microbiologists with an interest in anaerobic bacteriology and bacterial biochemistry to work on the intestinal bacterial flora in relation to chronic and malignant disease of the gastro-intestinal tract.

For further details of the work involved please contact the Director of the Bacterial Metabolism Research Laboratory, Dr. M. J. Hill (telephone 01-205 6144). The starting date will be November 1st, or earlier, when the laboratory transfers from Central Public Health Laboratory, Colindale, to the Centre for Applied Microbiology and Research, Porton Down.

The post will be for an initial period of 1 year with possibility of further extensions to 3 years.

Basic Grade Microbiologist

Current salary scale £4,839 - £7,110

to work on the intestinal bacterial flora in relation to chronic or malignant intestinal disease. The applicant should have an interest in anaerobic bacteria and in bacterial biochemistry. The laboratory is currently situated at Colindale, London NW9 but will move to the Centre for Applied Microbiology and Research on November 1st 1981.

For further details contact the Director of the Bacterial Metabolism Research Laboratory, Dr. M. J. Hill. Telephone 01-205 6144.

NHS Conditions and terms of employment will apply.

Applications for the above 2 Posts with full curriculum vitae stating date of birth, qualifications, experience and names and addresses of three referees should be made to

Mrs. M. Bushby, Personnel Officer, PHLS
Centre for Applied Microbiology and
Research, Porton Down, Salisbury,
Wiltshire SP QJG. Telephone Idmiston
(0980) 610391. Closing date
14th August 1981.

Public Health

Laboratory Service



Coláiste na hOllscoile Corcaigh University College Cork

IRELAND RESEARCH SCIENTISTS IN MICROBIOLOGY

required to work on the molecular Biology of Nitrogen Fixation in Root Nodule Bacteria

1 Geneticist/Molecular Biologist

preferably with a PhD to work on a project involving the genetic engineering of root nodule bacteria. Capabilities in recombinant DNA technology would be an advantage but is not essential as training will be provided.

2 Microbiologist/Microbial Physiologist

preferably with a PhD degree, to work on the physiology and regulation of CO₂ metabolism in rhizobium. This position is tenable in the first instance for one year with the possibility of renewal for a further year.

These positions are supported by the NBS and available from the 1st October 1981. Salaries will be on the appropriate national scales (IR £6,234 — post-doctoral max, including PRSI) with the point of entry negotiable.

Applications, containing full career details and the names of two referees should be sent as soon as possible to Dr. F. O'Gara, Microbiology Department, University College, Cork, Ireland. from whom further particulars are available. (9128)A

UNIVERSITY OF MELBOURNE

DEPARTMENT OF ZOOLOGY

RESEARCH FELLOW GRADE 1

(Extendible Tenure)

Applications are invited from biologists with expertise in the study of zooplankton. The appointee will be expected to study plankton from southern Australian and Antarctic waters in collaboration with staff of the Departments of Zoology and Botany. The appointee will be expected to give a small amount of teaching in undergraduate courses and will be encouraged to supervise postgraduate students. A research interest in trophic relationships or secondary productivity will be preferred. The position is available from 1 January 1982 for a period of up to three years.

Salary within the range \$A17,083 to \$A19,570 per annum.

Further details on the position are available from Professor G D Campbell, in the University.

Further information, including details of application procedure and conditions of appointment, are available from the Staff Officer, University of Melbourne, Parkville, 3052, Victoria, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 30 September 1981. (9116)A

TUMOR IMMUNOLOGY PRE/POSTDOCTORAL

position available immediately. Monoclonal Ab, characterization for human tumor Ag.

Send CV, three ref to: Dr Daniel Eskinazi, USC, PO Box 77912, GER 323, Los Angeles, CA 90007 EOE/MFH (NW765)A

THE UNIVERSITY OF BIRMINGHAM Faculty of Medicine and Dentistry

DEPARTMENT OF CARDIOVASCULAR MEDICINE Applications are invited for the post of

RESEARCH FELLOW

to work on a study of the biochemical role of myosin isoenzymes in the human heart during development and cardiovascular disease. The successful applicant will join the Molecular Cardiology Unit within the department which is investigating the contractile and regulatory proteins in normal and diseased myocardium.

The post, supported by MRC is available up to 30.9.83. Starting salary £6,070 to £6,880 with superannuation.

Further information from Dr P Cummins, Cardiovascular Medicine, Queen Elizabeth Hospital, 021-472 1311.

Applications (three copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ by 15th August 1981. (9157)A



Coláiste na hOllscoile Corcaigh University College Cork

Ireland

RESEARCH ASSISTANTSHIP GENETICS OF LACTIC STREPTOCOCCI

Applications are invited for a

RESEARCH ASSISTANTSHIP

(supported by the national Board for Science and Technology) to develop a transformation system in the lactic streptococci. Applicants should preferably have a PhD degree and research experience in genetics/ microbiology. The appointment is initially for one year from 1st October 1981 and is renewable.

Salary scale:

PhD degree,

IRE5,370 — IRE5,798; IRE4,417 — IRE5,073.

MSc degree.

Applications with Curriculum Vitae and names and a addresses of two referees should be sent as soon as possible to Dr. C. Daly, Dairy and Food Microbiology Dept., University College, Cork, Ireland.

POST DOCTORAL -RESEARCH POSITION

for Biochemist-Cell Biologist available September 1, for two to three years, to identify, isolate and characterize insulin-like growth factor and α -thrombin receptors. (J. Biol. Chem. Vol 256: 2767, 1981) from cultured cells and tissues.

Experience in membrane protein chemistry, monoclonal antibody production, and/or ligand receptor characterization is desirable but not essential. \$15,000 to \$20,000 yearly depending on qualifications.

Send curriculum vitae and names of three references to Dr James F Perdue, Lady Davis Institute for Medical Research — Sir Mortimer B Davis — Jewish General Hospital, 3755 Cote St Catherine Road, Montreal, Que Canada H3T 1E2. (NW760)A

LONDON SCHOOL OF **HYGIENE & TROPICAL MEDICINE**

(University of London) **EVALUATION AND PLANNING** CENTRE FOR HEALTH CARE ROSS INSTITUTE

A NURSE RESEARCH ASSISTANT

Required from 1st October 1981 to work in a multidisciplinary group concerned with health care in developing countries. Applicants should have had overseas as well as UK experience and preferably hold a degree or diploma in a relevant discipline. Salary in the range of £4,677 -£5,481 or £5,534 -£6,597 (under review) depending on experience and qualifications plus £527 London Weighting. The successful candidate will be required to travel overseas.

Application forms and further details from Dr J P Vaughan, EPC, 31 Bedford Square, London WC1. Telephone enquiries: 01-631 3216. (9124)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF **HAEMATOLOGY TECHNICIAN (GRADE 3)**

to assist a study of the sensitivity of leukaemic cells in culture to cytotoxic agents. Candidates must possess ONC in Medical Laboratory Science or appropriate equivalent as minimum qualification and experience in tissue culture techniques also an advantage. The post might be suitable for graduates.

This post is available for one year in the first instance.

Salary within range £4,672 -£5,474 per annum.

Application forms can be obtained from the Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref RV/855. (9173)A

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY **POSTDOCTORAL** RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral research assistantship (supported by SRC) to study stereo-

chemical effects on nuclear spin coupling between directly bonded carbon atoms.

Applicants should have experience in one or both of the following: (a) F T n m r and (b) synthesis with stable isotopes.

The appointment is for one year (from 1st October 1981, or up to 6 months later by agreement). The salary will be in the Research Support Grade 1A, the salary being not higher than £7,290 pa plus USS.

Applications (one copy), with curriculum vitae and names and addresses of at least two referees should be sent as soon as possible to Dr M J T Robinson, Dyson Perrins Laboratory, South Parks Road, Oxford OX13QY. (9088)A

EXXON CHEMICALS

a division of Exxon Corporation. is one of the world-wide leaders in the Chemical Industry. Its activities consist of manufacturing and selling a wide range of chemicals from primary petrochemicals to specialties.

Essochem Europe Inc. located at Machelen, near Brussels, is the coordinating headquarters for Esso Chemicals activities in Europe, Africa and the Middle East.

We have an immediate vacancy in our Environmental Affairs Division for a

TOXICOLOGIST

We offer: a challenging position involving participation in ongoing toxicology programs, including contract testing, consultation and advisory work for product lines;

attractive

Requirements:

a university degree in toxicology; 1/3 years of relevant industrial experience; ability to give sound professional advice at review management level on a wide range of toxicological issues;

fluency in English, any other European language being an asset: ☐ willingness to travel and/or relocate.

salary and benefits.

Please forward application with full curriculum vitae in English to:

ESSOCHEM EUROPE INC., Employee Relations Department, Nieuwe Nijverheidslaan 2. 1920 MACHELEN (BELGIUM).



MEDICAL RESEARCH COUNCIL **CLINICAL AND POPULATION CYTOGENETICS UNIT**

SCIENTIST GRADE II

Applications are invited for a SHORT-TERM NON-CLINICAL SCIENTIFIC post in this MRC Unit, tenable for 3 years. Candidates of immediately post-doctoral or equivalent status will be preferred.

The appointee will work with a group studying the biological effects of chemical mutagens and will be mainly involved with studies on induced DNA/chromosome damage in human cells. The post will be suitable for applicants interested in aspects of DNA repair and/or mutagenesis and interested candidates with some experience of cytogenetics are also invited to apply.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff. Further information may be obtained from Mr Alasdair Douglas, MRC Clinical and Population Cytogentics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Tel. 031-332 2471, with whom applications — including a full CV and the names and addresses of two professional referees - should be lodged (9178) Å by 18th September 1981.

Soil Scientist

£4,809 — £9,619 (under review)

Age Limit: Applicants must not be more than 55 years of age

on the closing date, i.e. born on or after 7th August 1926.

Closing date: 7th August 1981 Job Ref. SB76/81/NN. Tel. ext. 256

DEPARTMENT OF AGRICULTURE (NORTHERN IRELAND) AGRICULTURAL AND FOOD CHEMISTRY RESEARCH DIVISION

The succesful applicant will join a team engaged in research and advisory work in the various aspects of Soil Chemistry and Plant Nutrition and may also be required to undertake teaching duties in the faculty of Agriculture and Food Science, Queen's University, Belfast.

The main research objectives are to investigate factors controlling, and to study means of improving, the fertility of agricultural soils and the output of agricultural crops by related field, pot and laboratory studies.

Appointment will be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level.

Senior Scientific Officer

Applicants must possess a First or Second Class Honours Degree in Soil Science, Chemistry, Agricultural Chemistry or related discipline with at least 4 years relevant post-graduate experience.

Higher Scientific Officer

Applicants must possesss an Honours Degree as above with at least 2 vears relevant post-graduate experience.

Scientific Officer

Applicant must possess an Honcurs Degree as above.

Preference may be given to candidates with appropriate post-graduate experience in Soils and/or Plant Nutrition.

The Civil Service Commissioners, by taking into account such factors as the applicant's educational qualifications, extent and duration of previous relevant experience, may decide to interview only those who appear to be best qualified.

Salary Scales (under review)

Senior Scientific Officer £7,644 — £9,619 Higher Scientific Officer £6,075 — £7,999 £4,809 - £6,480 Scientific Officer

In addition there is a non-contributory pension scheme apart from a 11/2 % deduction from salary to cover widows' benefit in the case of male officers

Grading and starting salary will be related to qualifications and experience.

These posts are open to both men and women. Please write or telephone for an application form (using the extension number indicated and quoting the job reference) to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585). Completed forms must be returned to arrive not later than the closing date stated.



UNIVERSITY OF LEICESTER

DEPARTMENT OF GENETICS

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above position, funded by the Medical Research Council, to investigate the mechanism of iron in plasmid-bearing bacteraemic strains of Escherichia coli, with particular emphasis on the organisation and function of specific membrane components. The post is available at any time after 1 October 1981; it is tenable for a period of 14 months and may therefore interest applicants returning from abroad.

The salary is on Range 1A for Research and Analogous Staff, plus superannuation.

Applications, with curriculum vitae and the names of two referees, should be addressed to Dr P H Williams, Department of Genetics, University of Leicester, Leicester LE17RH, from whom further details (9134)Aare available.

university college of

Research Demonstrators

Applications are invited for vacancies of Research **Demonstrator** in the Department of Zoology Applicants, who should be in possession of a PhD degree or have submitted for such a degree, should have specialised in mathematical ecology including computing, and biology of marine invertebrates and/or zooplankton.

The appointments, which will be for one year in the first instance, from October 1, 1981 will be on a scale up to £6,880 per annum.

Closing date for applications, Friday, 14th August, 1981.

Research Demonstrator

Applications are invited for the vacancy of Research **Demonstrator** in the Department of Geology. Applicants should either be in possession of a PhD or have such a degree near to completion.

The appointment, which will be for one year in the first instance renewable on an annual basis for maximum of three years, will be on a scale up to £6,880 per annum, together with USS/ USDPS benefits.

Closing date for applications, Friday, 7th August 1981.

Further particulars and applications forms (2 copies) for both posts may be obtained form the Personnel Office, University College of Swansea Singleton Park, Swansea SA2 8PP, to which office they should be returned by the above dates. (9161)A

UNIVERSITY OF READING **LECTURESHIP** IN ZOOLOGY

Applications are invited for a Lecturership in Zoology, tenable for a fixed term of one year from 1 October 1981. The duties will involve lecturing, tutorial and demonstrating work in applied zoology and expertise in entomology, agricultrual pests or genetics is essential.

Applications (two copies) from persons able to begin on 1 October, giving the names of two referees, should be sent to Professor K Simkiss, Department of Zoology, The University, Whiteknights, Reading RG6 2AJ, by 1 August. (9126)A

NATIONAL INSTITUTE FOR **RESEARCH IN DAIRYING**

PHYSICAL SCIENCES DEPARTMENT A GRADUATE

is required to work for 18 months on the effect of heat on the proteins and enzymes of human milk. The work involves a study of the kinetics of their heat denaturation to improve knowledge of the effects of pasteurisation treatments on human milk

Qualifications: First or Upper Second Class Honours degree in biochemistry or food science.

Appointment, for a limited period of only 18 months, will be as Scientific Officer; current salary scale £4,809 to £6,480.

Application forms are obtainable from the Secretary, NIRD, Shinfield Reading RG2 9AT. Quote reference 81/22

Closing date: 14 August 1981 (9162)A

UNIVERSITY OF **EAST ANGLIA** Norwich

Applications are invited for the post of SENIOR RESEARCH

ASSOCIATE IN PHYSICS

to work with Professor D V Osborne on an experimental investigation of the surface of liquid helium. The experiments use the techniques of cryogenics and optics (including optical ellip-sometry), and experience in one or both of these areas would be an advantage. The programme is funded by SRC, and the appointment is for a period of three years, starting as soon as possible. Applications are invited from candidates who have completed (or are about to complete) a PhD in experimental physics. The salary will be on scale 1A, starting between £6,070 and £6,880 per annum, depending on the age and experience of the successful candidate.

Applications (one copy of a curriculum vitae and the names of two referees) should be sent to Professor D V Osborne, School of Mathematics and Physics, University of East Anglia, Norwich NR4 7TJ, if possible by 28 August 1981. - (9146)A-

UNIVERSITY **COLLEGE LONDON**

DEPARTMENT OF BIOPHYSICS **TECHNICIAN GRADE III**

required for general laboratory duties with tuition in electronmicroscopy and some photography. Grant supported.

Salary £4,672 plus £1,016 London Weighting.

Application form from Personnel Officer (Technical Staff FD2) University College London, Gower Street, London WC1E 6BT (9168)A

ST THOMAS'S HOSPITAL MEDICAL SCHOOL (University of London) London SE1 7EH DEPARTMENT OF ANATOMY **ELECTRON MICROSCOPY** MLSO/SENIOR MLSO

required in early October for the above Department. Proven ability in TEM Techniques and some know-ledge of SEM and X-Ray analysis an

Please apply, in writing, to the Senior Chief MLSO, Anatomy Department, giving the names of two (9132)A

CHARLES DARWIN **RESEARCH STATION** Galapagos

The Charles Darwin Research Station in the Galapagos offers two positions to begin as soon as possible.

A PLANT ECOLOGIST

with a strong background in quantitative plant community analysis and plant systematics. The candidate should work on assessing the distri-bution of feral plants in the Galapagos, on native plant-feral animal interactions and should advise Ecuadorian authorities on protection of endemic/indigenous flora and erradication of introduced plant species.

AN AVIAN ECOLOGIST

is needed to undertake conservationrelated research, a major focus of attention will be the threatened Hawaiian or Dark-rumped Petrel, and more general monitoring of the islands bird populations.

Both persons should furthermore assist in the education programs of the Station and advise Ecuadorian students in their research work. Therefore, Spanish or its rapid acquisition would be essential and a PhD would be preferred.

Salary is 19.000 sucres (Approx US\$630) per month; airfare to and from Galapagos at beginning and end of contract (one year, renewable), free housing and health insurance.

Please send curriculum, supporting letters and representative reprints to: Director, Charles Darwin Research Station, Casilla 58-39, Guayaquil, Ecuador

Deadline for submission of applications: 15 September 1981. (NW770)A

TUFTS UNIVERSITY SCHOOLS OF MEDICINE

DEPARTMENT OF PHYSIOLOGY Tufts University has instituted a search for

CHAIRPERSON

Department of Physiology. The Department of Physiology is expected to be a center of research and scholarly excellence, as well as providing leadership in physiology instruction for the School of Medicine, School of Dentistry, School of Veterinary Medicine, and the Sackler School of Graduate Medical Science.

Individuals interested in being considered for this position should direct their letters to Dr Seymour Reichlin, Chairman, Physiology Search Committee, Box 275, New England Medical Center Hospital, 171 Harrison Avenue, Boston MA 02111. Tufts has an affirmative action program, and encourages applications from women and members of minority groups.
(NW771)A

UNIVERSITY OF DUNDEE DEPARTMENT OF **BIOLOGICAL SCIENCES**

We require, to work with Dr Rowell and Professor W D Stewart, FRS, on biochemical aspects of nitrogen metabolism in cyanobacteria,

1 Ref. EST/47/81 A POSTDOCTORAL **RESEARCH FELLOW**

preferably a biochemist with interests in molecular biology or biochemical genetics. 2 Ref. EST/116/81

A RESEARCH TECHNICIAN

(probably on scale £4,672 £5,473) with interests in microbiology, biochemistry or

biology.
Further Details from Personnel Office, The University, Dundee DD1 4HN. Tel. 0382 23181 ext. 383. By August 13th 1981. (9143)A

UNIVERSITY OF SOUTHAMPTON **POST-DOCTORAL** RESEARCH FELLOW **Department of Biology**

Applications are invited from biologists with interests in Taxonomy to work with Dr F A Bisby and Dr R J White from 1 September 1981 to the end of July 1982 in the first instance. The SRC funded project involves building a data-base with morphological and chemical data on a tribe of the Leguminosae and experimenting with the provision of novel Taxonomic services for evaluation by a users' panel. The successful applicant will have an interest in the use of computer-based techniques in taxonomic problems.

Starting salary at the rate of £5,505 — £6,245 per annum + USS benefits.

Further particulars may be obtained from Mrs P C P Sears, Staffing Section, The University, Highfield, Southampton SO9 5NH to whom applications with curriculum vitae and names of two referees should be sent as soon as possible, and not later than 3 August 1981. Please quote reference number 1608/R.

-(9123)A.

CSIRO

AUSTRALIA

Principal Research Scientist/ Senior Principal **Research Scientist** (Research Fellow)

Division of Environmental Mechanics Canberra Act

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,400 employees - 2,500 of whom are research and professional scientists located in Divisions and Sections throughout Australia.

Field: Soil Physics.

General: Research in the Divisional deals with transport of energy, water salts and gases in the natural environment. The Division, located in Canberra, has 27 staff members, including 14 research scientists, working in areas of soil physics, solar radiation, fluid mechanics, micrometeorology and physical aspects of plant physiology

Duties: The soil physics program of the Division has concentrated on interaction between water and soil. The appointee will be expected to complement this program through theoretical work on porous medium physics and applied mechanics. The research will be directed in the first instance to studies of problems presented by systematic and random spatial variability of soils; to physical consequences of the interaction between soils and plants; and to the physics and physical chemistry of saline soils. The extension of theory to other areas of science and technology will be pursued.

The appointee will collaborate with other members of the program and with research officers of State instrumentalities.

Qualifications: A PhD or equivalent qualifications, and extensive experience and expertise in the areas of mathematical and physical research in soil water.

Salary: Principal Research Scientist/Senior Principal Research Scientist (Research Fellow) \$A29,638 - \$A38,921 pa.

Tenure: A fixed term appointment of three years is envisaged. Superannuation benefits available.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number A4489 should reach: The Personnel Officer, Australian Scientific Liaison Office, Australia House, Strand, London WC2B 4LA by 24 August 1981. Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036

Current vacancies in CSIRO appear on PRESTEL page 252903.



Royal Postgraduate Medical School

(University of London)

DEPARTMENT OF MEDICINE Senior Research Officer

Applications are invited from post-doctoral biochemists to join a Medical Research Council-supported research programme studying complement function in health and disease, particularly to characterise the molecular mechanisms of complement activation by antigen-antibody complexes and the effects of other complement activators in various diseases. Experience in immunochemistry is desirable.

Salary will be at an appropriate point on Range 1A, £6,070 -£10,575 plus £967 London Allowance.

Prospective applicants should write to Professor D. K. Peters enclosing a curriculum vitae and the names of two referees. Please quote reference 2/227. (9118)A

CSIRO AUSTRALIA

Research Scientist/ Senior Research Scientist (Research Fellow)

Division of Tropical Crops & Pastures **Northern Territory** Darwin

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,400 employees - 2,500 of whom are research and professional scientists located in Divisions and Sections throughout Australia.

Field: Crop Scientist (Soybean adaptation).

General: The Charles Darwin Laboratories are in the main scientific base for CSIRO research in north-western Australia. They accommodate 15 scientists from the Division of Tropical Crops and Pastures and Wildlife Research, have good library and statistical services, and a computer node linked to the CSIRONET system. The agricultural research programs are concentrating on rice, sorghum and soybeans and on animal production from dryland and irrigated pastures. Most of the field experiments are done at the Kimberley and Katherine Research Stations.

Duties: To undertake agronomic/ecophysiological studies on the adaptation of soybeans to agricultural environments in northern Australia. This will involve an extensive program of field experimentation under irrigated conditions during both wet and dry seasons and under dryland conditions.

Salary: Research Scientist/Senior Research Scientist (Research Fellow) \$A19,662 - \$A28,564 pa

Tenure: The position is available for a three year term with a possible extension for a further two years. Superannuation benefits available

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number A5905 should reach: The Personnel Officer, Australian Scientific Liaison Office, Australia House, Strand, London WC2B 4LA by 24 August 1981. Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036.

Current vacancies in CSIRO appear on PRESTEL page 252903.

The Royal Marsden Hospital, Downs Road, Sutton, Surrey

Medical Physics Technician GRADE III

Salary scale £5,750 - £7,277 p.a. (increase pending)

We are looking for a technician to complete a team of three in an in vitro laboratory carrying out mainly routine tests involving radioisotopes. The work is rewarding and requires an interest in patient contact.

Applicants should hold either an appropriate science degree, ONC, HNC, HND or equivalent qualification and have had at least 3 years similar technical experience.

For an application form and job description please contact the Personnel Department Royal Marsden Hospital, Fulham Road, London SW3 - Tel: 01-352 8171 ext 446.

Closing date: 14th August 1981.

(9174)A

INSTITUT PASTEUR PARIS-FRANCE

Applications are invited for a position of staff and POSTDOCTORAL RESEARCH SCIENTIST

with experience in molecular genetics, recombinant DNA techniques, and animal cell culture.

The genetic engineering laboratory involved in medical and industrial applications, is located on the Institut Pasteur Campus and has contact with fundamental research units.

Knowledge of French is not essential.

Applications with curriculum vitae and requested salary should be sent to: Monsieur le Directeur du Personnel, Institut Pasteur Paris, 28 Rue Du Docteur Roux, 75724 Paris, Cedex 15, France (W385)A

PRINCETON UNIVERSITY THE DEPARTMENT OF

BIOMEDICAL SCIENCES ASSISTANT PROFESSOR **OF BIOCHEMISTRY**

invites applications for tenure-track positions at the Assistant Professor level. Candidates should have a strong background in molecular biology, molecular genetics or bio-chemsitry and demonstrated excellence in research. An interest in eukaryotic organisms is preferred.

Applicants should submit a complete curriculum vitae, including a list of references and a description of their future research program to Dr S J Flint, Biochemical Sciences, Princeton University, Princeton NJ

Princeton University is an affirmative action/equal opportunity employer. (NW769)A employer.

UNIVERSITY OF DURHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for the post of

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

tenable for three years from 1 August 1981, to work on the processing of seismic reflection data. The work will involve running the existing pro-cessing laboratory based on PDP-11 mini computer, documenting and expanding the existing software library, writing some systems software and processing seismic reflection data obtained on cruises off E. Greenland and the Lesser Antilles.

Applicants need not necessarily have a PhD, but equivalent experience and a strong computing background are essential. Salary is on the Research Scale 1A according to age, starting at £6,070 per annum at age 24, plus USS.

Applications (3 copies) naming three referees should be sent as soon as possible to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE. (9160)A

UNIVERSITY OF OTAGO Dunedin, New Zealand

LECTURER IN **PHYSIOLOGY**

Applications are invited from graduates with a research interest in membrane cellular and epithelial physiology for appointment as Lecturer in Physiology.

Salary within the scale: NZ\$19,140 - NZ\$23,520 per annum, according to qualifications and experience.

Further information may be obtained from Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H0PF or from the Registrar of the University. Additional detailed information may be obtained from Professor D W Taylor, Department of Physiology, University of Otago Medical School, PO Box 913, Dunedin, New Zealand.

Applications close on 31 August (9141)Ă

UNIVERSITY OF CANTERBURY Christchurch, New Zealand

LECTURER IN ASTRONOMY OR PHYSICS

Applications are invited for the above-mentioned position. The appointee must be prepared to take an active part in one of the experimental research fields of the Department; namely Astronomy, Solid State Physics or Atmospheric Physics.

Applications close on 31 October 198Î

The Salary for Lecturers is on a scale from NZ\$19,140 to \$23,520 per annum.

Further particulars and Conditions trom the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H0PF.

UNIVERSITY OF **EXETER**

DEPARTMENT OF PHYSICS POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited from suitably qualified candidates to take part in a research programme to investigate methods of studying air layers surrounding grinding wheels rotating at high speeds. The research programme, which is funded by SERC, is initially for one year. Some experimental experience in fluid flow measurement together with a strong theoretical interest in high speed fluid flow would be desirable. within the range £6,070 - £6,880 pa, placement depending on age and experience.

Applications including a curriculum vitae and names of two referees should be sent to the Personnel Office (Appointments), University of Exeter, Exeter, Devon by 25th August 1981. Please quote reference (9119)A No.7176.

Plant Biologist

The Plant Genetics Department of Pfizer Central Research is looking for creative individuals to participate in a multi-disciplinary research program for crop improvement. This department provides basic research support for the corn and soybean breeding programs of Pfizer Genetics, Inc.

Applicants should have strong formal training in plant biology, agronomy or crop science at the MS level. Experience in one or more of the following areas is desirable; plant tissue culture; genetics; stress physiology; field crop breeding methods; plant pathology.

In addition to an exceptional opportunity for professional recognition and growth, we offer a comprehensive salary/benefits package and attractive Connecticut shore living. Please send résumé and brief description of research interests to:

Ms. Lorraine C. Corr, Pfizer Central Research, Eastern Point Road, Groton, CT 06340.



An equal opportunity employer

(NW767)A

POST-DOCTORAL POSITION

Available immediately for studying changes in immune regulation in aged mice.

Send curriculum vitae and three letters to: Dr R M Gorcynski, Ontario Cancer Institute, Canada. c/o Nature Box xxx, 15 East 26th Street, Suite 1503, New York, NY 10010 (NW772)A

UNIVERSITY OF LEICESTER DEPARTMENT OF

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATESHIP IN ORGANIC CHEMISTRY

Applications are invited for a postdoctoral research associateship supported by the SRC, to work in collaboration with Dr P R Jenkins on the development of a synthetic approach towards the taxane group of natural products. Applicants should have interest and experience in synthetic organic chemistry.

The appointment, in Research Support Grade 1A, is initially for one year (from 1 October 1981) and is renewable for a second year. The starting salary is in the range £6,070 — £6,475 plus USS benefits.

Applications with curriculum vitae and the names and addresses of two academic referees should be sent as soon as possible to Dr P R Jenkins, Department of Chemistry, The University, Leicester LE1 7RH.

(9135)A

UNIVERSITY COLLEGE LONDON

RESEARCH ASSISTANT

NEUROPHARMACOLOGY

to study the release of neurotransmitters during the development of experimental epileptogenic activity. Applicants should have finished or be finishing their PhD programme. Salary from £5,675 pa + London Allowance + USS for 3 years.

Curriculum vitae to Dr R A Webster, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT.

STANFORD UNIVERSITY California

Applications are invited for an opening at the

ASSISTANT PROFESSOR

level in the newly-formed Program in the History of Science.

Candidates should have demonstrated ability in the area of 19th and 20th century physical science.

Curriculum vitae and the names of at least two referees should be sent before November 15, 1981 to: Professor Eric Hutchinson, Academic Secretary, Stanford University, CA 94305.

Stanford University is an equal opportunity employer. (NW762)A

Director of Technical Liaison

Cetus Corporation, a leader in the commercial application of biotechnology, is seeking a microbiologist/geneticist with practical experience over a broad base of biological applications to coordinate the scientific aspects of the Company's proprietary base. The successful applicant will be the liaison between inventors and the patent counsel, continually maintaining an overview of corporate science with the aim of identifying and developing new concepts and discoveries as they emerge, as well as playing a significant role in corporate scientific strategy. The scientific aspects of corporate licensing will also be a responsibility.

This important staff position will be attractive to mature scientists who wish to move from the laboratory to an administrative role without losing detailed, hands-on involvement in this rapidly developing field.

The successful applicant will join an outstanding team of scientists and managers in a highly motivated, goal-oriented atmosphere. Please send C.V. and salary expectations, in confidence, to Cetus Corporation, Box "NG", 600 Bancroft Way, Berkeley, California 94710. An equal opportunity employer.

(NW758)A

cetus

YOU HAVE GOT A BRIGHT IDEA

TO FIND OUT BIOLOGICAL OR CHEMICAL COMPOUNDS

WE HAVE THE CAPITAL TO DEVELOP THESE NEW DRUGS

We are not a pharmaceutical firm, but a swiss based independant financial group, and we are prepared to let you participate in a capital venture for the exploitation of your project.

Our object: the development of new biological or chemical products (which have previously had a pharmacological screening) up to the stage of clinical tolerance and effectiveness required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to: DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W368)A

Experimental Officer

This is an excellent opportunity to join a team dedicated to the discovery and practical application of synthetic and natural regulators of the immune system. The successful candidate will have ample opportunity to contribute to the development of this recently established project and to collaborate with scientists in other disciplines.

You should have a biological degree or equivalent qualification and a knowledge of immunological techniques and theory. Experience of tissue culture and radio-immunoassay techniques would be an added advantage.

Conditions of service, salary and relocation assistance are designed to attract and retain staff of a high calibre. Our modern research laboratories are situated in pleasant surroundings but within easy reach of main road and rail routes.

Please send full C.V. to:



Mr. R. Yates, Personnel Officer, Imperial Chemical Industries P.L.C., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire.

Closing date for applications: August 31st 1981.

(9179)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY Applications are invited for the post of DEMONSTRATOR/SENIOR

DEMONSTRATOR

in the Department of Biochemistry. The duties of the post will start on 1st October, 1981.

Salary within the range £5,285 — £7,700 per annum.

Applications, together with the names of three referees, should be received not later than 7th August, 1981, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/854. (9144)A

THE INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS (ICARDA)

has a vacancy for the post of LIBRARIAN

at its Research Station in Aleppo, Syria. Starting date: January 1, 1982. Qualifications and experience required: MSc or PhD degree in Library Science; fluent English and Arabic; working knowledge of agriculture; working experience in the Arab World.

Applicants should send their c.v. and write for further details to: The Personnel Officer, ICARDA, PO Box 5466, Aleppo, Syria. (W384)A

PROFESSORSHIP in the Clinical Smell and Taste Research Center at the University of Pennsylvania. Established scientist working in olfactory neurophysiology in Physiology and/or Otorhinolaryngology Department. Based in VA Hospital. Preferably with an MD degree. Write to Dr James B Snow, Jr, Department of Otorhinolaryngology and Human Communication, 5th Floor Silverstein, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104 and provide three letters of reference. The University of Pennsylvania is an Equal Opportunity/ Affirmative Action Employer.

(NW751)A

STUDENTSHIPS

THE HANNAH RESEARCH INSTITUTE (University of Glasgow) POST-GRADUATE STUDENTSHIP

Applications are invited for a PhD studentship tenable at the Biochemistry Department to study aspects of the regulation of lipid metabolism in the mammary gland.

Applicants must possess or expect to obtain a First or Upper Second Degree in Biochemistry and should write (including c.v. and names of two academic referees) to: The Secretary, The Hannah Research Institute, Ayr KA6 5HL, Scotland. (9130)F

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BOTANY
SRC CASE
STUDENTSHIP

Applications are invited from candidates with a first or upper second class honours degree in Botany or Microbiology for a three year CASE studentship on the Biology of Soft Rot Erwinias on Potato Leaves.

The research will be carried out at the University of Aberdeen (under the supervision of Dr J P Blakeman) and at the Scottish Crop Research Institute, Dundee (under the supervision of Dr M C M Pérombelon).

Applications, including curriculum vitae and names of two referees should be sent as soon as possible to Dr J P Blakeman, Department of Botany, University of Aberdeen, St Machar Drive, Old Aberdeen AB9 2UD. (0224) 40241) from whom further information may be obtained. (9133)F

THE CITY UNIVERSITY

DEPARTMENT OF OPTOMETRY AND VISUAL SCIENCE

TWO RESEARCH STUDENTSHIPS IN VISUAL SCIENCE

Applications are invited from graduates with a good honours degree for research towards a PhD on either: "Flicker and Movement Detection as a Function of Field Scan Direction in Television Systems," or "Photometry and Visual Performance Under Conditions of Chromatic Adaptation". The projects are suitable for graduates in Physics, Optics, Ophthalmic Optics, Physiology, Electronic Engineering or related communications subject.

Further details and application forms can be obtained from: Dr J E Saunders, Department of Optometry and Visual Science, The City University, Dame Alice Owen Building, 311-321 Goswell Road, London EC1V7DD. (9139)F

UNIVERSITY OF CAMBRIDGE Scott Polar Research Institute

NERC RESEARCH STUDENTSHIP

Applications are invited from mathematicians, mathematical physicists or oceanographers for a PhD studentship in one of the following

1) The physical oceanography of the marginal ice zone.

2) Sea ice rheology from submarine sonar profiles.

Applicants who have or expect to obtain a 1st or upper 2nd degree should contact Doctor V A Squire at Scott Polar Research Institute, Lensfield Road, Cambridge CB2 IER (tel 0223-66499 ext 411) as soon as possible. (9167)F

UNIVERSITY OF KENT AT CANTERBURY BIOLOGICAL LABORATORY

SERC CASE STUDENTSHIP

Applications are invited for an SERC CASE studentship leading to the degree of PhD and tenable from 1 October 1981 for research on lignin degradation and subsequent aromatic metabolism by cultures of the edible mushroom Agaricus bisporus. The project will be supervised by Dr R B Cain in collaboration with Dr D A Wood of the Glasshouse Crops Research Institute.

Applicants should have a first or second class honours degree in Biochemistry, Microbiology or Botany.

Applications, giving details of qualifications and the names of two academic referees should be sent to the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH from whom further details may be obtained. Closing date 7 August 1981. Please quote ref. PG14/81/N. (9140)F

SEMINARS and SYMPOSIA

GULBENKIAN INSTITUTE OF SCIENCE Oeiras, Portugal INTERNATIONAL SEMINAR ON:

PATENTING, TOXICOLOGICAL TESTING AND REGULATORY APPROVAL OF NEW BIOLOGICAL PRODUCTS

Conducted by:

Dr D. Mansel-Jones (Huntingdon Research Centre, Huntingdon, U.K.)

Mr R.S. Crespi (National Research Development Corporation, U.K.)

Professor J.R. Norris (University of Reading, U.K.) 21 September — 2 October, 1981

> Information and Registration Forms from: Professor N. van Uden, Gulbenkian Institute of Science, 2781 Oeiras Codex, Portugal.

(9159)M

ASSISTANTSHIPS

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CERAMICS, GLASSES AND POLYMERS

RESEARCH ASSISTANTSHIP

Applications are invited from men and women for the above post, to develop the technique of cryogenic scanning electron microscopy and apply it in the investigation of structure in dispersions of clays, oxides and other ceramic materials. The results obtained will be used to interpret the rheological, sedimentation and filtration characteristics of such systems. This project supervised by Dr B Rand is financed by SRC. Tenable for 3 years.

Initial salary up to £6,880 depending on age and experience. An honours degree in a physical science subject and research experience (preferably to PhD level) required. Experience colloid science or scanning electron microscopy desirable, though not essential.

Applications, including the names and addresses of two referees, should be sent to the Registrar and Secretary, the University, Sheffield \$10 2TN by 7 August 1981. Quote ref: R615/G (9171)P

UNIVERSITY OF OXFORD

Dyson Perrins Laboratory

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a post-doctoral research assistant, supported by MRC, to study the mechanism of action of polyene macrolides by nuclear magnetic resonance. Candidates should have experience of chemical or biological applications of high field NMR and/or an interest in biological organic chemistry.

Letters of application should reach Dr J M Brown, Dyson Perrins Laboratory, South Parks Road, Oxford OXI 3QY as soon as possible, and contain a full curriculum vitae and the names of two referees. Salary will be on Research Support Grade 1A up to £6,882 pa plus USS according to age and experience, and appointment for one year in the first instance, from 1st October 1981 or a mutually convenient alternative date. (9150)P

UNIVERSITY OF SOUTH FLORIDA MEDICAL CENTER POSTDOCTORAL RESEARCH ASSISTANTSHIP IN BIOCHEMICAL TOXICOLOGY

Position is available immediately for aggressive individual with doctorate in pharmacology-toxicology. Program involves pulmonary metabolism and response to certain drugs and industrial chemicals. Strong background in biochemistry and physiology a must.

Send CV and 3 letters of recommendation to: Mark R Montgomery, PhD, Research Service (151), VA Hospital, 13000 N 30th Street, Tampa, Fl 33612.

(NW768)P

GRANTS and SCHOLARSHIPS

THE UNIVERSITY OF SYDNEY

RICHARD CLAUDE MANKIN SCHOLARSHIP

Applications are invited for the above-mentioned scholarship from graduates wishing to undertake research related to water conservation.

The award is at postdoctoral level (\$A17,083 — \$A19,075 per annum). Travel grants for overseas Fellows may be payable. An award may also be made at postgraduate level (\$A4,620 per annum plus allowances where appropriate).

Further information and application forms are available from the Registrar (Scholarships Section), University of Sydney, NSW 2006, Australia, with whom applications close on 31 October 1981.

(9148)H

THE UNIVERSITY OF SYDNEY FACULTY OF AGRICULTURE

Applications are invited from graduates for

AGRICULTURAL SCHOLARSHIPS

offered by the University of Sydney and tenable in the Faculty of Agriculture for 1982.

The object of the Scholarship is to encourage and promote scientific study within the Faculty of Agriculture.

Research is undertaken in the following agricultural disciplines: Agronomy, Animal Husbandry, Biometry, Chemistry, Economics, Entomology, Extension, Genetic and Plant Breeding, Horticulture, Microbiology, Plant Pathology, Soil Science.

The Scholarships will provide a stipend of \$A4,620 plus other allowances.

Further information and application forms are available from the Registrar, Scholarships Section, University of Sydney, NSW 2006, Australia, with whom applications close on 31 October 1981.

(9147)H

CONFERENCES and COURSES

International Genetic Sciences, Inc. is organizing an annual conference on Introduction of Genetic Materials into Eucaryotic Cells: Present State and Future Prospects

Among the Lecturers:

H. Goodman (U. of Calif, S.F.), A. Graesman (Free University of W. Berlin), S. Itzhar (Volcani Center, Israel), A. Loyter (Hebrew U. of Jerusalem), D. Papahadjopoulos (U. of Calif, S.F.), F. Ruddle (Yale U.), W. Rutter (U. of Calif, S.F.), R. Rott (inst. of Virology, Gissen, W. Germany), R. Shimke (Stanford U.)

Among the topics to be included:

- 1. Transfer of Nucleic Acid into animal cells.
- 2. Introduction of genes into plant cells.
- 3. Use of Liposomes as a carrier of biological materials.
- Fusion mediated transfer of biological materials into animal cells.

Cost of \$450 includes room and meals. No registration fees. Number of participants limited to sixty. The conference to be held at the Harrison Conference Center, Glen Cove, L.I., N.Y. Arrival Nov.22, 1981; departure Nov.25, 1981.

Interested scientists, please send as soon as possible information including name, address, phone, present position and areas of interest. Send to:

International Genetic Sciences, Inc. 155-25 Styler Rd, Jamaica, NY, USA 11433 or call (212) 526 0400 (NW761)C

UNIVERSITY OF SUSSEX

MSc IN BIOCHEMISTRY

This full-time, one year course can provide a training in general Biochemistry. Alternatively, applicants may specialize in either Biochemical Endocrinology or Plant Biochemistry and Bioenergetics. Research projects comprise a major part of the course, and are related to research currently underway in the Biochemistry Group, much of which is of immediate relevance to developing countries.

Applicants should normally possess a degree, or its equivalent, in Biochemistry or in a Biological, Chemical or Medical Science. Enquiries for further information and applications for the 1981/82 or 1982/83 sessions should be sent to the Subject Chairman of Biochemistry, School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG.

(9153)C

Please mention

nature

when replying to these advertisements

UNIVERSITY OF KENT AT CANTERBURY Faculty of Social Sciences STUDIES IN DISABILITY

Applications are invited for two courses which will begin in January 1982: (i) Diploma in Social Work and Physical Handicap (recognised by CCETSW) (ii) Certificate in Disability Studies.

The Diploma Course is full-time and lasts for nine months and is intended for Social Workers and other professionals in the Health and Social Services.

The Certificate Course is parttime, with teaching confined to four mornings a week for one University term. In addition a dissertation has to be completed within nine months of registration. This course is for anyone who has an interest in or wishes to update their knowledge of disability, including professionals who are unable to attend the full-time course.

Teaching facilities on the Diploma and Certificate Course will be shared.

For informal discussions about either course, contact Dr Mike Oliver at University of Kent. Tel: 0227 66822, ext 7491.

For applications and further details write as soon as possible quoting Ref PG15/81/HSS to: Senior Assistant Registrar, Faculty of Social Sciences, The Registry, The University, Canterbury CT2 7NZ

(9155)(

Travelling Fellowships 1982-83

Applications are invited for Travelling Fellowships to be taken up in 1982-83. The scheme enables clinical and non-clinical biomedical research workers to undertake advanced specialised training overseas, or more senior workers (including academic or research staff on paid leave from permanent appointments) to collaborate with colleagues abroad, for periods of three months to two years (normal tenure one year).

Candidates must have been ordinarily resident in the UK, the Channel Islands or the Isle of Man throughout the three years preceding the application date. Medically or dentally qualified candidates should be of at least NHS Registrar (or equivalent) status with some research experience. Non-clinical candidates should hold a PhD/DPhil.

Further information and application forms may be obtained from: Training Awards Group, Medical Research Council, 20 Park Cescent, London W1N 4AL, (Telephone: 01-636 5422 Ext. 448).



The closing date for receipt of applications is 30 September 1981.

(9142)E

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF MATERIALS ENGINEERING RESEARCH FELLOWSHIP IN CORROSION ENGINEERING

As part of an enquiry into the feasibility of setting up an Australian National Centre for Corrosion Prevention and Control a Research Fellow is required to help assess both the actual need for, and the likely benefits to accrue from the establishment of such a Centre.

The appointee will probably already have some postdoctoral experience, but applicants with lower academic qualifications and a wide range of experience are encouraged to apply.

Appointment initially for 1 year within range \$A17,083-23,373. Enquiries to Dr B W Cherry, in the University.

Applications including Ref no 25362, curriculum vitae and 2 referees to the Registrar, Monash University, Clayton, Victoria 3168, Australia, with copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF by 15/8/81. (9115)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSICS

RESEARCH FELLOWSHIP

Applications invited for this SERC-funded post in Nuclear Structure Physics, tenable from 1st October 1981 until 31st January 1984. Successful candidate expected to participate in charged particle spectroscopy studies using polarized ³He beams from the Department's Radial Ridge cyclotron and heavy ions from NSF tandem accelerator nearing completion at Daresbury Laboratory. Daresbury experimental facilities include large scattering chamber and Scanditronix QMG2 magnetic spectrometer.

Salary on Research Fellow 1A scale £6,070 to £10,575 plus superannuation. Maximum starting salary £6,880.

Further particulars from Assistant Registrar (Sc and Eng), PO Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees should be sent not later than 15th September 1981.

Please quote ref: ND2. (9156)E

MASSEY UNIVERSITY Palmerston North, New Zealand

POST-DOCTORAL FELLOWSHIPS

Applications are invited for Post-doctoral Fellowships tenable in any Department of the University.

The academic activities of the University are grouped under the following faculties and schools. Opportunities for study and research are available in the following fields:

AGRICULTURAL AND HORTICULTURAL SCIENCE — Agricultural Economics and Marketing; Farm Management; Agricultural Engineering; Agronomy; Animal Science (Dairy, Sheep, Beef, Pigs); Horticultural Science and Plant Health; Poultry Science; Soil Science.

BUSINESS ADMINISTRATION — Agricultural Business; Business Studies and Business Law; Data Processing; Economics; Financial Management and Accounting; Personnel Administration.

FOOD SCIENCE AND BIOTECHNOLOGY — Food Technology; Biotechnology (i.e. processing of Biological Materials); Industrial Management and Engineering; Industrial Management and Industrial Mathematics.

HUMANITIES - English; History; French; German; Philosophy.

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SOCIAL SCIENCE — Computer Science; Economics; Education; Geography; Mathematics; Psychology; Regional Planning; Social Anthropology; Sociology; Social Work; Statistics.

VETERINARY SCIENCE — Physiology and Antomy; Veterinary Pathology and Public Health; Clinical Sciences.

Information concerning the research activities of the departments associated with these groups are given in the University Calendar. Intending applicants are advised to write to the Head of the appropriate department in the course of preparing an application.

The Fellowship will be tenable for one year, with possible extensions, and carries an emolument of NZ\$15,755 per annum inclusive of an allowance of up to NZ\$2,205 toward return fares.

Further details of the position and the University, together with conditions of appointment and information to be supplied by applicants, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on 15 August 1981.

(9125)E

UNIVERSITY OF ABERDEEN

DEPARTMENT OF MICROBIOLOGY POSTDOCTORAL

RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral Research Fellowship to join a group led by Professor W A Hamilton working on the fouling and corrosion of steel structures in the North Sea. This is a joint project with the School of Mechanical and Offshore Engineering at Robert Gordon's Institute of Technology and is financed by the SERC Marine Technology Directorate.

Appointment will be from 1st October 1981 and will be for one year in the first instance.

Salary within Range 1A £6,070 — £10,575 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by Friday 7 August 1981. (9083)E

UNIVERSITY OF KENT AT CANTERBURY BIOLOGICAL LABORATORY POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for an SERC-supported post of Postdoctoral Fellow to work with Dr C J Knowles on the formation of haemoproteins in Streptococcus species. A PhD in either biochemistry or microbiology will be required. Experience of protein purification or immunology would be helpful but is not essential. The post is available for up to 3 years from October 1981. The initial salary will be in the range of £6,070 to £6,880 pa.

Applications with a curriculum vitae and the names of two referees should be sent, as soon as possible, and not later than 21 August 1981, to Mr JS Cowie, Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH from whom further particulars may be obtained. Please quote ref A20/81/N (9152)E

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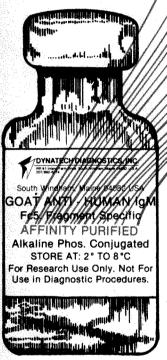
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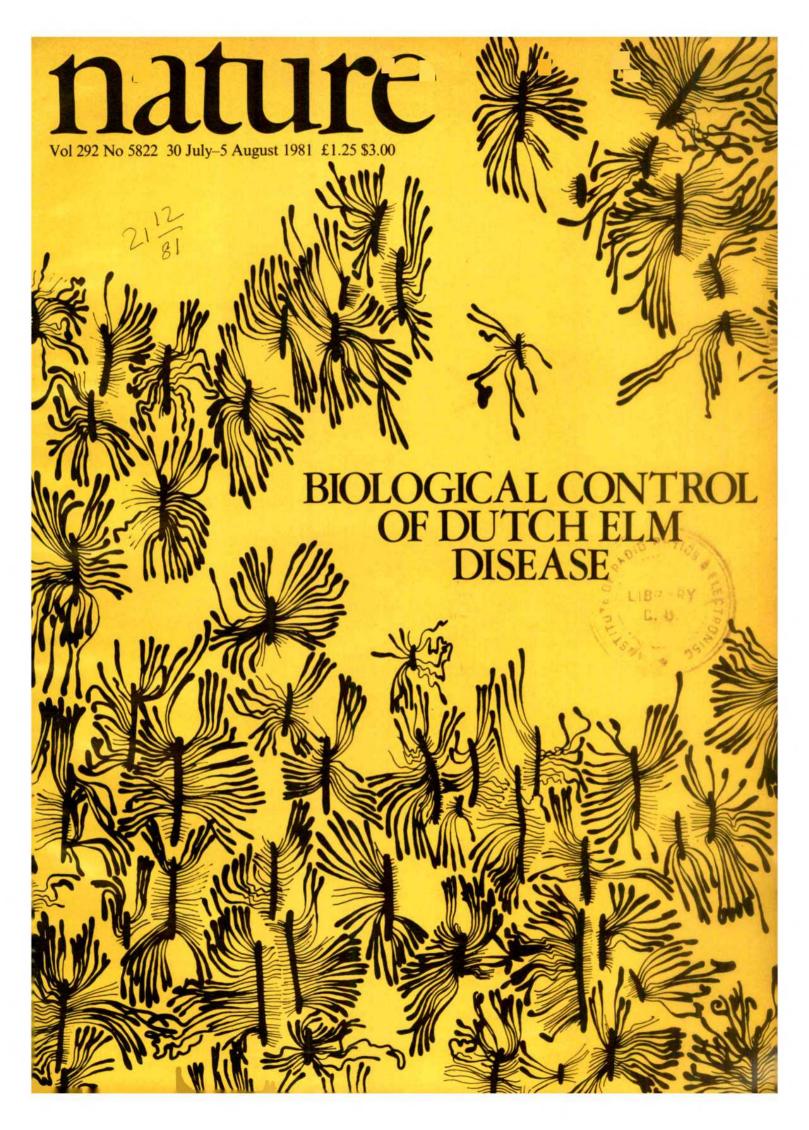
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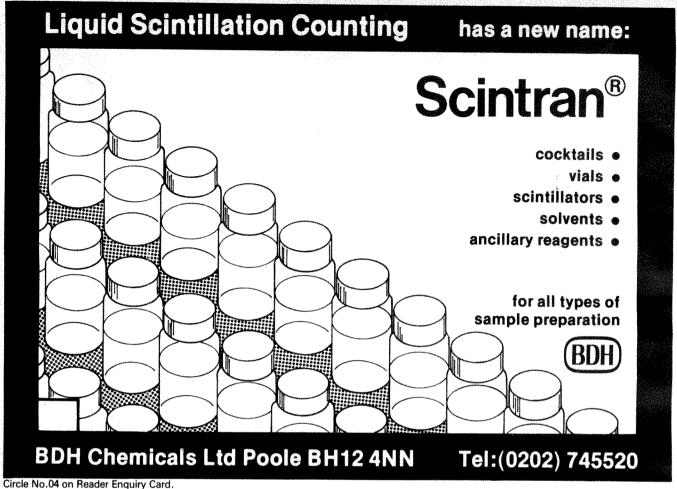


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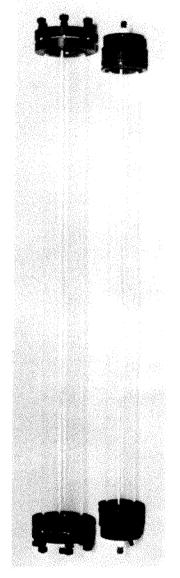
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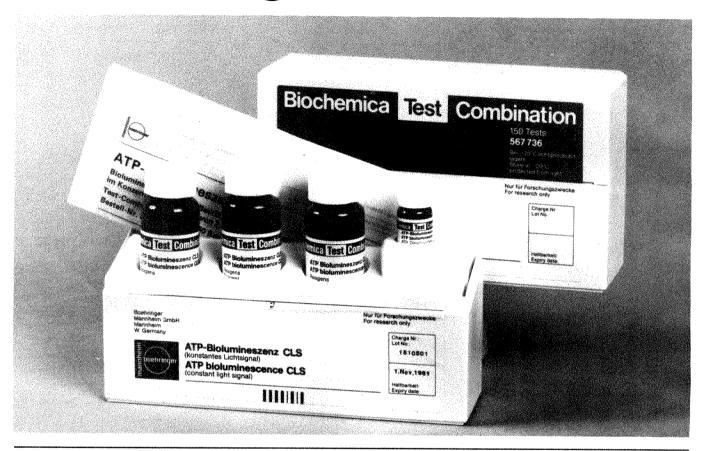
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EDITORIAL OFFICES

London

4 Little Essex Street, WC2R 3LF Telephone: (01) 836 6633 Telex: 262024 Telegrams: Phusis London WC2R 3LF

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Editorial Staff
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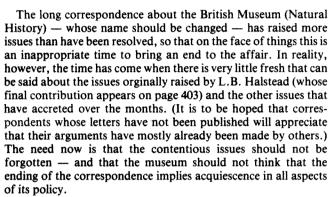
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nature

23 July 1981

Cladistics and evolution on display

How should museums of natural history exhibit specimens? Is cladistics a proper framework? And is it too agnostic—soft on Neo-Darwinism? The last contribution to the correspondence on these topics is on page 403.



The role of the technique of classification called cladistics in the museum's exhibition policy evidently appears to many at the museum to be one of the chief issues to have been raised. The letter from Dr Roger Miles, the director of the Public Services Department at the museum (which plans and mounts exhibitions), and his colleague Dr G.C.S. Clarke on page 402 defends the decision, in two of the recent exhibitions, that the relationship between specimens displayed should be described in cladistic terms - what first set Halstead off. The decision is justified on "educational grounds", presumably the argument that visitors to these exhibitions should be provided both with an understanding of how phylogenetic relationships may be inferred by objective means (which is one of the undoubted virtues of cladistics, at least when meaningful correlations are obtainable) and with a sense of the uncertainty running through phylogeny. This is consistent with the principles on which curriculum development in high-school science have been constructed in the past twenty years. But museums are not high-school classrooms, in which good teachers can ensure that students appreciate the basis on which their work is carried out. Most visitors to museums see what is displayed but once. To fail to record, for such people, that in the opinion of most palaeoanthropologists, Homo erectus is the most likely precursor of Homo sapiens, is to lose an important opportunity - and to avoid answering questions in many visitors' minds about the importance of recent discoveries in East Africa. And to fail to make explicit the principles on which these exhibitions were designed is to ask for trouble from people such as Halstead, caught up as they are with the argument about the validity of cladistics as a way of classification and as a cloak

Technically, cladistics has respectable origins, not least in the development of numerical taxonomy twenty years ago. Inevitably, however, the precision of a cladistic classification of fossil material is limited by the amount and variety of material available for analysis. Out-and-out cladists may hold that phylogenetic relationships not demonstrated by their techniques have no place in the interpretation of the fossil record and much of what they say makes philosophical sense. On present information, for example, there is no way of telling whether Archaeopteryx is the ancestor of all birds or (more probably) an early version of a bird. But pedantry can be carried too far, especially in public exhibitions. Although there may be cladistic doubt (represented by some such statement as "P < 0.1") about the relationship

between *Homo erectus* and *Homo sapiens*, do not the methods of classical palaeoanthropology, with all the appropriate qualifications, deserve a place in public exhibitions because they are stimulating, provoking?

A more serious difficulty, in the course the museum has elected to follow, is that the technique is best at sporting branching points in familial relationships, and says little about gradual transformations within a single population so profound that they are tantamount to a qualitative transformation. No wonder that Halstead fears that cladism is a refuge for those who believe in punctuated equilibrium. But, while he may be able to marshal substantial evidence for the moderate assertion that many Marxist evolutionists are cladists, the proposition that all cladists are Marxists — which has not seriously been suggested by anybody, but only inferred — has no place in logic. It would in any case, as Sir Andrew Huxley said in his attack on *Nature* on 27 May, be intolerable that such a reading of events should be held to be part of the case against cladistics.

The other serious objection to the modelling of public exhibitions within the framework of cladistics is that the framework is inherently agnostic. When the numerical correlations are too small to be significant when assessed against arbitrary criteria, relationships cannot be asserted. Although, even in exhibitions mounted for the general public, statements such as that "the case for thinking that Homo erectus was the precursor of Homo sapiens is not proved" may have a place, part of the trouble at the museum (to which there has been no reply) is that agnosticism has gone too far. Did evolution happen? The museum has cladistically not committed itself. Is Darwinism a "proper" scientific theory, in some sense of that word? The museum has withdrawn the obscure film that suggested to anybody who paused in passing that it is all a gigantic muddle; people will be looking, in the months ahead, at the museum's strategy for retreating from this gaffe. The difficulty, with which everybody must sympathize, is that all museums of natural history are used not merely by those who wish to answer questions in their minds about evolution and the like but also by those who believe that Darwinism is an abomination.

Several other issues remain to be resolved. The aesthetics of the museum's plans for the future have been questioned, and rightly. Is the new building really necessary? Is the push-button public exhibition the only acceptable modern style? Most certainly not. Was Sir Andrew Huxley's attack on Nature (for publishing alleged irrelevancies) just? Readers must judge for themselves. It is understandable that a trustee of the museum should defend members of the museum staff from supposedly (but imagined) personal attacks at the celebration of the museum's centenary. The issues raised are not, however, irrelevancies, even if not all of them are relevant to the museum. The defence would have been more persuasive if there had been less of it. And it was unfair to suggest that Nature's interest was by sensation to enlarge its circulation.

In all the argument of the past several months, perhaps the most important issue has been curiously neglected. Like many other invaluable institutions, the Natural History Museum is staffed by scientists who are public servants. This circumstance is not usually restrictive. Members of the museum's staff ordinarily function as

scholars without impediment. On the question of the public policy of the institution for which they work, however, civil service employees are less than free. In the past several months, many among the museum's staff have sympathized with many of the complaints made against their institution, but have been gagged. The problem is not novel. Scientists working in defence establishments may, for example, differ from official policy on defence procurement, but may be unable to say so publicly. Those working on the development of the Anglo-French supersonic aircraft Concorde may have considered at the time that the project would be a waste of money and resources, and would by now have been justified by events. In such circumstances, however, it is understandable (if reprehensible) that laboratories should require their employee-scientists to keep their silence. The case for insisting on such a policy at an institution such as a natural history museum, whose public reputation stems from the supposed academic independence and integrity of its members, is by comparison non-existent. It is to be hoped that the museum's trustees will now give this problem the attention it deserves, and urgently. For the correspondence of the past months shows that there is a great depth of seemly and legitimate professional interest in Darwinism, cladism and related matters. And it is not long until next April, when it will be time to mark the centenary of Charles Darwin's death.

Too much government

The British government has once again slipped into the trap of taking too detailed an interest in the management of technology, this time in the development of the British nuclear power programme. Last week, the Department of Energy published its reply (see page 398) to the Select Committee on Energy's report which, among other things, advocated that attention should be paid, even at this late stage, to the building of heavy water reactors on the Canadian pattern. In doing so, the committee was keeping faith with the traditions established by its predecessors (principally the now defunct Select Committee on Science and Technology) in hankering after reactor designs that nobody with direct responsibility for generating electricity wants to build. (Nearly a decade ago, the committee was pushing for the steam generating heavy water reactor, a complicated device using heavy water as a neutron moderator and pressurized ordinary water as a coolant, which was in 1977 discovered to be too complicated to fabricate.)

The Department of Energy rightly said last week that nothing has happened in the past four years to suggest that Canadian heavy water reactors would be economic in British circumstances, and thus rejected the committee's chief technical recommendation. As the department responsible for the Central Electricity Generating Board, the principal owner of reactors in the British electricity supply network, the department is well within its rights in making this opinion public. But it goes too far when it declares that the next thermal reactor ordered for the British network "should be the Pressurized Water Reactor". By this declarative statement of technical policy, the Department of Energy has committed the British government not merely to a technical policy that it (or its successor) may regret, but to a role in the management of technology that it should not be playing.

The circumstances are clear. The nationalized industry with a near-monopoly right to generate electricity south of the Scottish border is the Central Electricity Generating Board, much criticized by the Select Committee on Energy for its overoptimistic forecasts of electricity demand and for its imprecise calculations of the relative costs of generating electricity from different fuels. Constitutionally, the board has an unambiguous technical responsibility for deciding how best to generate electricity. If, with the passage of time, the board is found to be incompetent, either technically or in its economic judgements, the Department of Energy has a right, even a responsibility, to fire the board and appoint another. But if the department also sets itself up as the body that decides what kinds of reactors should be built,

its freedom to exercise its own constitutional responsibilities must be compromised. Worse, the government itself, and not the generating board, will become the target for the torrent of complaint that will be mobilized at next year's public inquiry at the proposal to "recreate Three Mile Island in Suffolk" (for where the generating station is destined). The result will be that the government's essential quasi-judicial role as the arbiter between the generating board and public protesters will also be undermined. How can it be otherwise when the department in its reply to the select committee uses forms of words such as "the government believes" that the next British reactor ordered should be a pressurized water reactor?

This complaint is not mere pendantry. Forms of words matter, and in this case all too clearly reveal that the Department of Energy has not clarified for itself its relationship with the electricity-generating industry. The result is that the department's announcement of the form of the public inquiry about the first British reactor of this type has a hollow ring. Last week's document says that the inquiry is to be "wide ranging", that documents describing the studies that will have been made of the safety of the British reactor should be published well in advance, and that objectors should have time in which to prepare their case. The department rejects the select committee's proposal that a time limit should be fixed for the inquiry. All this is sensible enough if the objective is to reconvert British public opinion to nuclear power. The snag is that the department, having announced in advance its "belief" in the generating board's plan, will be accused at the inquiry of being a prejudiced judge in the case. This is a surprising error for a government which, at its election two years ago, advocated that government should be less obtrusive.

Over-civil servants

The trouble with the British government's response last week to the inter-departmental review of the scientific civil service published nearly a year ago (see Nature 287, 264; 1980) is that much of what it says has been said before. Yes, steps will be taken to help people recruited into the public service as scientists to move to administrative work. Yes, arrangements will be worked out to make the careers of those who stay as working scientists more interesting and challenging. Yes, steps will be taken to see whether government departments could make fuller use of scientists on short-term appointments. Yes, steps will be taken to define the roles of chief scientists in government departments (and then to ensure that these roles are carried out). Yes, steps will be taken to improve the literature handed out to potential recruits to the British scientific civil service (and departments will be asked to explain themselves to the Civil Service Department if they make a habit of recruiting highly qualified people to relatively lowly jobs). Yes, yes; the future will indeed be different from the past and better, more enlightened, more flexible, more challenging.

Brave words do not necessarily come true, as the negligible response of successive governments to the report of Lord Fulton's Royal Commission on the Civil Service more than a decade ago has shown. Briefly, however, the British civil service (and any other public service) has two overriding problems to solve in its relationship with scientists. First, how is the appalling lack of scientific understanding in the formulation of general policy to be made good? And how are those employed exclusively as scientists to be kept interested and alert? The simplest course would solve both problems. Let the recruitment of professional scientists (PhDs for example) to the public service be at the outset for an initial period of seven years. Let it be understood that those dispensed with at the end of this period are given a substantial gratuity, say a year's salary. And let it be understood that those kept on should either help to make policy or function as professional scientists, with the proviso that while they may be required confidentially to advise the departments that employ them on technical matters, they should be free to take part in the affairs of the intellectual professions that gave them skill (see above).

US industry moves into biotechnology

Du Pont sets up new lab, others to come

Washington

Reluctant to commit themselves to the commercial promise of the "new biology" only a few years ago, the United States chemical giants are now queuing up to jump in the deep end. Last week the largest of all, Du Pont, announced plans for a new \$85 million life sciences complex, designed for 700 scientists and technical personnel, to be built near the company's headquarters in Wilmington, Delaware.

One of Du Pont's closest rivals, Monsanto, has already established what the company describes as a "free-standing molecular biology center", which will have "a larger component of basic research than at most industrial companies". According to Monsanto chairman, Mr John Hanley, genetic engineering is likely to become a major branch of the company in the future; "we're up to the eyeballs in molecular biology", he says.

Both companies already have substantial contracts with outside research institutions. Last month, for example, Du Pont announced a five-year, \$6 million agreement with Harvard Medical School (see Nature 16 July, p. 191) to conduct research on molecular genetics, with the company receiving exclusive licences to market any resulting products. Monsanto signed a similar deal with the same institution, though with a slightly different focus, six years ago; recently it has been working with Genentech on bovine and porcine hormones, looking at ways of producing more meat with less feed.

At the same time, however, major chemical companies such as Du Pont and Monsanto are building up their in-house capacity at a rate which is already producing major recruitment difficulties.

Du Pont's life sciences complex is part of a plan to raise from 10 to 20 per cent the proportion of gross revenues which the company earns from sales of pharmaceutical, biomedical and agricultural products. According to the company's new chairman, Mr Edward Jefferson, it intends to spend 21 per cent of its \$570 million research budget next year on the life sciences, and that figure is expected to increase over the next five years.

The new complex will include a 250,000-square-foot facility for health sciences research and a 100,000-square-foot expansion of existing plant laboratories at the company's experimental station in Delaware. Completion is

scheduled for December 1983, and the company intends to expand the scope of current research not only in molecular genetics, but also in fields such as immunology, neurobiology, cardiology, gerontology, plant biology, plant pathology and entomology.

Du Pont's announcement seems to have been partly precipitated by rumours that the company's current attempts to take over the oil company Conoco could interfere with Du Pont's recent moves to diversify away from petroleum-based products. Mr Jefferson has denied such rumours, claiming that the company intends to continue its aggressive thrust into the life sciences for their own sake—and for the profit they may bring.

Several petroleum companies are themselves beginning to enter the fields. Atlantic Richfield has set up a genetic engineering laboratory which company officials describe as "our most academic laboratory"; novel arrangements being discussed include two-year research appointments, and lengthy sabbaticals for research workers who wish to spend time at universities.

Waiting in the wings is the Exxon Research and Engineering Company, the research wing of Exxon Corporation, with an annual research budget of \$489 million—almost half that of the National Science Foundation. Company officials declined

to comment last week on the company's plans, saying that no announcement was expected for several months; however, there are rumours that Exxon is working on an arrangement that it likes to compare with the position of the Bell Laboratories in telecommunications research.

The broad potential impact of life sciences research in medicine is revealed in a report, entitled *Forecast of Emerging Technologies*, soon to be released by the Food and Drug Administration, of a study in which 190 experts both inside and independent of the agency were asked to predict which new medical technologies were likely to emerge within the next fifteen years.

Almost a quarter of the 168 technologies listed involved basic areas of genetic engineering and computer technology. Eight technologies accounted for one-third of the citations, headed by hybridoma technology, nuclear magnetic resonance imaging and the use of recombinant DNA to produce interferom.

Meanwhile at least one university has decided to provide financial support to faculty members attempting to exploit the result of their research in molecular biology. The Michigan State University Foundation has announced that it is contributing \$100,000 to help set up a new company, called Neogen, which is expected to specialize in the genetic engineering of plants.

David Dickson

Research council plans academic rescue

Britain's Science and Engineering Research Council is to come to the aid of outstanding academics threatened with redundancy as a result of cuts in the universities' income. Last week, the council decided that it would help academics in departments marked for closure to transfer to other universities, and would pay their salaries for up to ten years if necessary. The council is calculating that the universities should then have reorganized themselves and that the University Grants Committee should be able to take up the bill again.

The council is particularly anxious that good individuals and groups should not be lost simply because they happen to work in those universities faced with the largest cuts in their income. It will be looking for academics that it considers especially valuable who are about to lose their jobs and may also consider setting up special transfer fellowships for which threatened academics will be eligible to apply.

As yet, the council has little idea of how many individuals it will want to support or how much money it should set aside. Estimates will have to wait at least until the autumn, according to Sir Geoffrey Allen, chairman of the council, when universities have a better idea of where economies will fall. In the meantime, the council has increased the number of replacement

fellowships, by which a senior academic returns to research leaving a tenured post for a younger person, from 15 to 20 per year. The council, which will pay the net cost for five years, expects to spend £500,000 a year on the scheme

Sir Geoffrey's main concern is that the cuts in university income will further undermine the dual support system for research, whereby the University Grants Committee provides funds for staff and well equipped laboratories and the research councils award grants for specific projects. The gradual undermining of the universities' income over recent years has already weakened the research base, according to Sir Geoffrey, who is anxious that the restructuring of the university system caused by the cuts should be designed to strengthen the research base to near its former level rather than weaken it further.

He would like to see individual universities concentrate their efforts to a greater extent than implied by the University Grants Committee in its advice to them, preferring a smaller but stronger research base to a larger, weaker one. Although those views are shared by the University Grants Committee, which has attempted to confine the misery caused by the cuts by being selective, Sir Geoffrey believes that the selectivity could and should go further.

Judy Redfearn

French universities

Decision postponed

The 74 French universities, many of them created in the reform which followed the student rebellion of 1968, are in suspense. Does the new Socialist government love them or not?

The Minister of Education, M. Alain Savary, is keeping his own counsel. He has received representations from all sectors of the universities, from unions to presidents, since his invitation to the universities to appeal against the recently-published list of habilitations — a list of qualifications that each university is allowed to offer. The list was conceived largely by the previous Giscardian minister, Mme Alice Saunier-Seité, and eliminated many old courses and refused the introduction of some new ones. A second, supplementary list was promised for last Saturday — but it did not materialize.

Now, says the ministry, it will appear at the beginning of August, when conveniently most of France is on holiday. And at the end of the vacation, M. Savary is to call unions and university presidents to a discussion, where he will attempt to reach agreement on new rules for creating university research and teaching programmes.

The implication may be that the new list will not be everything the universities want — which is approval for all the courses they propose. For defying Savary's call for self-restraint, the universities have thrown themselves solidly behind their original lists, so heavily pruned by Saunier-Seïté.

Nevertheless, they could have done little else, for Savary has given them no guidelines for what to keep and what to cut.

In the end, it may be the level of political pressure that has been brought on him that will determine which courses are approved. For example, at the Université de Haut-Bretagne (Rennes II), it appears that a course on the Breton language will be restored in the second list because of pressure from a group of Breton parliamentarians. After all, the government is pledged to respect and increase regional autonomy. And the new emphasis on the oceans (France has for the first time a minister of the sea) may save a course of oceanography at the Université de Toulon et du Var.

Savary certainly declared himself appalled at the state of university politics when he arrived in office, and it has not all been attributable to his predecessor. According to one senior scientific official, the university system had got out of hand after 1968, and needed to be trimmed. Ninety-five per cent of Saunier-Seïté's cuts were well judged, he thought.

However, such outspoken officials and heads of department may find themselves the symbolic sacrifices of the new government, to satisfy the multitude while the new politics is being thrashed out. At the beginning of this month, one of the teaching unions (SGEN-CFDT) called for the resignation of certain heads of department who were still actively supporting Saunier-Seïté's point of view; and now it seems the Council of Ministers has a list of 27 names before it. These counterrevolutionaries may have to go, it is implied.

Robert Walgate

UK nuclear energy

Maintaining course

The British government remains convinced of the wisdom of its nuclear policy in spite of criticism from the House of Commons Select Committee on Energy in a report published earlier this year (see Nature 19 February, p.621). In a reply to that report, released last week (Nuclear Power, Cmnd 8317, HMSO, £2.30), the Department of Energy reiterates its belief that Britain could need 20 GW of new electricity generating capacity by the year 2000 and should go ahead with the programme, outlined in 1979, to order one new nuclear reactor a year for the next decade (15 GW in all). But it agrees with the select committee that the programme should be flexible and that each new order should be assessed on its merits.

The government is, however, sceptical of the select committee's suggestion that the £15,000 million that the programme is expected to cost might be better spent on conservation, saying that the relative benefits of investing in new plant and in conservation are too difficult to estimate. But it has accepted the recommendation that the planning margins of the Central Electricity Generating Board (CEGB), 28 per cent, should be reconsidered and has charged the Electricity Council with the task. On the contentious question of estimating future electricity demand, it says that it must rely on its own estimates and those of the Central Electricity Generating Board, the latest of which assume a growth rate in the economy of 1 per cent a year until the end of the century. Some of the estimates are due to be updated.

The select committee was particularly disturbed when it came to assess the nuclear programme on economic grounds. It shared the concern of a subsequent Monopolies and Mergers Commission report on CEGB over the way the board has estimated future capital costs from historic costs. The government agrees that this is an unreliable way of appraising future investment and, taking its cue more from the Monopolies Commission than from the select committee, has asked CEGB for improvements in its investment analysis. It has also asked for reassessments of assumptions about future coal prices and availability, power station construction times and costs and the performance of advanced gas-cooled reactors.

No change is planned, however, to the

structure of the nuclear power industry, nor does the government intend to implement the select committee's recommendations for broadening its advice on nuclear matters. But the Secretary of State is already looking at ways of attracting suitable staff to the Nuclear Installations Inspectorate.

The government is still set for a public inquiry in 1982 into the siting of Britain's first pressurized water reactor, intended to start off the new programme, at Sizewell in Suffolk. Although the precise details of the inquiry have yet to be released, Sir Frank Layfield, QC, who chaired an inquiry into local government finance in 1974, was appointed its inspector last week. The government will not, however, expect the inquiry to examine the merits of the pressurized water reactor relative to CANDU, the Canadian heavy water reactor. It rejected the select committee's recommendation for an urgent new appraisal of CANDU on the grounds that the costs of adapting it to British conditions would be too high at this stage.

Judy Redfearn

Science education funding

Pressing claims

Washington

In one of his first acts as the new president of the National Academy of Sciences, Dr Frank Press, formerly director of the Office of Science and Technology Policy under President Carter, has strongly criticized the cuts in federal support for science education that have been proposed by the Reagan Administration.

Dr Press's comments on the proposed cuts, which would virtually eliminate funding for science education from the National Science Foundation (NSF), were contained in a letter to the chairman of the House of Representatives Science and Technology Committee, Mr Don Fuqua. And the letter, together with similar complaints voiced by individuals and instituions from across the country, seems to have had an effect.

Last week, the House voted to approve a 1982 budget for NSF which included an additional \$35 million for science education in excess of the Administration's request. In the process, the Democrat-controlled body rejected by 264 votes to 152 an attempt by Republicans to remove this extra money from the NSF appropriations bill and reduce the foundation's total budget to the \$1,033 million proposed by President Reagan.

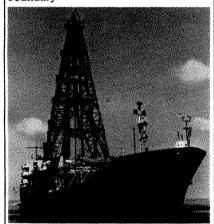
Although the House vote may prove to be critical in restoring the science education funds, there is still some way to go. Also last week the Senate appropriations committee approved a budget of \$1,044 million for NSF, which includes an additional \$20 million for science education; if accepted by the full Senate, the subsequent compromise with the

House would lie between this and \$35 million.

In his letter to Mr Fuqua, Dr Press points out that a study carried out last year for Mr Carter by NSF and the Department of Education demonstrated problems caused by the deterioration of science and engineering education that "are pervasive and complex and will require determined and concerted effort by all sectors of society for their solution". David Dickson

A brief respite

The Glomar Challenger, the work-horse of the Deep Sea Drilling Project, docked last week at Southampton in preparation for the next stage (Leg 81) of the project. The previous leg was spent in the Bay of Biscay, to determine the rifting and subsidence history of the continental margin. Leg 81 will be spent near Rockall, a few hundred miles to the west of Scotland, studying the variations of subsidence across the ocean-continent boundary.



Challenger on the crest of a wave

The Deep Sea Drilling Project is at present riding on a wave of enthusiasm for its scientific record, and has so far done well in President Reagan's financial obstacle race. Glomar Challenger's drilling operations are funded for at least another two years, with a 1982 budget of \$26 million. Of this, \$12 million will come from Britain, Germany, France, the Soviet Union and Japan, while the bulk of the United States contribution will be provided by the National Science Foundation.

Future plans are, however, less certain because of the debate within the foundation over the competing merits of Glomar Explorer - the converted ex-CIA submarine-dredger. Although vastly more expensive (its expected cost lies between \$500 million and \$1,000 million over ten years), the Explorer programme would be half financed by the oil industry on the strength of its considerable oilhunting potential. The scientific issues are complex, however; and a decision from Congress on whether or not to fund initial Explorer development is expected Philip Campbell in October.

EEC hormone legislation

First another report

Brussels

Progress of a sort was achieved by the European Community's agricultural ministers in Brussels last week when they discussed the problem of a total ban on hormone use in livestock breeding. They decided that Community law should ban stilbenes and thyrostatics as growth promoters in livestock breeding even though these hormones are already outlawed in all the member states.

And to serve as a figleaf for the lack of agreement on other growth promoters, it was decided to wait for a scientific report before dealing with the natural hormones oestradiol, progesterone, testosterone and the synthetic hormones trembolone and zeranol. A deadline of nine months has been set for this report but quite how it will help to solve what is essentially a political problem nobody is quite sure.

Details of how this scientific report will be compiled are also still hazy. Presumably, the same national experts who form the various scientific committees advising the European Commission on veterinary or nutritional matters will assess the present state of knowledge.

European consumer groups are planning to write to their ministers to demand that these experts are chosen for their impartiality. In fact, anyone nominated by a national government will have already contributed towards that government's position on hormones. The scientific report may therefore end up reflecting only the existing divisions of opinion.

The United Kingdom and Ireland are the strongest opponents of a blanket ban, but producers in other member states are lobbying effectively in the other direction. France, Denmark and Italy want legislation to be as severe as possible — and it was the ruling of an Italian court to ban veal sales which started the whole affair. The new French agriculture minister, Edith Cresson, stressed at the Council that France would not be bound by the conclusions of the scientific report. Belgium and Germany in theory already ban the use of all hormones.

Consumer groups are also incensed by the ministers' reluctance to thrash out the ways and means to enforce a ban of any kind. Natural hormones are notoriously difficult to detect and the various member states do not all have the same reputation for the effective policing of detectable substances.

What has been achieved is the right for any member country to prevent the entry from another EEC country of meat which contains hormones such as stilboestrol, diethylstilboestrol, dienoestrol and hexoestrol. And those member states with legislation which bans other hormones can carry on as before.

In nine months' time, the European

Community will still have to decide between a policy which favours the consumer approach of banning unless proved safe and one favouring the industry's attitude of carrying on until a substance is known to be dangerous to human health — which is the choice of the United States.

Jasper Becker

Third World development

Proper priorities

Washington

The US Agency for International Development (AID) is continuing to pick up the pieces following the failure of the Carter Administration to convince Congress of the need for a new Institute for Scientific and Technological Cooperation to act as a focus for research on the needs of Third World countries.

Earlier this month the agency announced that as part of a general reorganization, a new Bureau of Science and Technology was to be established. Furthermore the title of "assistant administrator for development support" will be changed to "senior assistant administrator for science and technology", a position that will have responsibility for the bureau.

One of the aims of this reorganization, according to agency officials, is to strengthen the research activities of AID which have in the past been of lower priority than more conventional technical aid programmes. This was one of the reasons quoted for establishing the Institute for Scientific and Technological Cooperation, a body which would remain under the same umbrella as AID, but operate independently with its own board of advisers.

The parallel between the goals of the ill-fated institute and the newly proposed bureau is strengthened by the fact that Mr Reagan's appointee to head the bureau, Dr Nyle Brady, had previously been successfully wooed by the Carter Administration as its proposed director for the institute. Dr Brady will have responsibility for the administration of a wide range of centrally-funded research and development programmes, some of which were to have been shifted from AID to the new institute.

The Carter Administration had originally proposed significant increases in research support for these areas which would have virtually doubled the AID budget for research and development to \$229.8 million in the fiscal year 1982. The Reagan Administration has reduced this dramatically to a request for \$134.5 million, with large cuts, for example, in support for agricultural research on food production in Africa.

Even with these cuts, however, AID officials point out that the Administration's request for science and technology programmes for 1982 is 40 per cent higher than the amount spent on

research by AID in 1980. A particularly significant increase is in energy research, scheduled to grow from \$3.4 million in 1980 to \$16.3 million in 1982.

The impact the increase in funds and the internal reorganization will have remains controversial. Those in Congress who argued most strongly for the institute are somewhat sceptical.

This scepticism is reflected in a report from the House Foreign Affairs Committee on the AID budget request, which has included as a separate item a proposed \$10 million (reduced from President Carter's requested \$18 million) for the support of scientific and technological cooperation with developing countries. The committee has reinserted this money into the broader category of "selected development activities".

In fact, much of this money will be spent in a way comparable to what the institute would have done, but through a different agency, the National Academy of Sciences. AID has agreed to provide \$35 million of its science and technology funds over the next five years to a new grants programme established under the academy's Board on Science and Technology for International Development.

The grants, which will be \$5 million for 1981 and increase to \$10 million by the middle of the decade, will be spent largely on supporting research activities in the developing countries on projects selected by an advisory board. "One of our principal goals is to help developing countries build up their research capabilities, precisely the request that they were making at UNCSTD (the United National Conference on Science and Technology for Development) in Vienna in 1979", says the board's staff director, Dr Victor Rabinowitch.

When the grants programme is added to its more traditional activities, such as arranging seminars on Third World problems, the board will begin to resemble a scaled-down version of the Institute for Scientific and Technological Cooperation.

Furthermore its relative autonomy from AID goes some way to meeting the objections of those who argued that, as put forward by the Carter Administration, the inclusion of the institute under the umbrella of development aid bodies would have stifled its flexibility. David Dickson

Polish economic reform

Self-governance wins

Warsaw

The latest Polish plans for economic reform, discussed in considerable detail during the recent extraordinary Party congress, include a major shake-up for Polish science and technology. The basis of the proposed new economic model is a switch from rigid central planning of industrial production to a degree of "selfgovernance" (samorzadność) - a word introduced into the Polish political lexicon

with the rise of the independent trade union movement last August.

The new system, which is to be introduced in four phases over the next 18 months, will allow for more public and expert discussion in advance of overall production plans and delegate to factories initiative for deciding how they fit in with the overall targets.

As in Hungary, the authorities will steer production by a system of financial incentives, but factory managements will have considerable responsibility for decision-making on modernization and technological innovation. One of the major charges laid against the previous Gierek government in the preamble to the new plan is that autocratic planning blocked technical innovation. At the same time, many of Mr Gierek's prestigious schemes are being dropped. The "Program-Wisla", for example, which was totally to remodel Poland's major waterway by the end of the century, has been cut back to its environmental and agricultural components. The schemes for hydroelectric dams and the straightening of rivers have been dropped sine die.

Other harm done by Gierek's policies will not be so easily remedied. Discrimination against the private farmer in the pricing and supply system ran agriculture down to a level where even the new rationing system cannot ensure supplies of basic home-produced foods. The abolition of the small pharmaceutical cooperative factories in the 1970s has meant that Poland has had to shop abroad for a wide variety of drugs whose production runs were too small for the state pharmaceutical enterprise Polfa - and when hard currency ran out, so did the supply of even the most essential drugs. The international relief operation to ensure vital supplies now being mounted jointly by Solidarity and the Polish Ministry of Health is a strange contrast with the anxiety in the mid-1960s in the United Kingdom that Polish drugs might undercut the domestic products.

Although the new plan pays lip-service to the strengthening of scientific and technical ties with other (especially Comecon) countries, it is also clear that for the present Poland will have to rely mainly on homebased technology.

A reorganization of the responsible ministries is also on the cards. One is for a State Committee for Technical Progress responsible to the Prime Minister. The new body would be unique in the Comecon bloc in dealing only with technology, and may point to an ultimate break-up of the Ministry of Science, Higher Education and Technology. Straws in the wind include the appointment last month of a mining specialist, Jerzy Nawrocki, as Minister of Science, Higher Education and Technology, and the plans discussed at the congress for a complete review of the country's industrial research institutes and the closure of those not cost effective.

Whatever the final form of the reorgani-

zation, one change is certain — a revision of the present practice of financing science by "problems" ostensibly related to economic needs and organized into a cumbersome hierarchy of priorities. Among likely innovations are direct research contracts between industry and the universities or academy institutes and the allocation of funds for basic research directly to the institutions concerned.

For the moment, however, virtually all such funding will come in non-convertible zloty. Hard currency for the purchase of equipment, reagents or journals from the West is simply not to be had; according to one leading physicist, only two subscriptions to Nature are now authorized for the whole of Warsaw.

British Technology Group

Mixed marriage

Last week, Britain's National Enterprise Board and the National Research Development Corporation finally merged. The British Technology Group, as the two are now called, has been in the offing since Sir Frederick Wood was appointed chairman of both the board and the corporation at the beginning of the year. His plan for a merger has now been accepted by the government, although legislation to give the group formal status will not be possible until 1983.

The plan is that the group will offer more streamlined services to innovators seeking backing for their ventures than either of the parent organizations. The merger will also eliminate competition between the board and corporation for the same projects.

The function of the former corporation to support research and development into potentially marketable innovations will be largely taken over by the group's technology transfer division. And the board's experience in raising finance to invest in industry will be incorporated into the new investment and operations divisions, the latter being responsible for returning investments to the private sector.

Both parent organizations have been widely criticized in the past. The board, which was originally set up in 1965 by the then Labour government with the potent title of Industrial Reconstruction Corporation, has since been substantially curtailed. And the corporation has been criticized mainly by academics for not being adventurous enough in supporting their innovations and persuading industry to take them up. The British Technology Group is intended primarily to marry the corporation's access to innovators with the board's financial expertise. There is to be a new liaison team whose prime responsibility will be to scour the universities more aggressively than in the past.

The group is also to have a new corporate development unit to develop strategies for investment. Areas of priority are advanced manufacturing and biotechnology. A

study team on robotics has already presented a strategy paper to the group which is expected to result in investments within the next few months. The group also has plans for investing in biotechnology in areas not covered by Celltech, the company set up by the board last year.

As yet the group does not know precisely how much public money it will have to spend. The precise structure of the new organization should become clearer in September.

Judy Redfearn

Tidal energy

The big dam

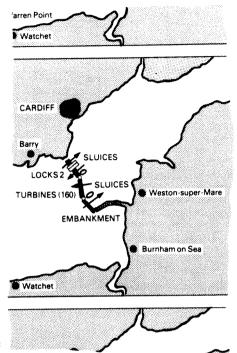
The economic feasibility of a tidal barrage for generating electricity across the Severn estuary in south-west England will depend on how many nuclear power stations are attached to the national grid. In most circumstances, however, a barrage would be a good investment, according to a report by the Severn Barrage Committee published last week. The committee, set up in 1978 under Sir Hermann Bondi, then chief scientist at the Department of Energy, and whose deliberations have cost £2.3 million, has also concluded that a barrage is technically feasible but has called for detailed studies of its potential environmental, industrial and social impact.

The outlook for a barrage across the Severn estuary, which has one of the greatest tidal ranges in the world, has improved considerably in the past two years, according to the committee. Approximately 40 per cent more energy should be obtainable from an inner barrage, the scheme favoured by the committee, than was originally thought. And electricity generation should be possible nine rather than 16 years after the start of construction.

The committee estimates that the most cost-effective scheme would cost about £5,600 million and would generate 13 TWh a year from an installed capacity of 7,200 MW. That scheme is for an inner barrage from Brean Down to Lavernock Point consisting of large prefabricated concrete units housing turbines and sluices. A more ambitious barrage built further out to sea, costing £8,900 million and generating 20 TWh from 12,000 MW capacity, would be less economic and involve greater risk during construction. The inner barrage scheme, on which the committee has based most of its calculations, has the added advantage that a second barrage could be added at a later stage. The two together would provide as much energy as the outer barrage at a similar overall cost.

Because of the variability in tidal energy, a barrage would reduce the need for other generating capacity only by about 1,000 GW, according to the committee, most savings being in fossil fuel. And as nuclear power is also capital-intensive and low on fuel costs, the attractiveness of a barrage will to a large extent depend on future plans

for nuclear power. Under the Central Electricity Generating Board scenario, which assumes a fairly rapid growth in nuclear power stations, the barrage is marginally unattractive, says the committee. But it could provide an attractive alternative if nuclear plans are modified only slightly.



The committee seems convinced that a Severn barrage should at least be seriously considered as a possible alternative to investment in nuclear power. Before a decision is finally taken, however, it recommends further studies of the effects on water levels, wildlife and local amenities and the capacity of British industry to cope with such a project. If the government decides to take investigations further, the next step should be an acceptability and preliminary design study costing £20 million and the building of prototype concrete units at a cost of £25 million.

Judy Redfearn

US nuclear weapons

Lasers purify

Washington

A Washington environmentalist group is trying to prevent the Department of Energy from carrying out an expanded programme of research into laser isotope techniques for separating plutonium for US nuclear weapons from spent nuclear fuel.

A classified research programme into laser isotope separations, using copper vapour lasers for uranium enrichment, has been carried out at a relatively low level of funding at the Department of Energy's Lawrence Livermore National Laboratory and the Los Alamos National Laboratory since the mid-1970s — in both 1980 and 1981 total funding for the programme was \$5 million.

In testimony before a congressional committee in March which has just been declassified, the Department of Energy requested an increase in funds to \$155 million over the next three years to support this research and its application to plutonium enrichment. This money would include the construction of a pilot plant, and the department is also discussing plans to construct a \$200 million multipurpose plutonium isotope production plant.

In a letter sent last week to the Secretary of Energy, Mr James Edwards, a staff scientist and an attorney with the Natural Resources Defense Council say that the plutonium laser isotope separation programme "carries grave nuclear weapons proliferation risks" and is "totally inconsistent with the fundamental objective of President Reagan's nuclear non-proliferation policy".

They point out that although the department has identified several other applications for the process — such as the enrichment of fuel-grade plutonium as fuel for the breeder reactor research programme — "it appears that these applications would not serve as adequate justification for the program, particularly in light of the severe proliferation dangers".

In their presentation to the congressional committee, Department of Energy officials said that the purpose of the expanded programme was to make available for weapons use approximately 70 tonnes of plutonium by reprocessing commercial spent fuel and then enriching the plutonium to weapons-grade by laser isotope separation. It would be possible to convert fuel-grade plutonium produced by the department's nuclear reactor in Hanford, Washington, to weapons-grade.

One of the purposes of the laser isotope separation process is to enrich plutonium to weapons grade by reducing the concentration of the major contaminant isotope Pu-240, which is not spontaneously fissile. According to testimony given to Congress by F. Charles Gilbert, assistant secretary for nuclear materials at the Department of Energy, plutonium is placed in one of four categories depending on the concentration of the Pu-240 contaminant: reactor-grade for 19 per cent or greater, fuel-grade for between 7 and 19 per cent, weapons-grade for less than 7 per cent, and supergrade for between 2 and 3 per cent.

The laser enrichment process has two non-military uses. The first is the separation of the Pu-238 isotope, which is used in space satellites and heart pacemakers, as well as for a heat source in thermoelectric generators. The second is the separation of the Pu-241 isotope which decays to Am-21 which is used in smoke detectors and for logging wells. According to the Department of Energy, the non-weapons applications at present require less than two per cent of the output of the Savannah River plant, which is the current source of supergrade plutonium.

David Dickson

CORRESPONDENCE

Man versus tsetse

SIR - I must contend with your leading article "Fighting tsetse flies and people" (Nature 2 July, p.1).

In it you state that the eradication of the tsetse fly in certain parts of Africa (by such organizations as ILRAD) would be a revolutionary advancement for its people. Cattle would then be able to graze over a much larger area of land, so more food would

This, in itself, is quite true — for man. But the tsetse fly is one of the last remaining barriers against mankind's inevitable destruction of the natural habitats for literally millions of animals of the continent. Would not this "advancement" seal the fate of these millions?

Animals in the wild cannot compete with organized grazing. It's like asking one man to stop a moving steamroller by standing in front of it — he would be crushed. The question is: do we have the right to do this, to crush other forms of life?

As the most powerful animal on this planet, man is entrusted with a duty to preserve that which cannot defend itself. Although the African lion is strong, it can't stop a steamroller.

ADAM J. BECKER

Jamesville New York, USA

Author — Anon

SIR — Drs Gillman and Lichtigfeld (Nature 25 June, p.608) suggest that the present system of assessment of scientific papers is endangered by a cabal of corrupt referees, and propose a completely open system. Referees may be prejudiced and incompetent but they are not a secret society. Vigilant editorial control rather than public exposure seems the most appropriate way to eliminate bad referees.

Since referees may be influenced by the known standing of the author or the author's institute. I propose that the anonymity afforded to referees should be extended to authors. If papers were submitted with the author's name, address and acknowledgements on separate sheets of paper and these were retained by the editor when the manuscript was forwarded to the referees, conscious or unconscious bias would be avoided.

P.M. GAYLARDE

Royal Free Hospital, London NW3, UK

From the museum

Sir - We should like to explain a matter that puzzled you in your editorial of 2 July (p.1): the place of cladistics in the exhibition policy of the British Museum (Natural History).

Exhibitions have a general tendency to conceal background controversyl. This should not be seen as a conspiracy on the part of designers but is, in fact, a phenomenon that is apparent in the majority of educational media. Every lecturer or textbook writer has to decide the extent to which he will emphasize a straightforward story at the expense of alternative views. The emphasis chosen depends on the circumstances; the less

knowledgeable the audience, the less appropriate it is to elaborate the story. Knowing how to make this choice is particularly difficult in exhibitions since the limits of educational attainment are even less well understood than in other more generally practised media. In public museums there is an overriding need "to deliver an interesting and stimulating message to laymen"2

One of the essentials for good communication in a museum is to set objects within some kind of context so that specimens take their place in a coherent line of thought and do not seem to be a series of unrelated items. Without such a context, the specimens have little meaning and odd facts that are gleaned tend to be quickly forgotten; in educational terms, the exhibit would be a failure.

In two of our recent exhibitions, Man's place in evolution and Dinosaurs and their living relatives, the context for the specimens has been that of phylogeny and the fact that we chose to present phylogeny in cladistic terms has been seen by some critics as an attempt to promote cladistics at the expense of more traditional views. Recent correspondence in Nature and elsewhere emphasizes the fact that a heated debate is in progress about the relative merits of cladistic and traditional approaches to phylogeny and systematics. Exhibitions have no part in this debate between scientists, but it is clear that whichever system is used in an exhibition, one faction or another will be provoked into stern response. Accepting that one of the competing systems had to be chosen, we judged on educational grounds that cladistics was the most appropriate because of its clear and overt reasoning. Once a layman has been introduced to the system he is in a better position to understand why and how the scientists reach their conclusions. No one would pretend that the use of a cladistic system makes the study of phylogeny easy, but it can make the steps in the argument more apparent.

Some critics feel that the exhibitions should have said more about the existence of alternative systems and we are making some changes to meet that criticism, but the danger is that the exhibits in question may become displays about phylogenetic systems rather than about fossil man, or dinosaurs. A separate exhibition about systems of classification which is scheduled for future display may help to give a fuller presentation of current views.

The recent controversy has highlighted how difficult it is to maintain a balance in exhibitions between directness and fairness to every point of view - exhaustive can be exhausting to the visitor. The application of educational principles to museum exhibitions is a subject that has been sadly neglected in the past and the credibility of museums as a means of informal education has suffered in consequence. We should like to encourage serious discussion and research in this field.

R.S. MILES G.C.S. CLARKE

Department of Public Service. British Museum (Natural History), London, SW7, UK

1. Lewis, B.N. Mus. J.80, 151-155 (1980)

Wittlin, A.S. Curator 14, 138-150 (1971).

Suiting everyone

SIR - You object most strongly (Nature 12 March, p.75) to the phrase "If the theory of evolution is true . . .". Having myself tried more than once to explain the work of a scientific institute in everyday language, I have some sympathy with the author of the museum guide. When all is said and done, scientific knowledge is provisional and fallible, as, implicitly, you agree. Your objection seems to be that this particular form of words gives a handle to those opponents of evolutionism whose views you regard as not "respectable". specifically to creationists. I am not a creationist and accept the evidence that evolution by natural selection has taken place. ultimately producing Man. Nevertheless I sometimes find biologists, when writing for a popular readership, to be overly dogmatic on this point. The creationist movement on this continent (which is not simply a revival of fundamentalism and obscurantism) is partly inspired, I believe, by a healthy reaction to this dogmatism.

To represent our scientific theories as final when they are not is in the long run to do our own cause a disservice, and is, in any case, dishonest. Can any evolutionist provide a clinching argument that chance mutations acted on by natural selection alone are the mechanism of evolution? I think not, and, indeed, I myself find it at least as easy to believe that there has been a purposive element in evolution. Did you really mean to imply that it is not "respectable" to believe "that the course of events may be determined by literally supernatural infuences"?

In your last paragraph, you almost seem to be proposing a noble myth, the purpose of which will be to keep all us scientists working happily at our research. If we recognize too clearly the logical status of our theories, you argue, the scientific enterprise will be undermined. But Popper's ideas, as you admit, have been around since 1934, and many branches of science have been uncommonly vigorous since then. Your argument has something in common with the old discredited argument that Christianity is socially beneficial and should be taught to the young whether it is true or false. It is this kind of argument that plays into the hands of creationists, by making more plausible their claim that evolutionism is not a scientific theory, but a kind of religion.

The most helpful ideas in this situation, I find, are not Popper's, nor dogmatism, but Kuhn's. Almost all of modern biology is "normal science" within the paradigm of Darwinism-Mendelism. Always there are some facts that do not fit a paradigm, and one day they may become so bothersome we will have to change it. In the meantime, it provides a frame of reference in which we work and by which we make sense of a wide range of data and experience. Darwinism-Mendelism is the best paradigm we have (in its field) and, as Darwin said, gives grandeur to our view of life, but it should not fight its critics by claiming, like them, to be based on divine revelation.

A.H. BATTEN

National Research Council of Canada, Victoria, British Columbia, Canada

Too tolerant

SiR — I agree with you (Nature 28 May, p.271) that tolerance of religious fundamentalism in the Natural History Museum has gone too far. But you underestimate the menace to science, and to the propagation of rational thinking by education, presented by this tolerance. I see two problems — first the conflict between science, in the broadest sense of the word, and mythology. The second is the egalitarian and anti-elitist attitude prevailing among many educated people which has brought about the woolly-minded tolerance you describe.

Homo sapiens indulges in two main kinds of thinking about his world which must go back to the very emergence of the first intelligent, tool-making primates. The appearance of the first stone tools, adapted for specific uses, implies the existence of empirical thinking involving designing an implement for future use, testing it practically and modifying it in the light of experience. From this technological thinking, based on trial and error, must ultimately have developed scientific thinking, based on observation, hypothesis, prediction, experimental test and modification.

On the other hand myth-making must have emerged at an equally early time to allow primitive men and women to explain the vast range of often frightening phenomena outside their understanding.

We might also describe this duality of thought processes in terms of deductive and inductive thinking. In the former the grand, explanatory ideas come first (usually being taught as dogma) and are then used to interpret everyday natural and social phenomena. In the latter the explanatory general hypotheses are developed specifically to explain a body of facts or phenomena. I am now using the term "myth" to describe all preconceived ideas or beliefs reached deductively (before examining the evidence properly) and "science" to describe the opposing, inductive method of explaining things (which also involves testing the hypotheses devised).

The conflict between these two modes of thought seems to be as basic now as it was in Darwin's time and to have extended into many more fields. Myths are believed in for a variety of reasons, both reputable and otherwise, but it seems reasonable to suppose that they reflect psychological or emotional needs. Their great variation suggests this, the zeal with which they are believed being the only common factor. The dedicated communist, Christian, Buddhist, believer in flying saucers or in a tribal god each believes his creed deductively (usually having been taught it a priori) and each is equally convinced of its truth, and of the folly or evil of those who disagree. The only common factor seems to be a strong, often overwhelming, perhaps unconscious desire to believe in some set of ideas which explain phenomena one is interested in or worried about. This is the exact opposite of the scientific method.

It is essential to realise that what is being described here is a mental attitude and not categories of rational and irrational subjects to think about. Of course it is possible to be a communist or a Christian on inductive, rational grounds if one has studied as much of the evidence as possible, considered alternative explanations for it and found them less convincing. Likewise it is unfortunately true

that a scientist can elevate the status of his favourite theory in his own mind to that of a myth — and believe it implicitly, emotionally and against all opposition. It is the mental attitude, not the subject matter, which produces both myth and science.

It follows that there can be no place for myths either in science or in any proper education programme that tries to avoid indoctrination. "Creation science" is a myth which depends on a dogmatic and deductive faith in the existence of a God who has intervened frequently on Earth to make new living things out of nothing and, this being so, the Natural History Museum may be said to have betrayed both its scientific status and its educational role by implying that this myth is somehow on the same intellectual plane as the theory of evolution.

Where will this process end? Another interesting if minor example of a new myth about human origins appeared in The Sunday Standard of 31 May. A female body builder, when explaining why she pursued this hobby, is reported as saying "We were never meant to be the weaker sex, and being seen as such is a fairly recent evolutionary development. Originally it was women who went out hunting to find food; they were the strength of the family." This relatively harmless myth, though it bears no relation to the facts of archaeology and anthropology, may be assumed to serve a useful purpose to the lady concerned by giving her the comfort of an ideological justification for what most people probably regard as a bizarre hobby. But to the Natural History Museum it must surely now be a valid alternative hypothesis, or even a theory, about the early development of man and society. Perhaps the Public Services Department will consider adding a new panel on "Ms Cheshire's hypothesis" to its display

on human evolution? Or is it the popularity of a myth which qualifies it for serious attention in Cromwell Road now? If so then a panel describing Von Däniken's ideas about the bringing of civilization to Earth by spacemen in prehistoric times is surely required.

My explanation for this excessive tolerance, which is an irresponsible failure to distinguish between scientific thinking and irrational myth-making, is that it is an effect of modern egalitarian thinking. This is quite widespread among some university staff, extending to advocating equal tolerance for the "views" of all people, no matter how well or ill-qualified in the subject concerned. I have actually heard it argued in faculty meetings in this university that it is no longer necessary to have professors as more than administrative departmental heads, to have them leading and guiding their staff in research and academic standards by their example; everyone, it seems, should now be "capable of standing on his own feet". Is this phenomenon sometimes due to a real fear of being thought elitist, "better" than one's fellows, and to a failure to realise that better trained or informed is not the same thing as absolutely better?

It cannot be a coincidence that no equivalents of the "creation science" myth appear in disciplines like engineering or navigation where equal tolerance for them would produce catastrophe. The tolerance of myths about human origins by the staff of the Natural History Museum will not cause their building to fall down about their ears but it has certainly undermined the institution's reputation as an authoritative laymen's guide to the world of science.

E.W. MACKIE

Hunterian Museum, The University of Glasgow, UK

Halstead's defence against irrelevancy

SIR — In his Presidential Address to the British Association in 1977, Sir Andrew Huxley "wondered what topic could, in the second half of the twentieth century, generate emotions as strong as those which arose over evolution in the early 1860s". If the recent correspondence in the columns of *Nature* is anything to go by then it undoubtedly remains this selfsame topic.

Previously I drew attention to what was taking place at the British Museum (Natural History) which occasioned serious disquiet in the scientific community. I suggested a possible explanation, but whether this is rejected or accepted does not affect the substantive issues I raised. So far, no serious attempt has been made by anyone in authority to respond to the accusations that have been levelled.

The present debate began in 1978, when the trend of the new policies of the museum's Public Services Department and the proposed presentation of dinosaurs merely to illustrate cladistics were discussed at a symposium at Reading (see refs 1,2). In 1979 the exhibit "Dinosaurs and their living relatives" opened and the accompanying booklet spelt out clearly exactly what cladistics involved. The crude didactic method of its presentation contrasted dramatically with the popular but nonetheless scholarly approach of the museum's book "A New Look at Dinosaurs" by Alan Charig, published at the same time³.

Then in 1980 came the exhibit "Man's place

in evolution", again with its accompanying booklet in which it was again emphasized that we can never know how species arose in the past and that we assume that new species arise only by splitting from previous species. But the booklet includes the most amazing assertion of all, that no fossil species can be considered the direct ancestor of any other. I referred4 to the place of Homo erectus and the Petrolona skull which sits on the morphological boundary between Homo erectus and archaic Homo saniens. The review article on fossil man by Cronin et al. 5 and also recent work on planktonic organisms by Prothero and Lazarus⁶ have dealt with this matter effectively. The museum has now, in 1981, removed the labels which previously stated categorically that the Homo erectus people were not our direct ancestors.

It is a fact that the dinosaurs are used to illustrate the principles of cladistics and not their natural history. Similarly, the evolution of man is being used as a vehicle for cladistics, even though this has involved crudely distorting the evidence. It is worth recalling the words that Andrew Huxley used in his Presidential Address to the British Association in 1977.

"Scientists whose findings contradict fashionable social theories are accused of distorting their results through political prejudice and it is suggested that on sensitive topics we should base our beliefs not on what is actually found to be the case, but on the supposed consequences of holding particular beliefs — in effect

that we ought to replace science by wishful thinking. I regard any such attempt to deflect scientific conclusions for political or social motives, however well-meaning, as a betrayal of science.

The situation at the British Museum (Natural History) is suspiciously comparable to that against which Huxley's criticisms were directed.

It was, however, something even more astonishing than all this that stung me, a palaeontologist and evolutionist, into activity⁷. This was the assurance given in writing in 1978 by the Head of the Public Services Department to the creationists 8 that the museum in its new exhibition on evolution (to be opened in 1981) would make it quite clear that the theory of evolution was not a scientific theory. It transpired that this attitude stemmed from the writings of Sir Karl Popper. The museum was now concerned with "concepts of science" as adumbrated by Popper, and the articles of Platnick and Gaffney⁹ and even more recently Tassy¹⁰ emphasized the scientific (in the sense of Popper) nature of cladistics. In marked contrast, palaeontology, the historical side of evolution, was dismissed because it comprised unique events which were by definition unrepeatable and so not subject to test and hence not part of science. This is the view of Patterson 11 which he claimed comes from Popper.

The notion that the process of evolution by natural selection is non-science was based on the idea that "survival of the fittest" is a tautology and hence unfalsifiable. On this point Popper 12 wrote:

"The fact that the theory of natural selection is difficult to test has led some people, anti-Darwinists, to claim that it is a tautology . . Since the explanatory power of a tautology is obviously zero, something must be wrong

"I mention this problem because I too belong among the culprits.... I have changed my mind about the testability and the logical status of the theory of natural selection; and I am glad to have an opportunity to make a recantation.

Ruse 13 and Flew 14 have also dealt with this. (The museum, although initially keeping faith with the creationists, has since withdrawn the film loop concerned, following the adverse review of the exhibition by Barry Cox 15 (see also Miles 16).

Popper 17, following my attack 7 on the ideas

being peddled in his name, has emphatically dissociated himself from his self-proclaimed disciples in the following terms:

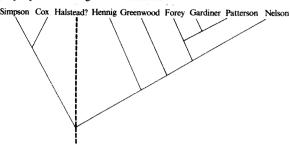
"It does appear that some people think that I denied scientific character to the historical sciences, such as palaeontology, or the history of the evolution of life on Earth. This is a mistake, and I here wish to affirm that these and other historical sciences have in my opinion scientific character; their hypotheses can in many cases be tested. It appears as if some people would think that the historical sciences are untestable because they describe unique events. However, the description of unique events can very often be tested by deriving from them testable predictions or retrodictions.

With the philosophical aspect in better perspective, it became possible to consider what might lie behind the attitudes to evolution and palaeontology being unveiled by the museum's Public Services Department. My suggestions on this stimulated a certain degree of controversy.

Sir Andrew Huxley in his opening of the museum's new exhibit "Origin of Species" stated that he had "been quite unable to comprehend the suggestion that cladism is

How to classify the cladists

Sir - May I propose a cladogram of cladists?



The cladogram is, of course, based only on derived characters appearing late in ontogeny; it makes no claims as to ancestry. (Apologies to Halstead, but he does display a confusing array of derived traits.) London, UK **BRIAN LEITH**

somehow antagonistic to evolution, or that cladism is linked to the theory that evolution progresses by fits and starts, or that cladism is more Marxist than other styles of classification"

That Hennigian or classical cladistics is antagonistic to the gradualist type of evolution is surely evidenced by Rosen, Nelson and Patterson 18:

"Hennig established a criterion of demarcation between sciences and metaphysics at a time when neo-Darwinism had attained a sort of metaphysical pinnacle by imposing a burden of subjectivity and tautology on nature's observable hierarchy. Encumbered with vague and slippery ideas about adaptation, fitness, biological species and natural selection, neo-Darwinism (summed up in the "evolutionary" systematics of Mayr and Simpson) not only lacked a definable investigatory method, but came to depend, both for evolutionary interpretation and classification, on consensus or authority.'

This sounds antagonistic to me.

The latest brand of cladism, the transformed variety, has undoubtedly slid into what seems to me to be an overtly anti-evolutionist stand. Patterson 19 writes:

'Hennig's 1966 book, as the title Phylogenetic Systematics suggests, was based in evolutionary theory. But as the theory of cladistics has developed, it has been realised that more and more of the evolutionary framework is inessential and may be dropped. Platnick (1980) refers to the new theory as 'transformed cladistics' and the transformation is away from dependence on evolutionary theory. In my view, the most important outcome of cladistics is that a simple, even naive method of discovering the group of systematics - what used to be called, the natural system - has led some of us to realise that much of today's explanation of nature, in terms of neo-Darwinism, or the synthetic theory, may be empty rhetoric. Eldredge and Cracraft (1980) provide a detailed criticism of the transformational approach in biology, and the ensuing interest in storytelling about adaptive change.

The link between cladistics and the theory that evolution progresses by fits and starts

- 1. Halstead, L.B. Nature 275, 683 (1978).
- Halstead, L.B. Nature 276, 759-760 (1979).
 Halstead, L.B. Nature 276, 759-760 (1978).
 Charig, A. A New Look at Dinosaurs (British Museum (Natural History), London, 1979).
 Halstead, L.B. Nature 288, 208 (1980).
 Cropin, L.E. Poers, N.T. Science, C.B. B. Park, V. M. S. Science, C.B. B. Science, C.B. Science, C.B. B. Science
- 5. Cronin, J.E., Boaz, N.T., Stringer, C.B. & Rak, Y. Nature 292, 113-122 (1981).
- 6. Prothero, D.R. & Lazarus, D.B. Syst. Zool. 28 (1978).
- Halstead, L.B. New Scientist 87, 215-217 (1980). Miles, R.S. Creation News Sheet 28 (1978).
- Platnick, N.I. & Gaffney, E.S. Syst. Zool. 26, 360-365 (1977); 27, 137-141, 381-388 (1978).
- Tassy, P. Int. Symp. Conceptual Methods in Palae-ontology, Barcelona, 65-73 (1981).
 Patterson, C. Evolution (British Museum (Natural
- History), London, 1978).

12. Popper, K. Dialectica 32, 344 (1978).

(punctuated equilibria) is emphasized in particular by Cracraft²⁰ and is certainly the type of association that a normal reader would obtain from reading Eldredge and Cracraft's Phylogenetic Patterns and the Evolutionary Process²¹

The concept of punctuated equilibria is undoubtedly linked to Marxism, according to Gould²², who claims to be a Marxist. Gould²² sees "notions of gradualism arising largely out of a pervasive political bias [and] . . . the replacing of gradualism with the flip-like style of change which has been appreciated within Marxist philosophy for a long time.'

The current drive against the concept of gradualism is motivated primarily by Marxists this does not mean that there are not plenty of non-Marxists who agree nor does it mean that the views may not turn out to be true. What is objectionable is the distortion of scientific data for ideological purposes.

Finally, I am not opposed to either a cladistic or a Marxist approach being presented. My objection is to the attempt to impose highly controversial concepts on to the general public not by argument or discussion but simply by unsubstantiated assertion. Whalley and Gibbon²³ have pointed out that the current policies being pursued by the museum are diametrically opposed to the 1972 paper to the trustees, on which they are supposedly based, where it was emphasized that the new exhibition scheme "should point out areas of doubt and speculation"

Whatever view is taken of my linking hypothesis, this does not in any way affect the factual account of what has been happening in the museum. It is to be hoped that the minor changes made in the past few weeks herald a new awareness by the trustees of their responsibility towards maintaining integrity in the natural sciences.

L. BEVERLY HALSTEAD

University of Reading, UK

- 13. Ruse, M. Phil. Sci. 44, 638-661 (1977); New Scientist 90, 828-830 (1981).
- Flew, A. A Rational Animal (Clarendon, Oxford, 1978).
 Cox, B. Nature 291, 373 (1981).

- Cox, B. Natine 291, 330 (1981).
 Miles, R.S. Nature 291, 530 (1981).
 Popper, K. New Scientist 87, 611 (1980).
 Rosen, D.E., Nelson, G. & Patterson, C. in Phylogenetic Systematics (University of Illinois Press, 1979).
 Patterson, C. Biologist 27, 234-240 (1980).
 Cracraft, J. in Phylogenetic Analysis and Palaeontology (Columbic University Press, 1970).
- (Columbia University Press, 1979).
- Eldredge, N. & Cracraft, J. Phylogenetic Patterns and the Process of Evolution (Columbia University Press, 1980).
 Gould, S.J. Sci. Nature 3, 5-12 (1979).
- 23. Whalley, R. & Gibbon, K. Palaeont. Ass. Circ. 105, 4 (1981).

NEWS AND VIEWS

Thalassaemia: from theory to practice

from Bob Williamson

THE international symposium on recent advances in thalassaemia, held in Sardinia from June 7th to 11th, was divided quite sharply into two parts. Several molecular biologists presented the latest data on the gene defects causing the various types of thalassaemia found throughout the world, following which doctors from the countries where the disease is common exchanged experiences on the use of this knowledge in programmes for population screening and antenatal diagnosis. The meeting was the first in three years to involve most of the molecular biologists and all of the leading figures involved in control of haemoglobinopathies, and allowed some interesting conclusions to be drawn.

Since the first demonstration that most cases of a-thalassaemia (with depressed a-globin synthesis) are due to gene deletion, the exact gene defects in each of the haemoglobinopathies have been analysed one after another. As the thalassaemias, and in particular β -thalassaemia (in which the synthesis of the β -globin chain synthesis is impaired), protect carriers against malaria, it is not surprising that many independent mutations have spread in populations in malarial areas. One interesting possibility put forward by Herman Lehmann (University of Cambridge) is that a-thalassaemia might not really protect against malaria at all, but rather mitigates the effects of β-thalassaemia (or sickle cell disease) and so is often found in association with it.

Genetic engineering techniques have now allowed a complete understanding of the causes of the major forms of β -thalassaemia in Italy, Greece and Cyprus. In β° -thalassaemia, A. Bank (Columbia University) showed that a point mutation has occurred in many patients at the 5' intron-exon junction of the large intron so that splicing of the nuclear RNA transcript cannot take place at all. Patients homozygous for this point mutation cannot make messenger RNA, and therefore make no β -globin. Fortunately, the splice junction is also a restriction site for the enzyme HphI, and therefore a direct gene analysis (of the patients, or antenatally) should be relatively simple.

In the case of β^+ -thalassaemia, the form most common in Cyprus (and therefore in London), B. Forget (Yale University) reported results from his laboratory and from that of R. Williamson (St Mary's, London) which show that the mutation is within the small intron and creates a new splice site which can act as a preferred alternative to the correct one. This explains why patients are still able to make a small amount of β -globin. Yet another type of mutation, causing premature termination of the β -globin protein chain, has been identified by Y.W. Kan (University of California, San Francisco). It is now clear that the majority of β -thalassaemia cases, as well as those of α -thalassaemia, are a result of either a single point mutation or a gene deletion of one of the globin structural genes. Therefore, hopes that control mutations would be common among the thalassaemias do not appear to be fulfilled. Even cases of 'hereditary persistance of fetal haemoglobin', reported by D. Weatherall (University of Oxford) and S. Ottolenghi (University of Milan), show linkage to the β -globin gene, and therefore are probably caused by a similar type of mutation.

The one new application of molecular biology to antenatal diagnosis was reported by Y.W. Kan (and originated from John Wilson, University of Georgia). He noted that the point mutation causing sickle cell disease, in the codon specifying amino acid 6 of the β -globin chain, eliminates a restriction enzyme site for Ddel. Wilson has used this in a direct analysis of DNA from patients and from the amniotic fluid of a fetus at risk. Previous DNA antenatal diagnoses have depended upon linkage between a restriction site polymorphism and the gene defect, and therefore have required a family study (Kan and Dozy Proc. natn. Acad. Sci. U.S.A. 75; 5631, 1978; Little et al. Nature 285; 114, 1980). If the defect itself can be examined directly at the nucleotide level, it simplifies matters

Bob Williamson is Professor of Biochemistry at St Mary's Hospital Medical School, University of London.

enormously for the specialist in molecular medicine, as both the theoretical problems of gene cross-over and the practical difficulties involved in family studies and the establishment of paternity all vanish.

Perhaps the most interesting view of the meeting concerned the attempts of clinicians in Italy and Greece to implement community programmes to control the disease. Pride of place must surely go to A. Cao (University of Cagliari), the organiser of the meeting, whose efforts in southern Sardinia have cut the incidence of thalassaemia from 1 in 250 live births to 1 in 800. This dramatic threefold reduction is not due exclusively to prenatal diagnosis, but it was very illuminating to learn that even in Sardinia, as a result of intensive popular education programmes, termination of pregnancy where prenatal diagnosis shows the fetus to be affected by this very severe disease is accepted in 95 per cent of cases. This surely indicates that community attitudes to very serious diseases are changing even where one might think that religious attitudes would prevent a successful programme.

D. Loukopoulos (University of Athens) is attempting a similar programme in Greece, but the fact that the country (and the problem) is so much bigger, and the absence of a unified health service and education programme, have meant that his programme has had less impact. However, he is now carrying out over 500 antenatal diagnoses a year, and in some regions in Greece the incidence of the disease is falling. He commented that the fact that affected children are now recieving blood transfusions (up to 20 a year) and very expensive support therapy to remove the iron from their bodies means that there is enormous financial and clinical burden on Greek paediatric departments which did not exist when most children died in the first two years of life. Therefore, the very fact that clinical care has improved (although still quite stressful for the patients) has created a dramatic need for better antenatal diagnosis.

B. Modell (University College Hospital, London) then reported upon the reaction from the Cypriot community in London to the availability of antenatal diagnosis. There has not been a single case of β -thalassaemia within the Cypriot community during the past two years, as a result of intensive community education programmes and the awareness of both the community itself and those general practitioners in North London who serve it.

Parents who were at risk of having an affected child had virtually stopped reproducing before antenatal diagnosis, but now are having a normal number of children, as it can be guaranteed that all of these will be healthy. In this way, it has been shown that far from resulting in a decrease in the number of normal children

being born, antenatal diagnosis with abortion of affected pregnancies has resulted in a large increase in normal children, which is what both the families at risk and the community wish.

However, antenatal diagnosis (whether using fetal blood or DNA) can only deal with the situation in advanced countries at the moment, and even in Britain, Italy and Greece there are still seriously ill children with thalassaemia. Bone marrow transplantation for blood diseases such as the leukaemias is now an established, if risky, clinical practice in several centres, and D. Thomas (University of Seattle) discussed whether this procedure might also work for thalassaemia. The best results in bone

marrow transplantation are achieved with HLA-compatible brothers or sisters, and these certainly exist in thalassaemic families. However, transplantation should be carried out early, when the child is well, and not when the tissues have already been damaged by high levels of transfusion and the iron deposits this leads to. The question facing clinicians in the immediate future is whether to risk the life of a relatively well child with thalassaemia by giving a marrow transplant, knowing that if it works (a 75 per cent chance) the child will be healthy permanently — or to hope for other, less heroic treatment to be developed, knowing that if you guess wrong, the child may die in late teens of a very unpleasant disease.

Minor body mass determination

from David W. Hughes

SPACECRAFT missions to both asteroids and comets have been much in the news recently. One of the aims of these investigations will be the accurate determination of the masses of minor bodies in the Solar System.

From theoretical considerations, the mass of a typical short-period comet has been estimated to be about 1016g. It seems that the direct determination of a comet's mass by observing the perturbation that it induces in the orbit of another celestial body is quite out of the question. One of the closest approaches to Earth of any recorded comet was that of periodic comet Lexell, which on 1 July 1770 came to within 2.25×10^6 km, about six times further away than the Moon. Laplace recorded in his Traité De Mécanique Céleste (Paris, 1805) that this close encounter had no measurable effect on the length of the sidereal year and thus the mass of this comet must be less than 2×10^{-4} the mass of the Earth (which is 6×10^{27} g).

Asteroids are generally bigger than comets and therefore the problem is slightly easier. A great many positional observations are available over a long time period for the largest two, Ceres and Pallas, beginning with the years of discovery, 1801 and 1802. These observations provide information about the accumulated gravitational effects which each of the two bodies have produced in the orbital motions of the other. The total effects are small. Schubart (Astr. Astrophys. 30; 289, 1974) integrated the data for 1802-1970 and found the mass of Ceres to be $(1.17 \pm 0.06) \times 10^{24}$ g. Pallas was smaller — $(2.6 \pm 0.8) \times 10^{23} g$ — and so the error in its mass determination was relatively larger.

A more recent determination of the mass of a minor body has been attempted by analysing the radio-tracking data of a spacecraft as it flies close to the body. The Viking Orbiter 1 made several flybies to within 80-300 km of the Mars satellite Phobos. Radiometric tracking from Earth measured the spacecraft – Earth distance and the geocentric velocity of the spacecraft. The latter was achieved by recording the Doppler shift in the S and X band frequencies transmitted by the spacecraft. Analysis of the gravitational perturbations induced in the orbit of the spacecraft led to a mass determination of $(9.9 \pm 0.6) \times 10^{18}$ g for Phobos — an object which is about 21.5 km across.

The problem of measuring the mass of a comet by using the tracking data of an in situ spacecraft has been analysed by D.K. Yeomans, M. Ananda, W.L. Sjogren and L.J. Wood of the Jet Propulsion Laboratory, Pasadena (see J. astronaut. Sci. 29; 19, 1981). In a representative experiment, the authors decide to determine the mass of the short-period comet Tempel 2 during a rendezvous mission. A solar electron propulsion system (SEPS) has been used to transfer the spacecraft to the comet and the data are collected near perihelion when the comet becomes active. A single slow flyby on the sunward side of the cometary nucleus is assumed.

The main relative spacecraft—comet acceleration is due to the gravitational attraction between the two bodies. There are, however, four additional accelerations to be considered. Comets near perihelion give off a lot of gas and dust, at a rate that varies with heliocentric distance and is probably uneven, the material coming from a series of relatively short-lived active regions on the spinning cometary nucleus. The magnitude of the acceleration effect can thus vary stochastically about some mean value. It also depends on the orientation of the solar array panels, obviously

David W. Hughes is in the Department of Physics, University of Sheffield.

being larger when these are face-on to the flux as opposed to being 'feathered'. Solar radiation pressure also influences the spacecraft and again the effect depends on the area of the spacecraft presented to the radiation flux. Radiation pressure is generally larger than the gas and dust drag but is more easily accounted for as it can be considered constant over short time periods. There is also a tidal acceleration due to the unequal distance between the spacecraft, the comet and the Sun. Finally, spurious accelerations would be present, generated by the spacecraft due to the incomplete shut down of SEPS engines, altitude and articulation control subsystem gas jets. The operation of tape recorders and resonant vibrations of large solar panels also cause problems.

Two primary measurements are taken. Doppler tracking using the Deep Space Network of tracking stations enables the velocity to be found to an accuracy of 0.1 cm s⁻¹ over a count time of 1 min. Spacecraft—comet ranging using, for example, X band (10 GHz), a pulse length of 2 μ s and a pulse repetition frequency of 250 Hz can give an accuracy of 20 – 30 m when the spacecraft is within 1,000 km of the cometary nucleus.

Yeomans and colleagues find that the Doppler tracking data are more useful than the spacecraft-comet ranging data when it comes to determining the mass of the comet. Accuracy is improved by having both. The sensitivity of the mass determination to the stochastic nature of the gas and dust emission indicates that an accurate on-board accelerometer would also improve the accuracy.

Returning to Tempel 2, if the rendezvous spacecraft flies by the comet at a minimum distance of 100 km at a velocity of 2 m s⁻¹ and data are collected for about 8 days from both a Doppler and a ranging system, a conservative estimate is that the mass can be measured to just better than 15%.

Leeuwenhoek's specimens discovered after 307 years

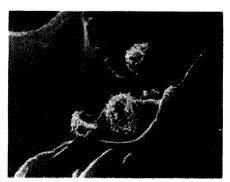
from Brian J. Ford

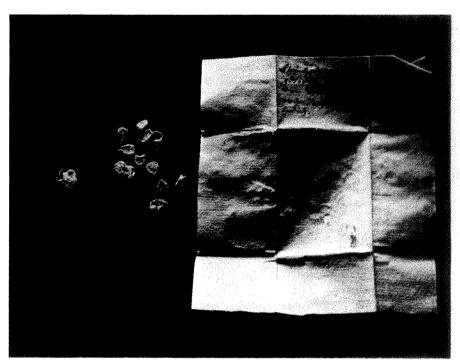
AFTER not being seen since 1674, original specimens sent by the "father of the microscope", Antony van Leeuwenhoek, have been found to be still in existence. A systematic search through the four large files of his letters sent to the Royal Society, revealed nine little envelopes containing original material and fixed to the final pages of three of the letters. Only minute traces of one of the specimens ("tWitte van een schrijff-penne" — white from a writing-pen [quill]) remained, but the remainder were intact. The sectioned material was compressed into compact masses, but otherwise the specimens were in excellent condition.

Antony van Leeuwenhoek (1632-1723) lived and worked in Delft where he produced his own single-lens microscopes. His status as the founder of microscopy derives from decades of dedicated and accurate observation, faithfully recorded in lengthy letters most of which were sent to London. His observations of spermatozoa, bacteria and a host of histological specimens were without precedent. Many workers have since commented on the 'crudity' of his techniques, emphasizing that his material was examined whole, or 'torn apart', and it has become generally accepted that section-cutting did not begin until the middle years of the nineteenth century.

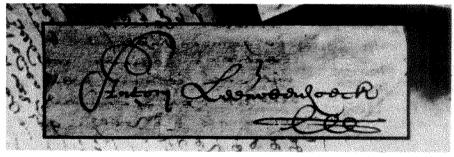
The discovery of his specimens reveals two examples of plant material cut as fine sections. They are "Pit van vlier" — elder pith, and "Kurk" — cork. The conclusions that can be drawn from these specimens about seventeenth-century microtechnique are many (a fuller account by the author appears in the current *Notes and Records of the Royal Society*) but the central finding is that van Leeuwenhoek, working at the dawn of microscopy, could prepare by hand sections that would be acceptable for laboratory use today.

Brian J. Ford is in the Science Unit, Cardiff.

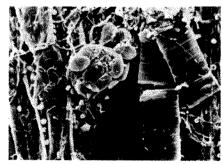




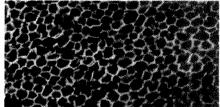
"Stuckjens vande gesicht senuwe van een koebeest over dwars afgesneden" — pieces of optic nerve of a cow cut in transverse section. The specimens were attached to a letter dated 1 June 1674, and signed by van Leeuwenhoek, as shown below.

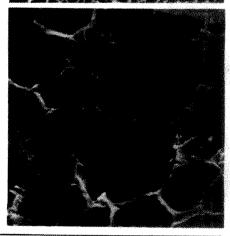


Right, the stereoscan microscope reveals the regular, fine cut of the sections of cork. Though the hand-cutting technique seems primitive, the results are good enough for present-day microscopy. Below right, the sections of cork shown reveal the room-like appearance from which the term 'cell' was derived in 1663.



Left, high-magnification examination of the cell walls reveals occasional fungus spores. Thread-like hyphae occur from time to time in the sections, but have caused no structural change over 307 years. Right, specimens of papery material which van Leeuwenhoek claims arose from water are attached to a letter dated 17 October 1687. Algal cells and diatom chains revealed in this scanning electron micrograph confirm his finding.





Mixed-species foraging groups

from Jared M. Diamond

A bird-watcher is walking through a tropical rainforest, dejected at having seen and heard almost no birds for the last hour. Then a faint sound becomes audible afar, gradually grows louder and approaches. Soon the forest comes alive with dozens of bird species busily foraging together and calling simultaneously. The feverish excitement lasts for a few minutes, dies down as the flock moves on, and the forest is again silent and seemingly devoid of birds. A mixed-species foraging party has passed.

Analogous scenes repeat themselves daily on all the continents and in all the oceans, in the treetops, on the ground and underwater. The most familiar mixed foraging parties involve birds; the most spectacular ones, African open-country grazing mammals. Further examples are being discovered among fish, cetaceans and primates, and in at least six cases birds and primates have been found regularly associated with each other. The growing literature on mixed foraging parties (flocks, herds, or schools) has focused on four main questions. Are flocks chance aggregations or always composed of the same individuals? Who leads, and who follows? Why do different species associate? Why do the species sometimes resemble each other in appearance and call far more closely than one would expect from their taxonomic relationship?

Recent papers on mixed bird flocks of the neotropics1-4 have revealed how unexpectedly initimately the lives of flock members belonging to different bird species are intertwined. These authors introduced to the study of mixed flocks techniques for recognizing individual birds by capturing, colour-banding and releasing them. Using these methods they found that the core of a flock consists of individuals of different species that forage together daily for many years, probably for their whole adult lives. Up to a dozen core species are each represented by one pair or family, whose territorial boundaries coincide among the core species and which may roost at night in the same tree. Even territorial confrontations are shared: when one flock happens to meet the neighbouring flock at the territory boundary, the Bluish-slate Antshrikes, Dusky-throated Antshrikes, Dot-winged Antwrens and White-flanked Antwrens of one flock simultaneously display and call to their conspecifics in the other flock. Individuals of additional flock species with smaller territories than the core members join the flock only when it enters their territory. Species with larger territories than the core members forage first with one flock, then with a neighbouring one a few hours later. Some species switch between

two distinct types of flock that occur in the same area: a slowly moving flock in the understory basically consisting of insectivorous antbirds, and a rapidly moving flock in the canopy of omnivorous tanagers and honeycreepers.

Are some species leaders and others followers? Usually there is a leader species (the 'nuclear species'), which is conspicuous in its plumage, frequent vocalizations and nervous behaviour such as wing- or tail-flicking, and which forages in groups of several individuals⁵. Nuclear species of bird flocks are often tits in Europe, North America and west Africa. babblers in tropical Asia, thornbills and fairy-wrens in Australia, gerygone warblers in New Guinea, and antbirds and tanagers in the neotropics (see, for example, ref. 6). Other species follow the conspicuous noisy whirlwind of the nuclear species group. In most bird-mammal associations, the mammal is the leader, the bird the follower, but tamaring monkeys of the neotropics are thought to follow tyrannid flycatchers.

Flocking seems to serve several purposes simultaneously, and different motives may predominate in different cases. Some of the leading theories are the convoy theory, gang theory, beater theory, pirate theory and feeding efficiency theory.

Convoy theory. Flocks may be an antipredator device, like the World War ship convoys that were so effective against U-boats^{7,8}. The more individuals in a flock, the more eyes and ears there are to detect a predator, the lower is the chance of a given individual being the victim of an attack, the harder it is for the predator to focus on any target amidst all the activity and the better is the flock able to mob or ward off the predator. Observations that support the convoy theory include that lone birds peer about more, and spend less time feeding, than birds in a flock9. On African plains, near-sighted zebras with acute hearing associate with far-sighted species such as ostriches, wildebeest and giraffes, so that each complements the other's detector capabilities. Near-sighted gleaning birds tolerate the proximity of far-sighted salliers that occasionally rob them of food (antwrens tolerating antshrikes in the neotropics, tits tolerating drongos in Africa): the improved predator detection is worth the lost food.

Gang theory. Flocking may permit flock members access to resources within a territory whose owner would be able to expel the members if they were solitary. Thus a flock is like a gang of marauders overwhelming a victim by their numbers.

Jared M. Diamond is Professor of Physiology at University of California Medical School, Los Angeles. For example, on Caribbean reefs the Three-spot Damselfish can inhibit single Stripe Parrotfish from feeding in its territory, but not parrotfish schools¹⁰. Similar cases are reported for Australian honeyeaters, Australian corvids and North American mockingbirds^{11,12}.

Beater theory. Tiger hunters follow a line of beaters who create a disturbance and flush out tigers. Similarly, some flock members, in the course of their own foraging, flush prey that a different species can capture. For example, gleaning and bark-feeding birds disturb resting insects into flight, where a sallying bird may catch them. Drongos, which are sallying insectivores, are notorious for following large or active animals so that they can catch insects disturbed by these beaters. Beaters used by drongos include elephants, giraffes, buffalo, ostrich, baboons and occasionally humans in Africa; monkeys in Borneo and Celebes; and babblers in New Guinea. In the neotropics, monkeys are used as beaters by the hawk Harpagus bidentatus. Africa's Ground Hornbills follow baboons that are turning over logs. African arboreal monkeys that eat fruit husks are joined by squirrels that eat the fruit kernels, ungulates that eat the fruit falling to the ground and hornbills catching insects flushed out by the monkeys.

Pirate theory. From using other species as beaters to flush out food that the beaters could not take, it is a small step to the role of pirate: seizing food that another flock member has caught. Drongos practice occasional piracy on other birds in New Guinea and African flocks¹³; antshrikes victimize antwrens in neotropical flocks. Why does the victim tolerate the pirate? Probably because the pirate is a noisy, farsighted sallier that detects and warns of predators.

Feeding efficiency theory. Species that flock together have similar diets, although they differ in foraging technique, height and substrate9,14,15. At first it seems in defiance of competition theory that species with similar diets should flock, and that flocking should vary inversely with food density 16,17. Yet flocking promotes feeding efficiency in several ways. The more individuals searching, the more likely is a good feeding patch to be found. Animals can learn new foraging techniques from allospecifics18: Morse saw a Blue Tit fly up to a Lesser Spotted Woodpecker pecking at bark and begin pecking next to it. Perhaps more important, by travelling together. species with similar diets can keep track of where food has already been gathered and is no longer worth seeking¹⁷. Imagine five janitors who clean a large hall and are paid per kilogramme of trash collected. One janitor uses his hands, one a pitchfork, one

a broom, one a vacuum-cleaner and one a carpet-sweeper. Each janitor is better adapted than the others to collecting certain trash items, but there are many items that can be collected by any of several janitors. A good strategy for all janitors is to forage together, thereby constantly assuring themselves of trash-rich areas and avoiding areas partly cleared by other janitors.

For whatever reason species do forage together, it is striking that flock members of widely different families often resemble each other in appearance, vocalizations, or both. As examples of this co-evolutionary phenomenon, termed social mimicry, Moynihan¹⁹ noted that flocking tanagers in the mountains of Panama are mainly black and/or vellow, in the northern Andes brilliant blue or blue and yellow, and in the south-central Andes blue-grey dorsally and brown ventrally. A prime example of this tendency for birds of a feather to flock together is the 'brown and black flock' of New Guinea, first noticed by the flamboyant Italian explorer Count Luigi d'Albertis in 1880 and rediscovered much later by Bell²⁰. The flock involves up to 22 medium-sized, omnivorous bird species from seven families (birds of paradise, babblers, whistlers, drongos, cuckooshrikes, honey-eaters and flycatchers). All these species are either mainly rufous or mainly black; in three, the male is black, the female rufuous. The flock is led by a group of the babbler Garritornis isidori, of which one individual is the actual leader and has a distincitive 'leader' call while his/her conspecifics have a 'follower' call. At least five other species in the flock mimic one or other of the babblers' calls.

Why should species from such different families converge in plumage? We are not dealing here with detailed mimicry of plumage patterns, but merely broad resemblances in overall colour. Moynihan points out that interspecific signals are required so that mixed flocks can form and maintain cohesion. The convergence in these signals may be for economy: it is much easier for a flock member to stay with the flock and avoid wandering off with some non-flock species if it need only remember to look for brown or black birds, instead of keeping 21 different colour patterns in mind.

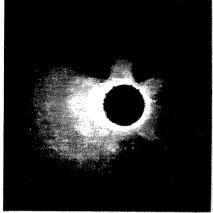
- Gradwohl & Greenberg Auk 97, 385 (1980).
- Munn & Terborgh Condor 81, 338 (1979). Powell Auk 96, 375 (1979).
- Buskirk et al. Auk 89, 612 (1972).
- Moynihan Smithson misc. Collns 143, 1 (1962)
- Bell Emu 80, 227 (1980). Hamilton J. theor. Biol. 21, 295 (1971).
- Pulliam J. theor. Biol. 38, 419 (1973). Morse Ibis 120, 298 (1978).
- Robertson et al. Ecology 57, 1208 (1976)
- Paton Emu 80, 213 (1980).
- Merritt Auk 97, 869 (1980)
- Greig-Smith Ibis 120, 284 (1978).
- Wiley Auk 88, 881 (1971). Austin & Smith Condor 74, 17 (1972).
- Morse Ecol. Monogr. 40, 119 (1970). Cody Theor. Opulat. Biol. 2, 141 (1971).
- Rubenstein et al. Ibis 119, 10 (1977). Moynihan Evolution 22, 315 (1968); Am. Nat. 177, 372
- 20. Bell Emu 76, 95 (1967)



THE CHEMISTRY OF THE SUN

Although in the year 1866 a great many people were familiar with the spots on the sun, those who had been favoured by a sight of a total eclipse, and many more who had read the accounts of total eclipses, knew that there was a great deal more of the sun than one generally sees. From the time of Stannyan, who observed the prominences at Berne, down to the year 1842, let us say, several eclipses had been observed. and very beautiful coloured phenomena had been recorded by different observes. Red things had been seen projecting round the dark moon during the time of eclipse, and although many held them to be beautiful effects produced by the passage of the moon over the sun, or even clouds in the atmosphere of the moon coloured by the strange way in which the solar light then fell upon them, a larger number of people, on the other hand, insisted that these things must really belong to the sun. Now if that were so, it was perfecly clear that we should not be contented with merely observing the chemical nature of the spots.

We have a round dark moon, which in this case is represented as entirely covering the sun; then these different prominences and luminosities, this wonderful set of streamers, or whatever you like to call them, which seem to veil, or to render less distinct, something else which is lying beyond them. You will see here that some of these prominences are red, and others have a yellow tinge, and that, quite independent of the colour of the prominences, we have the most exquisite coloured effects. Sometimes the radial structure is not so marked, and reveals



Eclipse of 1870. Photograph of the corona taken at

indications of structure further away from the sun. You see wonderfully delicate tracery, lines being seen now in one part and now in another. In the photograph taken during the eclipse of 1870 we see that the luminosity of the solar atmosphere was excessively irregular, by which I mean that in one part we get a very considerable excess of light, quite independent of the sharply defined prominences. whereas in other portions the atmosphere of the sun at the same height is not nearly so luminous. Now in none of these cases have we been able to see the thing which struck us most clearly the moment the artificial eclipse system was set at work. It is a good indication of the extreme difficulty of making observations during eclipses, and how important it is that one should have a method which makes us independent of

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Origin of the galaxies

from Joseph Silk

COSMOLOGISTS generally believe that galaxies formed as a consequence of the growth of tiny density fluctuations in the very early Universe. The remarkable uniformity of the cosmic microwave background radiation has provided convincing evidence for the high degree of regularity of the Universe at an early epoch but within the past year two groups of astronomers, at Florence and Princeton, have reported evidence for a very weak anisotropy on an angular scale of 90°, which is probably produced by similar matter fluctuations to those from which the galaxies evolved.

One of the most tantalizing issues in cosmology concerns the origin of these fluctuations. It is thought that the initial conditions of the big bang might provide an answer. Perhaps the Universe is the way it is because of the way it was. Surely the resourceful cosmologist can improve on this hollow echo of creationism, for if the initial conditions were very different, then we would not be here to take stock of the situation. This concept of the observer's role in determining the state of the Universe has been elevated by Robert Dicke and Brandon Carter to the status of a fundamental cosmological principle: the anthropic principle. Its power arises

Joseph Silk is in the Astronomy Department at University of California, Berkeley

because the growth of fluctuations is inevitable from the beginning of time.

Consider the history of a fluctuation destined eventually to form a galaxy. It grew in amplitude throughout much of the early expansion of the Universe. The earliest epoch that the cosmologist can usefully discuss is the Planck instant, a mere 10-43 second after the singularity. At this time, our galaxy-sized fluctuation must have had an infinitesimal but nonzero density contrast. These inhomogeneities are best considered as fluctuations in the spatial curvature, or slight wrinkles in the geometry of space-time. The magnitude of such primordial wrinkles must be about 1 part in 103, if fluctuations in the energy density are considered; for fluctuations in the matter component alone, the associated wrinkles attain a considerably smaller but well defined value. Were the fluctuation amplitudes much smaller, galaxies would not have formed by now; were they much larger, eventual collapse in the early Universe would have formed separate topologically disjoint universes, bearing little relation to our observed universe.

Although this argument purports to show that the initial conditions may have been unique, it certainly does not provide any explanation. One is left with the following conundrum. The very early

Universe was uniform to a high degree. However to form galaxies, fluctuations must have been present at an exceedingly small but non-zero level. This situation is aesthetically unappealing - granted that the early Universe must have been very nearly uniform, one would obviously prefer to begin with a perfectly homogeneous and isotropic expansion, and fluctuations see develop spontaneously at some later epoch. No one has been able to discover a means of generating fluctuations over a sufficiently large scale to form galaxies in the standard big bang cosmology. To some cosmologists, this failure has provided sufficient reason to reject the standard model. Some have opted for an initially cold big bang in the hope that cold matter may be more susceptible to fragmentation. Others have taken a stronger stance, and rejected the big bang entirely.

A fascinating resolution of this cosmological dilemma has been recently proposed by Ya. B. Zel'dovich of the Space Research Institute, Moscow (Mon. Not. R. astr. Soc. 192; 663, 1980) and Alexander Vilenkin of Tufts University (Phys. Rev. Lett. 46; 1169, 1981). Recent applications of elementary particle theory to the very early Universe imply the possibility of a novel mechanism for the spontaneous generation of the seed fluctuations from which galaxies eventually formed. The origin and nature of elementary particles can be understood in terms of a class of theories known as the grand unified theories (GUTs), which seek to unify the electromagnetic, weak and strong nuclear forces into a single theoretical framework. Above the grand unification energy of about 1015 GeV, these fundamental forces of nature all play an equal part, and matter is completely symmetrical in all its properties. The very early Universe provides a natural laboratory for testing GUTs, since particle energies attained then greatly exceed those produced in any manmade particle accelerator. One of the most elegant consequences of GUTs is that they predict (at least in order of magnitude) the observed baryon asymmetry of the Universe. The breakdown of symmetry occurs some 10-35 second after the big bang, when particle energies have dropped below the grand unification energy. This produces a slight excess of particles over antiparticles which is crucial to the existence of an environment in which life could evolve, for the fact that we and our surroundings consist exclusively of matter rather than antimatter implies that in the very early Universe, when the cosmic blackbody radiation copiously produced particle-antiparticle pairs, the amount of matter exceeded that of antimatter by about 1 part in 109. Our very existence is therefore a consequence of GUTs and the breakdown of symmetries as the Universe expanded and cooled, for a Universe which precisely conserved matter-antimatter symmetry would have undergone considerable annihilation; little residual matter (or antimatter) would remain.

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Zel'dovich and Vilenkin argue that GUTs may have profound implications for the large-scale structure of the Universe. The breakdown of symmetry at 10-35 second can be regarded as a phase transition. A useful analogy is an isotropic ferromagnet, which when cooled below a critical temperature (the Curie point) spontaneously develops magnetism. The direction of magnetization is indeterminate and random, and may vary in different regions or domains. The GUTs phase transition may involve the spontaneous generation of a domain structure. The symmetry breakdown is believed to be effected via an intermediary class of particle - the Higgs particle - and its associated fields. These are important in the unification of strong and electroweak interactions, being responsible for the masses of the elementary particles once the symmetrical state is broken. As the Universe cools, the symmetry breakdown occurs because particles and radiation are no longer energetic enough to create the full panoply of states of all types of particle predicted by GUTs. The end state of the matter is characterized by a vacuum state of lowest energy. In the classical physics regime, the vacuum has approximately zero energy, although small fluctuations are present. The fluctuations are variations in the energy of a reservoir of virtual pairs of particles and antiparticles that are released under extreme conditions of temperature or gravitational stress. The vacuum state after the GUTs phase transition has a finite energy density associated with the Higgs field. It is believed that the Universe began with a very large vacuum energy density of almost equal magnitude but opposite sign, forming a false vacuum state, so that one ends up after the phase transition in the true vacuum of zero energy.

In fact, the true vacuum state is not unique. Although the minimum energy state of the Higgs field has a universal value, implying the same energy density everywhere, the direction of the associated field is indeterminate. Multiple true vacuum states are possible, depending on the degree of complexity of the Higgs field. The phase transition can result in the formation of different domains of vacua, corresponding to the multiple vacuum states. The direction but not the magnitude of the Higgs vacuum expectation value differs in each domain. The domain boundaries are characterized by gradients in the Higgs field, and therefore contain energy density; they also consist of residual false vacuum that has not undergone the phase transition. When the Higgs particles have decayed, the Universe contains the remnants of these boundaries in the form of topological structures of false vacuum that may contain a very considerable energy density.

Theory indicates that possible forms for

the domain boundaries are topological discontinuities that can be either two-, one-, or zero-dimensional in character. There are two-dimensional walls, one-dimensional strings, or monopoles, which are point-like singularities. An important characteristic of the energy density associated with the monopole's structure is that it effectively behaves as a rest-mass, and assumes a progressively more important gravitational role as the radiation is redshifted away. The walls and strings suffer very little friction from the ambient radiation and matter, and the tendency of these initially highly convoluted structures to straighten out causes them to move at near the speed of light on the horizon scale. Density fluctuations are generated on and below the horizon scale, and larger and larger fluctuations develop as the horizon scale grows.

In fact, expanding walls can be excluded by simple astrophysical considerations. Zel'dovich, Kob'zarev and Okun pointed out (Zh. Eksp. Teor. Fiz. 67; 3, 1974) that such walls should be extremely massive, and would grossly perturb the cosmic microwave background radiation. However strings are quite another matter. Their one-dimensional structure implies that their influence in the present epoch is relatively modest. Strings may still lead to sizable density fluctuations. The process of generating density fluctuations continues after the phase transition at 10⁻³⁵ second, perhaps until the present epoch if the strings can survive. At formation, the strings are very tangled, and both infinitely long strings and closed loops are present. Dissipation tends to reduce the tension in the strings on scales smaller than the horizon, and the strings gradually straighten out. New strings are likely to form as intersecting strings form closed loops; this provides a principal means of straightening the strings. The expansion of the Universe stretches the strings on scales larger than the horizon, and the characteristic dimension of a string at any enoch is in fact the horizon scale.

The density fluctuations generated by the strings can provide the seeds for galaxy formation. The mass of a galaxy is first contained within the horizon at an epoch of about 10 yr and the mass of a galaxy cluster after about 104 yr. Strings will be present on the horizon scale moving at light speed and their gravitational pull will induce density fluctuations. Zel'dovich argues that the density of infinite strings remaining from the GUTs phase transition amounts to about 0.1 per cent of the mean cosmological density. This implies that the expected amplitude of density fluctuations is of the order of 0.1 per cent. Such fluctuations survive the radiation even when their growth is inhibited by the frictional effect of radiative viscosity. After the decoupling epoch, the fluctuations grow in amplitude uninhibited by the radiation, accreting the surrounding matter. Galaxy formation eventually occurs once the fluctuations become of very large amplitude and collapse.

How inevitable is such a scenario? It seems that in general types of GUT, these topological singularities (either walls, strings or monopoles) will form as the symmetry breakdown occurs, depending on the degree of complexity of the Higgs field. The only requirement is that the Higgs field possess a finite correlation length, beyond which its expectation value in different locations is completely uncorrelated. This provides our best hope for understanding galaxy formation in an initially homogeneous and isotropic universe. Galaxies may therefore have originated from a tangle of strings spontaneously produced some 10-35 second after the big bang. The strings are best visualized as topological knots of high energy density that may be either of infinite length or closed loops. As the Universe expands, the strings untangle, continuously generating sizable density fluctuations via their gravitational effects on scales less than the instantaneous horizon scale.

Cosmologists are not entirely convinced by this scheme, since it involves rather specific assumptions about elementary particle theory concerning the nature of the Higgs field that have not received widespread acceptance. One difficulty is that not only strings are produced by the GUTs phase transition. Massive monopoles are

predicted by the simplest GUTs, and these particles could dominate the present mass density of the Universe, contrary to observation, unless the theory of the very early Universe is carefully adjusted. Perhaps strings will only meet universal acceptance if more direct proof of their existence is forthcoming. This is most likely to emerge from their effect on electromagnetic radiation, in the large-scale angular anisotropy of the cosmic microwave background radiation. Another intriguing possibility is that the gravitational lenses, recently identified with close pairs of quasars having strong spectral similarities, may be due to the gravitational deflection of light by cosmological strings.

Bose-Fermi equivalence and soliton theory in solid-state physics

from R. K. Bullough

NONLINEAR oscillators and nonlinear wave mechanisms are providing the key to our understanding of an increasing number of fundamental processes in physics, chemistry and biology. Some years ago nerve axon pulse propagation was modelled by the nonlinear Hodgkin-Huxley or FitzHugh-Nagumo equations. Now nonlinear wave equations are providing insights into, for example, the way chemical concentration waves may implement the changes in anatomical structure demanded by a mutant gene, or cell division in the emerging embryos of sea urchins.

Although most of these nonlinear systems can be handled by the theoretician only by use of large computing machines, there is a large and growing class of nonlinear wave equations which so far have been applied in theoretical physics rather than theoretical biology, and which can be solved analytically, that is, in terms of the familiar mathematical functions. Many of these systems have soliton solutions—the subject of this article.

A word first on nonlinearity; even at school we learn to write down the equation of motion for the angular displacement u of a simple pendulum in the form $Ml d^2u/dt^2 = -Mg \sin u$. Then immediately linearize this equation by replacing $\sin u$ by u and so gain the harmonic oscillator equation d^2u/dt^2 + (g/l)u = 0 which has $\cos(g/l)^{1/2}t$ or $\sin (g/l)^{1/2}t$ as solution. But the linearized theory is applicable only if u is small. So what happens if the pendulum is rigid and can turn right over so that u is 2π or bigger? And what is the situation if we couple such oscillators together in a regular array to form a model crystal? In linear approximation we gain the socalled Klein-Gordon (KG) equation, $u_{xx} - u_{tt} = m^2 u$, which is familiar (the

notation is the convenient one $u_{xx} \equiv \frac{\partial^2 u}{\partial x^2}$, etc. and m here is a number playing the part of a mass). But the nonlinear analogue is the sine-Gordon (s-G) equation $u_{xx} - u_{tt} = m^2 \sin u$ (the name is no accident!). The s-G has soliton solutions but the KG does not.

This word 'soliton' was coined by Norman Zabusky and Martin Kruskal (Phys. Rev. Lett. 15; 240, 1965) who were concerned with a very different-looking wave equation, the Korteweg-de Vries (KdV) equation, $u_t + 6uu_x + u_{xxx} = 0$, and in essence they observed that its solitary $u = \frac{1}{2}V \operatorname{sech}^2 \{\frac{1}{2}V^{1/2}\}$ solution. (x - Vt) asymptotically preserved (or exchanged) both its form and its speed V in collision with other such solitary waves travelling at different speeds. Because of this particle-like property, these solitary waves were dubbed solitons. We now know that this behaviour typifies a whole class of nonlinear wave equations, outstanding examples being the KdV, the modified KdV (MKdV) $v_1 + 6v^2v_x + v_{xxx} =$ 0, the nonlinear Schrödinger equations (NLS) $i\psi_t = \psi_{xx} \pm 2\psi |\psi|^2$ (with ψ complex) and the s-G. Clearly the solutions of such equations could be superb examples of 'elementary excitations' in some context of many-body physics.

With this in mind, solid-state physicists, in particular, have been quick to use solitons to explain the high, anisotropic and essentially one-dimensional, non-ohmic electrical conductivity of certain organic polymers like TTF-TCNQ at temperatures $T \sim 3$ K, or the central peak observed in the neutron-scattering spectrum from crystalline SrTiO₃ in the displacive regime, or the central peak in the spec-

R. K. Bullough is Professor of Mathematical Physics in the Department of Mathematics, The University of Manchester Institute of Science and Technology. trum of the effectively one-dimensional ferromagnetic crystal CsNiF₃ for $T \approx 10$ K, and so on. Already in 1978 the conference report 'Solitons and Condensed Matter Physics' (eds A. R. Bishop and T. Schneider; Springer) was able to review a wide range of such applications.

The physical insight displayed in many of these investigations is admirable. Still it seems a pity that the word soliton is so often misused: sometimes it simply means a solitary wave (a pulsed solution with argument (x - Vt)) or even just a lump; jacobian elliptic functions like cn(x; k) (which tends to $\cos x, k \to 0$, or $\operatorname{sech} x, k \to 1$) are called 'multisolitons' or a soliton 'lattice'. However, there is a mathematical structure surrounding the solitons and their collision properties which does not surround the simpler solitary waves; and this mathematical structure may be relevant to the physical problem in hand.

A case in point is the interesting paper entitled 'Soliton Lattice in Polyacetylene. Spin-Peierls Systems and Two-Dimensional Sine-Gordon Systems' by Baruch Horovitz (Phys. Rev. Lett. 46; 742, 1981)—as well as a frequent misuse of the word soliton in this way in a series of papers by K. Maki and his collaborators who are referenced in the Horovitz paper. In his paper, Horovitz treats three physical systems: a fermion system concerned with the insulator-to-metal transition at ~1% doping observed in polyacetylene, which is explained in terms of a charge carrying soliton 'lattice' or soliton 'droplets' of that lattice; a 'lock-in' phase transition in a boson system engineered by quantum corrections to the classical s-G: and the first-order phase transition observed in the magnetic spin system TTF-BDT (Cu) [tetrathiafulvalene-biscis - (1, 2 - perfluoromethylethylene - 1,,2 dithiolato)-copper] in a strong magnetic field (~120kG) at low temperatures

(~10K). The attraction of the analysis is that all three very different physical problems are shown to be mathematically equivalent. So it is a pity, and I think misleading to the physics, that the author does not make proper use of the mathematical structure surrounding the theory of solitons.

In his first example the author uses Peierls's idea (subsequently extended by Fröhlich as an incipient theory of superconductivity) that the electrons in a onedimensional lattice with a half-filled band can lower their energy if a gap in the electron energy spectrum is induced by a suitable periodic distortion of the ion lattice. If the lattice distortion moves (as a phonon), the ions' charges stay locked to their sites; but if the lattice and its distortion have incommensurate periods, the electrons can travel freely with this phonon and constitute a resistanceless current. In practice this moving 'condensate' is pinned—typically by a periodic potential like $\cos \phi(x, t)$ depending on the condensate phase ϕ . In this particular case the current is carried by the soliton 'kink' 'antikink') solutions, (or $4 \tan^{-1} \left[\pm \exp \left\{ m \left(x - Vt \right) / (1 - V^2)^{1/2} \right\} \right]$ of the s-G. Such a theory describes the nonohmic conductivity of TTF-TCNQ for T < 4.2K quite well (see Cohen et al. Phys. Rev. Lett. 37; 1500, 1976; and Trullinger et al. Phys. Rev. Lett. 40; 206, 1978, and earlier papers). Note that the kink $\phi = 0$ as $x \rightarrow -\infty$, but jumps rapidly from 0 to 2π in a small region of order m^{-1} about (x -Vt); the antikink jumps from 0 to -2π . Horovitz looks for a periodic lattice of kinks and antikinks in which the lattice ion displacement field $\Delta(x)$ swings (on some scaling) between $\pm 2\pi$ about the lattice sites. A sequence of s-G kinks and antikinks might describe just such a situation; however, such a multisoliton solution of the s-G at rest is not stable.

The mathematical problem in all three cases can be reduced to solving a Diractype eigenvalue problem for two-component spinor eigenfunctions $\psi_n(x)$ in the presence of the ion displacement field $\Delta(x)$ such that the total energy $E_0 = \Sigma' \varepsilon_n + \int \mathrm{d} x \, \Delta^2(x)/2 \, n \lambda$ is minimized; the $\Sigma' \varepsilon_n$ is the contribution of the eigenenergies of occupied electron states and λ a coupling constant. The eigenvalue problem takes the form $\hat{L}\psi_n(x) = \varepsilon_n\psi_n(x)$ in which \hat{L} is a Hermitean 2×2 matrix differential operator: $\hat{L} \equiv i\sigma_3 \partial/\partial x + \Delta(x)\sigma_1$ where σ_3 and σ_1 are Pauli matrices.

Experts in soliton theory will immediately recognize this eigenvalue problem as essentially that used by Zakharov and Shabat to solve the NLS in 1972. Likewise it solves the MKdV $v_t - 6v^2v_x + v_{xxx} = 0$. It looks uninteresting for solitons because the eigenvalues must be real (\hat{L} is Hermitean) and we know that solitons are associated with complex eigenvalues. However, the point of all this here is the following: it is known that for the MKdV

(or for the NLS or the s-G for that matter) a 2×2 matrix \hat{B} depending on v, v_x, v_{xx} and the ε_n can be found so that the MKdV equation is equivalent to the operator equation

$$\hat{L}_{t} = [\hat{B}, \hat{L}] \equiv \hat{B}\hat{L} - \hat{L}\hat{B}$$
 (1)

The pair of matrix operators (\hat{B}, \hat{L}) which makes equation (1) the MKdV is the familiar Lax pair for the MKdVnamed after Peter Lax who first found this form for the KdV in 1968. To introduce it here we have extended $\Delta(x)$ by letting it depend on the parameter t and set $\Delta(x, t) \equiv v(x, t)$ so that $\Delta(x, t)$ follows the MKdV 'flow'. It is easily checked that equation (1) is the MKdV flow if, and only if, the ε_n s do not depend on t even though the scattering potential $\Delta(x, t)$ in \hat{L} and the eigenfunctions ψ_n now do so. The MKdV is thus an isospectral flow in this sense. If a nonlinear equation like the MKdV can be put in the form of equation (1), we can expect to solve it for appropriate boundary conditions by the so-called inverse scattering method. This method generalizes the Fourier transform for linear problems to the present class of nonlinear problems via the isospectral transform $\hat{L}\psi_n = \varepsilon_n \psi_n$. If the equation has soliton solutions we can find them through the complex eigenvalues ε_n in this way.

The key point is that the ε_n s remain stationary under the flow. Thus if $\Delta(x+t)$, of single argument x+t, is a particular solution of the MKdV for any t, we might guess that it will minimize the contribution of the electron eigenenergies ε_n to the total energy E_0 for all t; furthermore, we know that under the MKdV flow with appropriate boundary conditions, $\int v^2 dx \equiv \int \Delta^2 dx$ is also a constant of the motion. Thus we guess that $\Delta(x+t)$, or scaling t, $\Delta(x+\gamma t)$ minimizes E_0 for an appropriate choice of γ .

Horovitz took a different route: he converts the spinor eigenvalue problem to a Schrödinger problem with scattering potential $\Delta^2 - \Delta'(x)$ where $\Delta'(x) \equiv \mathrm{d}\Delta/\mathrm{d}x$. He argues, incorrectly in my view, that this is of KG type and uses results in perturbation theory taken about the s-G equation by linearizing this equation about its kink solution to set $\Delta^2(x) - \Delta'(x) = U''(\phi(x))$ and to infer that $U(\phi(x)) \equiv \Delta_1^2(1-\cos(\phi(x)))$. This potential is the s-G potential in its Hamiltonian

$$H = \int \left\{ \frac{1}{2} \phi_{x}^{2} + \frac{1}{2} \phi_{x}^{2} + m^{2} (1 - \cos(\phi(x))) \right\} dx$$
 (2)

so Δ_1 is a mass m (Hamilton's equations for a field $\phi(x,t)$ generate the s-G from equation (2)).

Those familiar with the history of soliton theory will, however, see in the transformation $\Delta^2(x,t) - \Delta(x,t)_x = u(x,t)$ the celebrated Miura transformation which moves a solution $\Delta(x,t)$ of the MKdV to a solution u(x,t) of the KdV $u_t - 6uu_x + u_{xxx} = 0$! It is a curious fact, associated

more with the theory of jacobian elliptic functions than with solitons, that the KdV has the solution $u(x + \Delta_1^2 t) \equiv u(\xi)$ given by $u(\xi) = \Delta_1^2 \cos \phi(\xi)$ providing $\phi'(\xi) =$ $2\Delta_1 \sin \left\{\frac{1}{2}\phi(\xi)\right\}$ so that $\phi'' = \Delta_1^2 \sin \phi$. Certainly this is the equation of motion (in the 'time' variable $\xi = x + \Delta_1^2 t$) of a pendulum capable of large angular displacements; and it has the static kink solutions $\phi = -\pi + 4 \tan^{-1} \exp \Delta_1 \xi$ in which the pendulum turns from upside down very slowly through angle 2π back to upside down. Nonetheless, it derives from a wave equation, the MKdV, very different from the s-G, and the physical associations it conveys are also very different.

Of course, like a good physicist, Horovitz still finds a correct mathematical solution for his lattice which for large period does indeed behave like a series of alternating kinks and antikinks of the s-G. He then finds that this lattice is stable for large doping levels (large values of the phonon-electron coupling constant λ) but becomes unstable for small λ . The formation of droplets of this lattice is suggested to herald the observed insulator-to-metal transition observed in polyacetylene.

In a similar way his so-called bose problem appeals to the fact that if the s-G hamiltonian equation (2) is linearized about a kink and the resulting small oscillations quantized as 'phonons', this quantum correction is essentially the energy of the fermion soliton lattice with a change in sign. Thus there is a first-order phase transition as the coupling constant λ is reduced. Similar considerations apply to his spin problem where $\pm \frac{1}{2}$ spins are carried by the solitons.

The moral of this interesting paper seems to be that good physical insight leads to good physics. But soliton theory is now so important in many areas of nonlinear theoretical physics that a good understanding of it has become an essential technical prerequisite for the practising theoretician. This is particularly true in high-energy physics where mathematical structure may be the only guide. It is a remarkable fact that the so-called spin $-\frac{1}{2}$ xyz lattice model can be mapped exactly, in the continuum limit of zero lattice spacing, onto the massive Thirring model (a relativistically co-variant fermion field theory) and that this maps exactly onto the quantized s-G model (a co-variant boson theory). This formal fermion-boson equivalence, as well as the remarkable mass spectrum of the quantized s-G, has stimulated much work on nonlinear field theories, especially the gauge and supersymmetric gauge theories, in high-energy physics. It has also recently stimulated a quantum version of the inverse scattering method for solving some of these quantized problems exactly. Apparently it was these ideas on fermion-boson equivalence that motivated Horovitz's line of analysis in his interesting paper on solid-state physics.

REVIEW ARTICLE

Actions of cholera toxin and the prevention and treatment of cholera

Jan Holmgren

Institute of Medical Microbiology, University of Göteborg, S-413 46 Göteborg, Sweden

The drastic intestinal secretion of fluid and electrolytes that is characteristic of cholera is the result of reasonably well understood cellular and biochemical actions of the toxin secreted by Vibrio cholerae. Based on this understanding it is possible to devise new techniques for the treatment and prophylaxis of cholera to complement those based on fluid replacement therapy and sanitation.

CHOLERA patients have characteristically watery diarrhoea which leads to dehydration and metabolic acidosis. If untreated, this fluid loss rapidly leads to death. The disease is caused by an intestinal infection with *Vibrio cholerae*. These bacteria adhere to and colonize the small intestine and secrete an exotoxin—cholera toxin—that binds to receptors on the mucosal cells and stimulates intestinal adenylate cyclase activity. The resulting increase in cyclic AMP then causes diarrhoea and fluid loss by inhibiting uptake of sodium chloride by the villi as well as by stimulating active chloride secretion by crypt cells¹.

Koch, who identified *V. cholerae* as the causative agent of cholera, had in 1887 already proposed that the disease was toxin-mediated but it was not until 1959 that the Indian scientists De² and Dutta³ convincingly demonstrated the existence of a cholera toxin. This was purified^{4,5}, and its effect on the adenylate cyclase-cyclic AMP system soon established⁶⁻¹⁰. Since then, activation of adenylate cyclase by cholera toxin has been shown to occur in most mammalian cell types, the structure-function relationship of the toxin has been defined, the cell membrane receptor identified and, most recently, the mode of action of the toxin on adenylate cyclase explained in considerable detail.

This knowledge of the cholera toxin has made it a useful tool for cell biologists, biochemists and physiologists interested in aspects of cell membrane and cyclic nucleotide research that are essentially unrelated to cholera. It has also suggested various possibilities for novel approaches to prevention and treatment of disease. My main intention here is to describe the cellular action of cholera toxin and to discuss the possible exploitation of this knowledge in the design of vaccines, receptor-prophylactic agents, and antisecretory drugs against cholera.

The cholera toxin molecule

Cholera toxin is a protein with two types of subunit: a single 'heavy' subunit of molecular weight (MW) 28,000 noncovalently attached to a 58,000-MW aggregate of 'light' subunits (Fig. 1).

The demonstration that choleragenoid, a protein immunologically related to cholera toxin and able to bind to intestinal epithelium without having toxic activity⁴, contains the same 'light' subunits as the toxin but lacks the 'heavy' subunit strongly suggested that the 'light' subunits are responsible for cell binding (B subunits) and the 'heavy' subunit for the direct toxic activity (A subunit)^{11–14}. Experiments with toxin subunit fractions, prepared by gel filtration in acidic buffer and dialysed to allow renaturation and reassociation of subunits, confirmed that B subunit bound strongly both to the cell and to the isolated cell receptors, but was nontoxic. Purified A subunit neither bound to nor was toxic for intact cells. However, A reassociated with B had both binding and toxic activity in various whole-cell systems ^{11–13,15,16}.

The requirement for membrane binding by the B subunits can be circumvented by using disrupted cells; in these conditions purified A subunit and also its A_1 fragment (see Fig. 1) can activate adenylate cyclase^{17–19}. Furthermore, the characteristic lag period of 10–60 min observed in intact cells before any effect of cholera toxin on adenylate cyclase is seen^{6–10}, essentially disappears in broken cells. Reduction of the disulphide bond between the A_1 and A_2 regions, but not the physical separation of the two fragments, seems to be necessary for the activity of A on adenylate cyclase²⁰.

The cholera toxin receptor

The first event in the action of cholera toxin on cells is the rapid, tight binding to receptors on the cell surface. Studies with $^{125}\text{I-labelled}$ toxin have shown that binding occurs almost instantaneously, is saturable and initially reversible 15,21 . The number of binding sites per cell varies widely with the cell type but affinity of binding varies very little $(K_{\rm A} \sim 1 \times 10^9 \, {\rm mol}^{-1})$ indicating that the receptor is the same for various cell types $^{21-23}$.

It is now known that the membrane receptor for cholera toxin is a specific ganglioside (Fig. 2). Van Heyningen $et\,al.^{24}$ observed that a crude ganglioside mixture inactivated cholera toxin; J.H. $et\,al.^{25}$, Cuatrecasas²¹ and King and van Heyningen²⁶ showed that this inactivation resulted from specific binding between the toxin and a single ganglioside, G_{M1} . G_{M1} neutralized cholera toxin in about equimolar proportions and gave a specific precipitation band with cholera toxin in gel-diffusion tests²⁵.

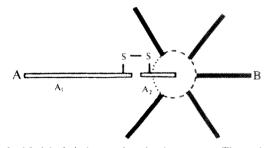


Fig. 1 Model of cholera toxin subunit structure. The toxin has one 28,000-MW A subunit and five 11,600-MW B subunits $^{11-16,29,62,102-107}$. The B subunits, which contain 103 residues 9 and 86 (refs 105, 106), are aggregated in a ring by tight, noncovalent bonds. The A subunit is linked to and partially inserted in the B ring through weaker noncovalent interactions 45 . A subunit, although synthesized as a single polypeptide chain (one gene) 108 , is usually 'nicked' between its two cysteine residues by bacterial protease(s) and thus splits into fragments A_1 (MW \sim 21,000) and A_2 (MW \sim 7,000) when treated with thiol-reducing agents $^{11-15,62}$. Reduction of whole toxin releases the A_1 fragment from the A_2 -5B complex, indicating that A is attached to the B ring by its A_2 portion 102,109 . Electron micrographs show the outer diameter of the B ring to be 90–100 Å, and the size of the separated A and B subunits as \sim 35 ×55 Å and \sim 24 × 30 Å (refs 110, 45).

Subsequent studies in several laboratories have provided further evidence that ganglioside $G_{\rm M1}$ is the natural biological receptor for cholera toxin.

- (1) Studies of various cell types, including small intestinal mucosal cells of different species, have demonstrated a direct relationship between the cell content of $G_{\rm M1}$ and the number of toxin molecules that the cells can bind^{22,27,28}. In each cell type studied there have been about five molecules of $G_{\rm M1}$ per toxin binding site²⁷, which supports data from *in vitro* fixation studies²⁹⁻³² that each toxin B subunit binds to one $G_{\rm M1}$ molecule.
- (2) Exogenous G_{M1} ganglioside can be incorporated into the cell membrane, there to act as a functional receptor. This was first shown by Cuatrecasas³³, who observed an increased cholera toxin-binding capacity and lipolytic responsiveness of fat cells which had been soaked in G_{M1} . Using ³H-labelled G_{M1} , J.H. et al. ²² demonstrated the incorporation of G_{M1} into epithelial membrane of small intestine from humans and other species and showed that the increase in G_{M1} was associated with a corresponding increase in the capacity of the intestine to bind cholera toxin. In vivo tests in rabbits showed parallel increases of G_{M1} and susceptibility of the gut to the diarrhoeogenic action of the toxin²². Incorporation of G_{M1} into transformed cells deficient in this ganglioside has restored cell responsiveness to cholera toxin^{34,35}.
- (3) Pretreatment of cell membranes with cholera toxin has been found to block specifically the membrane $G_{\rm M1}$ from reacting with galactose oxidase³⁶.
- (4) Incubation of certain tissues with V. cholerae sialidase increases the number of toxin-binding sites in proportion to the additional $G_{\rm M1}$ produced by the enzyme from more complex gangliosides in the membrane; cellular sensitivity to the toxin is simultaneously enhanced^{27,113-115}. However, V. cholerae sialidase has failed to create new receptors for cholera toxin in intestinal epithelium²² even though after extraction, the intestinal gangliosides are normally hydrolysed by this enzyme²². Thus intestinal epithelium seems to possess a means of preserving its structural integrity from enzymatic attack.
- (5) Chemical modifications of cholera toxin by various reagents have consistently and proportionally affected binding to cells and to plastic-adsorbed G_{M1} ganglioside³⁷.

Membrane penetration and activation of adenylate cyclase

As already indicated, the lag between cell binding of cholera toxin and activation of adenylate cyclase mainly reflects the time taken for the toxin A subunit to penetrate the cell membrane. In comparison, the generation of A₁ and the subsequent effect on adenylate cyclase are rapid events that occur in <1 min (ref. 17). The cell penetration process is poorly understood; the time it takes is markedly influenced by the incubation temperature and the composition of the cell membrane (J.H. et al., unpublished results). Lateral diffusion and capping of cholera toxin in the membrane have been demonstrated and have been proposed to be critical for action of the toxin^{38,41}. Lateral diffusion seems to have a much greater influence on the lag time in $G_{\rm MI}$ -poor cells than in those with more receptors. Thus, cell incorporation of exogenous G_{M1} significantly shortened the lag period in C6 cells which bind 7,000 toxin molecules per cell⁴², while no such effect was seen for mouse thymocytes (J.H. et al., unpublished results) which bind 150,000 toxin molecules per cell and exhibit a much shorter lag period³⁷

Moss et al.⁴³ and Tosteson and Tosteson⁴⁴ have shown that both cholera toxin and its B region can create pores in synthetic lipid bilayers containing G_{M1} ; however the observed capping of cholera toxin in viable cells suggests that the initial G_{M1} -toxin complex makes contact with an integral membrane protein. As discussed in detail elsewhere⁴⁵, the association of toxin with the membrane G_{M1} receptors might induce a conformational change in the B subunits, resulting in exposure of otherwise hidden hydrophobic B-subunit regions which then fuse with hydro-

phobic protein or lipid components of the plasma membrane. This could result in entry of the A subunit into the cell by one of at least three possible mechanisms. First, it is possible that the B subunits form a hydrophilic channel through the membrane, allowing A to pass; second, a channel for A subunit, may be built by the B subunits and an integral membrane protein; the third possibility is that a hydrophobic interaction between the A subunit and an integral membrane component may transpose A to the inner face of the membrane where intracellular glutathione would reduce the A_1 - A_2 disulphide linkage, thereby releasing A₁. Membrane-bound cholera toxin is partly endocytosed^{27,46}. Recent studies have suggested that for diphtheria toxin (the action of which resembles that of cholera toxin although the metabolic and clinical effects are very different⁴⁷), adsorptive endocytosis is required for membrane penetration and cytotoxicity at physiological pH; lysosomotropic agents protect the cells, probably by neutralizing the acid pH in lysosomes, thus preventing the acid-dependent conformational change of endocytosed toxin in secondary lysosomes that is necessary for the translocation of the diphtheria toxin A fragment^{48,49}. Lysosomotropic agents, such as ammonium chloride and chloroquine, should also be tested for their effect on the activity of cholera toxin.

The intracellular biochemical events that lead to activation of adenylate cyclase are known. Gill⁵⁰ showed that, in broken cell preparations, activation depends on NAD, undefined cellular cytosol factors and ATP, in addition to the A₁ fragment and cell membrane. Moss *et al.*⁵¹ showed that cholera toxin, like diphtheria toxin, has ADP-ribosyltransferase activity, that is, it catalyses the reaction:

NAD + acceptor protein \rightarrow ADP-ribose-acceptor protein + nicotinamide + H⁺

The protein that is ADP-ribosylated by cholera toxin has recently been identified as the guanyl nucleotide-binding component of the membrane-bound adenylate cyclase 52.53. Cassel and Selinger 4 have shown that adenylate cyclase is active while GTP is bound to the GTP-binding component but reverts to an inactive state as GTP is hydrolysed to GDP by GTPase; cholera toxin blocks the GTPase action which stabilizes adenylate cyclase in an active conformation. Alternatively, cholera toxin may stimulate adenylate cyclase by enhancing an exchange reaction in which stimulatory GTP replaces inhibitory GDP at a rate higher than that of hydrolysis of GTP to GDP 55

B subunit cholera vaccine

Can recent knowledge about cholera toxin be used to improve prevention or treatment of disease? Although the development of the highly successful water and electrolyte substitution therapy was essentially an empirical process, three rather more specific approaches have given promising results in animals and are now being used in clinical trials.

The first of these is the development of an oral cholera vaccine based on enterotoxin B subunit. In contrast to clinical cholera disease, which gives rise to long-lasting immunity. the wholecell cholera vaccines give only partial immunity for <6 months. Animal studies have shown that antitoxic and antibacterial cholera immunity cooperate synergistically in the gut giving a multiplicative protective effect by interfering with separate pathogenic events—toxin binding and bacterial adhesion and colonization. The whole-cell vaccines probably fail both because they lack any toxin-derived antigen and because the injection route may be relatively inefficient in stimulating local immunity in the gut mucosa.

Purified cholera B subunit, which spontaneously reassociates to the pentamer ring⁶⁰, is a logical 'toxoid' immunogen against cholera, especially for oral immunization. It is immunologically unrelated to A subunit^{13,29,62} and is a much stronger immunogen⁶³. Furthermore, isolated antibodies to B subunit have considerably higher cholera toxin-neutralizing activity than antibodies to the A subunit^{63,64}. The separation of B

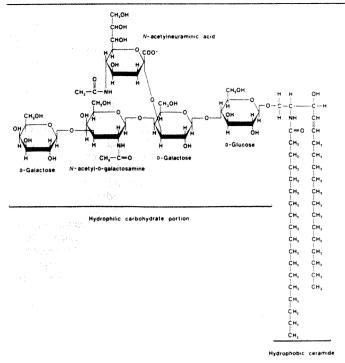


Fig. 2 Structure of the cholera toxin receptor, G_{M1} ganglioside. The oligosaccharide moiety of G_{M1} carries the binding determinants for cholera toxin, and studies have shown that both the terminal galactose and the sialic acid residues, positioned as in G_{M1} , are required for receptor activity 25,11 ; the removal or masking of any of these residues effectively inhibits the cholera toxin binding activity 25 . Decarboxylation of the sialic acid residue destroys the receptor activity of G_{M1} (ref. 30 and L. Svennerholm et al., in preparation), while oxidation with sodium periodate or exchange of the N-acetyl group by N-glycolyl does not affect activity (ref. 34 and L. Svennerholm et al., in preparation). The lipid moiety of G_{M1} is also important. A sufficiently long hydrocarbon chain is needed for stable fixation of cholera toxin 31,112 . Lyso- G_{M1} , in which the fatty acid has been replaced by an acetyl group, has been shown both to have intact toxin-binding activity 31 and to be even better than G_{M1} in promoting subsequent toxin-membrane interactions necessary for activation of adenylate cyclase 34 .

subunit from A excludes any risk of reversion to toxicity but does not lead to significant loss of protective antigen determinants⁶⁰. B subunit is particularly well suited to oral immunization because it retains the ability to bind to the intestinal epithelium, which has been shown to be important for stimulating mucosal immunity in animals, including local immunological memory⁶¹. Finally, as will be further discussed, B subunit given orally may also provide nonimmunological protection by blocking receptors before stimulating local immunoglobulin A (IgA) antitoxin formation. Purified B subunit is a strong protective antigen against experimental cholera in rabbits when given either alone or in combination with somatic antigens⁶⁰. It was recently tested⁶⁵ for its ability to stimulate mucosal immunity in humans; a single oral administration stimulated a marked local secretory IgA antibody response in 80% of the recipients. The response was comparable to that evoked in cholera patients by the natural disease⁶⁶. In further studies, two oral immunizations with a mixture of B subunit and whole-cell vaccine have produced secretory IgA antibody responses in intestine both to the toxin and the bacterial cell-wall lipopolysaccharide antigens in almost all people vaccinated⁶⁷. These results are promising but protective efficacy of an oral combined B subunit-whole cell vaccine in people living in areas in which cholera is endemic will require evaluation by field trials. The production of sufficient amounts of B subunit should not pose any problem, due to the recent development of affinity chromatography purification using a G_{M1} column⁶⁸.

Genetic methods may also supply *V. cholerae* strains which selectively lack the gene for the 'toxic' A subunit. By retaining the ability to colonize the intestine and produce immunogenic B subunit, such strains could be useful as live oral cholera vaccines. It must be borne in mind, however, that any living vaccine of this sort will require thorough testing for stability, and lack of side

effects before it can be used in humans. In that respect it is disappointing that the first such V. cholerae strain (Texas star)⁶⁹, produced diarrhoea (although usually very mild) in about 20% of recipient American volunteers in spite of producing no detectable holotoxin when grown in vitro⁷⁰.

Receptor-specific interference with toxin binding

The second rational approach to cholera prophylaxis is based on the identification of $G_{\rm M1}$ as the toxin receptor. In theory, the oral administration of large amounts of $G_{\rm M1}$ or a structural analogue could be used to prevent binding of the toxin to cell receptors. Alternatively, cell receptors could be blocked by the nontoxic B subunit.

Development of cholera was prevented by giving $G_{\rm M1}$ to rabbits³⁰, but for prophylactic medical use, enough $G_{\rm M1}$ would have to be given to ensure an excess in relation to the amount of toxin produced by the cholera vibrios in the gut. Most clinical isolates of V, cholerae produce considerably less than $1~\mu g~ml^{-1}$ of cholera toxin when grown in vitro, and toxin concentrations in diarrhoeal fluid of patients have not been found to exceed $0.2~\mu g~ml^{-1}$ (refs 71, 116). Assuming a daily production of $\sim 10~nmol$ of cholera toxin in $\sim 5~l$ of small intestinal juice, neutralization of the toxin should be accomplished by a similar amount of $G_{\rm M1}$. To avoid the incorporation of oral $G_{\rm M1}$ into intestinal cells, which would increase their susceptibility to cholera²², the toxin could be adsorbed onto medical charcoal⁷¹, or covalently coupled to cellulose powder, for example (L. Svennerholm et al., in preparation).

As G_{M1} cannot deactivate toxin that has already bound to the intestine, it would be expected to have a preventive rather than a curative effect. However, in a recent clinical trial the amount of G_{M1} -charcoal that completely bound the free, luminal V, cholerae enterotoxin also reduced purging in the early stage of disease⁷¹. The most likely explanation for this observation is that, because of the constant and rapid regeneration of gastrointestinal cells, there are always new cells available to bind cholera toxin or to be protected by an agent that binds the toxin. This is probably the first instance in which a specific receptor has been used to interfere with an infectious disease. However, it should be emphasized that the observed effect of G_{M1} -charcoal was too transient and incomplete to be practically useful; rather, G_{M1} given orally would possibly be useful for prophylaxis in high-risk groups such as family contacts of cholera patients.

As the B subunit of cholera toxin binds tightly to G_{M1} receptors but has no toxic activity, it might be possible to block the intestinal receptors by occupying them with purified B subunit protomer. It has been shown that pretreatment of the gut of rabbits with 0.5 μ g of B subunit per cm completely protected the animals from experimental cholera after challenge with high doses of active cholera toxin^{22,72,73}. A human trial is conceivable now that it is technically possible to prepare purified B subunit in sufficient quantities⁶⁸; as little as ~100 μ g of B subunit could theoretically block all available receptors, based on the determination of amounts of G_{M1} in human intestinal epithelium²². B subunit is both safe and specific, and might prevent manifestation of disease while inducing local antitoxic immunity and allowing the infection to elicit an antibacterial immune response. In contrast to prophylactic G_{M1} , B subunit would also block toxin which is secreted in close proximity to the microvilli.

Antisecretory drugs

The third approach, intended for therapy rather than prophylaxis, aims at specifically reversing the toxic action after the cholera toxin has become bound to the intestinal cells. During the past decade oral hydration therapy by means of appropriate glucose-electrolyte solutions has greatly simplified the treatment of dehydrating diarrhoea, making effective therapy feasible in situations where intravenous treatment facilities are limited or unavailable 74-76. This treatment takes advantage of the presence of an uptake mechanism for sodium in conjunction

with certain organic solutes, including glucose, which is not regulated by cyclic nucleotides and thus is unaffected in cholera and other forms of enterotoxic diarrhoea⁷⁷. In patients with mild or moderate dehydration the success rate with oral therapy is very high, usually more than 90%. However, in severely affected patients purging often occurs at such a high rate that balance cannot be maintained by oral fluid only, and intravenous replacement of fluid is essential for survival. Drugs that could reduce the rate of fluid loss in the severe dehydrating diarrhoeal diseases would obviate the need for much intravenous therapy and thus could play an important part in the management of severe diarrhoea. Such antisecretory drugs would need a high therapeutic index so that they could be used without extensive medical supervision, and they would have to be compatible with oral fluid therapy.

Based on present understanding of the biochemical events initiated by cholera toxin, possibilities for specific intervention include: (1) prevention of entry of A subunit (although not clinically useful as this event occurs before the onset of symptoms); (2) use of purines and their analogues to alter the NADase action of cholera toxin; (3) use of alternative acceptors, such as arginine or imidazoles, for the ADP-ribose generated by the toxin; (4) reversal of the ADP-ribose-adenylate cyclase association; and (5) removal of ADP-ribose by a specific ADPribosidase. While some of these approaches have reversed the action of cholera toxin in vitro in broken cell systems, kinetic and other considerations make the likelihood of success of inhibiting this stage of the secretory process in vivo extremely remote?

There seem to be considerably greater opportunities for inhibiting the action of cholera toxin subsequent to the ADP ribosylation step by interfering with cyclic AMP formation or metabolism, or by modulating electrolyte-translocating mechanisms of the intestinal membrane. Several drugs have been found to inhibit secretion induced by cholera toxin in experimental animals, for example, chlorpromazine⁷⁹ and certain related compounds⁸⁰, nicotinic acid⁸¹, aspirin⁸², indomethacin⁸³, ethacrynic acid⁸⁴, propranolol⁸⁵, lidocaine⁸⁶ and berberine¹¹⁷ (for review see ref. 118). Chlorpromazine is the best studied of these drugs. In mice, treatment intramuscularly or enterally with 1-4 mg of chlorpromazine per kg body weight completely inhibited the intestinal secretion caused by cholera toxin, Escherichia coli LT, prostaglandin E1 or dibutyryl cyclic AMP⁷⁹. In similar doses, chlorpromazine reversed fluid loss in piglets with enterotoxinogenic E. coli diarrhoea⁸⁷. In adult patients with severe cholera, treatment with chlorpromazine either perorally or intramuscularly, in a single dose of 1 or 4 mg per kg, rapidly and drastically reduced the purging volumes by -65% (ref. 88). The patients became mildly sedated, were more comfortable, and had no nausea or vomiting. A follow-up clinical trial has shown that treatment of children with 1 mg per kg chlorpromazine increased the success rate of oral hydration therapy in patients with severe cholera⁸⁹

The mechanism of antisecretory action of chlorpromazine is not fully known; however, it does seem to be confined to the intestinal epithelium^{79,90,91}. Chlorpromazine was selected for testing because of its ability to inhibit hormonal stimulation of cyclic AMP formation in various tissues^{92,93}; both the cholera toxin- and fluoride-stimulated adenylate cyclase activities of the intestinal mucosal membrane of chlorpromazine-treated mice are suppressed⁷⁹. However, the activity of the protein kinase of mucosal membranes is also reduced, suggesting multiple effects of chlorpromazine⁷⁹. In the brain, chlorpromazine and other phenothiazines are known to inactivate calmodulin and to interfere with transfer of calcium across the nerve-cell membrane94; if these effects occur in the intestine, they could lead to inhibition of secretion 91,119. The action of chlorpromazine on calmodulin could possibly be linked to an inhibitory effect on adenylate cyclase.

Perspective

It has been estimated that each year about 1,000 million episodes of acute diarrhoea occur in children under 5 years of

age in Asia, Africa and Latin America, resulting in 5 million deaths. In many developing countries one-third to one-half of infant mortality can be attributed to diarrhoeal diseases. Even in the older age groups diarrhoeal episodes are frequent causes of severe illness and death^{95,96}.

Cholera is not the most prevalent of the diarrhoeal diseases, but it causes the most severe fluid loss and thus is responsible for a large proportion of life-threatening illness and death during the cholera season in endemic areas. Furthermore, research during the past decade has also made it clear that cholera is a prototype of diarrhoeal diseases caused by other enterotoxinproducing bacteria, especially E. coli⁹⁷. Taken together, the enterotoxic enteropathies probably account for at least one-half of dehydrating diarrhoeal illnesses. Knowledge of the structure and function of cholera toxin has been the main stimulus and provided guidelines for recent research in elucidating the aetiology and pathogenesis of these related diarrhoeas. Diagnostic assays for *E. coli* LT based on toxin-induced cyclic AMP manifestations in animals or cell lines 98,99, or more recently, on immunological 100 or receptor-immunological methods 101 are based on similar methods for cholera toxin.

Although clean water supplies and safe disposal of human sewage would effect a dramatic reduction in cholera and other diarrhoeal disease, these two goals cannot be achieved in many areas for decades. If effective vaccines could be developed, they would no doubt be important components of national control programmes against diarrhoeal disease. The same holds true for drugs that could prevent or reduce fluid loss in enteric infections and thus alleviate the need for intravenous treatment.

Here I have described some theoretically possible approaches to counteract cholera toxin by immunological or pharmacological means. While each of these methods have been effective against cholera in animal studies, and, to the limited extent to which they have been tested, have also given promising results in man, much more research is needed to define their place, if any, in future prophylaxis and treatment of cholera. They should be regarded merely as starting points for further development. For example, in relation to vaccine development there is a need for better understanding of factors regulating the magnitude and duration of intestinal antitoxic as well as antibacterial immunity. With regard to receptor-prophylactic agents, basic research should be directed to identify specific binding or blocking agents for cholera vibrios which might cooperate synergistically with those for toxin. In the case of antisecretory agents, the aim should be to find drugs which effectively depress secretion but lack the sedative action of chlorpromazine. However, it is gratifying that cholera toxin research has now reached the stage where rational counteractive methods can be both formulated on the basic molecular knowledge, and, most importantly, be evaluated in controlled clinical and field trials.

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- Ouchterlony, Ö. & Holmgren, J. (eds) Cholera and Related Diarrhoeas: 43rd Nobel Symp., Stockholm 1978 (Karger, Basel, 1980).
- De, S. N. Nature 183, 1533-1534 (1959). Dutta, N. K., Panse, M. V. & Kulkarni, D. R. J. Bact. 78, 594-595 (1959).
- Finkelstein, R. A. & LoSpalluto, J. J. J. exp. Med. 130, 185-202 (1969). Richardson, S. H., Evans, D. G. & Feeley, J. C. Infect. Immunity 1, 546-553 (1970).
- Field, M., Fromm, D., Wallace, C. K. & Greenough, W. B. J. clin. Invest. 48, 24a (1969). Schafer, D. E., Lust, W. D., Sircar, B. & Goldberg, N. D. Proc. natn. Acad. Sci. U.S.A. 67, 851-856 (1970)
- Sharp, G. W. G. & Hynie, S. Nature 229, 266-269 (1971).
- Field, M., Fromm, D., Al-Awqati, Q. & Greenough, W. B. J. clin. Invest. 51, 795-804

- 10. Greenough, W. B., Pierce, N. F. & Vaughan, M. J. infect. Dis. 121, S111-S114 (1970).
- Lönnroth, I. & Holmgren, J. J. gen. Microbiol. 76, 417-427 (1973).
 Cuatrecasas, P., Parikh, I. & Hollenberg, M. D. Biochemistry 12, 4253-4264 (1973)
- 13. Holmgren, J. Proc. 9th Joint US-Japan Cholera Conf., Grand Canyon 1973, 196-213 Holmgren, J., Friec. 9th John OS-Japan Cholera Conf., Grand Canyon 1973,
 Heyningen, S. van. Science 183, 656-657 (1974).
 Holmgren, J., Lindholm, L., & Lönnroth, I. J. exp. Med. 139, 801-819 (1974).

- Finkelstein, R. A., Boesman, M., Neoh, S. H., LaRue, N. K. & Delaney, R. J. Immun. 113, 175-180 (1974).

- Gill, D. M. & King, C. A. J. biol. Chem. 250, 424-432 (1975).
 Heyningen, S. van & King, C. A. Biochem. J. 146, 269-271 (1975).
 Flores, J. & Sharp, S. W. G. J. clin. Invest. 56, 1345-1349 (1975).
 Mekelanos, J. J., Collier, R. J. & Romig, W. R. J. biol. Chem. 254, 5855-5861 (1979).
 Cuatrecasas, P. Biochemistry 12, 3547-3558 (1973).
 Holmgren, J., Lönnroth, I., Mánsson, J. E. & Svennerholm, L. Proc. natn. Acad. Sci. LLS 4, 72, 5230, 2524 (1975). U.S.A. 72, 2520-2524 (1975)
- 23. Fishman, P. H. & Atikkan, E. E. J. biol. Chem. 254, 4342-4344 (1979)
- Heyningen, W. E. van, Carpenter, C. C. J., Pierce, N. F. & Greenough, W. B. J. infect. Dis. 124, 415–418 (1971).
- Holmgren, J., Lönnroth, I. & Svennerholm, L. Infect. Immunity 8, 208-214 (1973).
 King, C. A. & Heyningen, W. E. van. J. infect. Dis. 127, 639-647 (1973).
- Hansson, H.-A., Holmgren, J. & Svennerholm, L. Proc. natn. Acad. Sci. U.S.A. 74, 3782-3786 (1977).
- 28. Fishman, P. H. & Atikkan, E. E. J. biol. Chem. 254, 4342-4344 (1979)
- 29. Holmgren, J. & Lönnroth, I. J. gen. Microbiol. 86, 49-65 (1975)
- Sattler, J., Schwarzmann, G., Staerk, J., Ziegler, W. & Wiegandt, H. Hoppe-Seyler's Z. physiol. Chem. 358, 159-163 (1977).
- Holmgren, J., Mânsson, J.-E. & Svennerholm, L. Med. Biol. 52, 229-233 (1974).
 Fishman, P. H., Moss, J. & Osborne, J. C. Biochemistry 17, 711-716 (1978).
 Cuatrecasas, P. Biochemistry 12, 3558-3566 (1973).
- 34. Fishman, P. H., Pacuszka, T., Holm, B. & Moss, J. J. biol. Chem. 255, 7657-7664
- 35. Moss, J., Fishman, P. H., Manganiello, V. O., Vaughan, M. & Brady, R. O. Proc. natn. Acad. Sci. U.S.A. 73, 1034-1037 (1976).
 Mullin, B. R. et al. Proc. natn. Acad. Sci. U.S.A. 73, 1679-1683 (1976).

- Holmgren, J. & Lönnroth, I. J. infect. Dis. 133, 564-574 (1976).
 Craig, S. W. & Cuatrecasas, P. Proc. natn. Acad. Sci. U.S.A. 72, 3844-3848 (1975).
 Revesz, T. & Greaves, M. Nature 257, 103-106 (1975).
- Sedlacek, H. H., Staerk, J., Seiler, F. R., Ziegler, W. & Wiegand, H. FEBS Lett. 61, 272–276 (1976).
- 41. Bennet, V., O'Keefe, E. & Cuatrecasas, P. Proc. natn. Acad. Sci. U.S.A. 72, 33-37 (1975).
- Fishman, P. H. in Secretory Diarrhea (eds Field, M., Fordtran, J. S., & Schultz S. G.) 85-106 (American Physiological Society, Maryland, 1980).
- 43. Moss, J., Richards, R. L., Alving, C. R. & Fishman, P. H. J. biol. Chem. 252, 797-798
- 44. Tosteson, M. T. & Tosteson, D. C. Nature 275, 142-144 (1978).
- 45. Holmgren, J. & Lönnroth, I. in Cholera and Related Diarrhoeas: 43rd Nobel Symp., Stockholm 1978, 88-103 (Karger, Basel, 1980).
- 46. Manuelidis, L. & Manuelidis, E. E. J. Neurocytol. 5, 575-589 (1976).

- Manueliois, L. & Manueliois, E. E. J. Neurocytol. 5, 5/5-589 (1977).
 Pappenheimer, A. M. Jr A. Rev. Biochem. 46, 69-94 (1977).
 Sandvig, K. & Olsnes, S. J. Cell Biol. 87, 828-832 (1980).
 Draper, R. K. & Simon, M. I. J. Cell Biol. 87, 849-854 (1980).
 Gill, D. M. J. infect. Dis. 133, S55-S63 (1976).
- 51. Moss, J., Manganiello, V. C. & Vaughan, M. Proc. nam. Acad. Sci. U.S.A. 73, 4424-4427
- Cassel, D. & Pfeuffer, T. Proc. natn. Acad. Sci. U.S.A. 75, 2669-2673 (1978).
 Gill, D. M. & Mehren, R. Proc. natn. Acad. Sci. U.S.A. 75, 3050-3054 (1978).
- Cassel, D. & Selinger, Z. Proc. natn. Acad. Sci. U.S.A. 74, 3307–3311 (1977).
 Lad, P. M., Nielsen, T. B., Preston, N. S. & Rodbell, M. J. biol. Chem. 255, 988–995
- (1980).
- Cash, R. A. et al. J. infect. Dis. 130, 325-333 (1974)
- Feeley, J. C. & Gangarosa, E. J. in Cholera and Related Diarrhoeas: 43rd Nobel Symp. Stockholm 1978, 204–210 (Karger, Basel, 1980).
- Svennerholm, A.-M. & Holmgren, J. Infect. Immunity 13, 735-740 (1976). Peterson, J. W. Infect. Immunity 26, 528-533 (1979).
- 60. Holmgren, J. et al. Nature 269, 602-604 (1977).
 61. Pierce, N. F. J. exp. Med. 148, 195-206 (1978).
- Ohtomo, N., Muraoka, T. & Tashiro, A. Proc. 10th Joint US-Japan Cholera Conf., Kyoto 1974, 115-118 (Tokyo, 1974).
- Svennerholm, A.-M. in Cholera and Related Diarrhoeas: 43rd Nobel Symp., Stockholm 1978, 171-184 (Karger, Basel, 1980).
- Peterson, J. W., Hejtmancik, K. E., Markel, D. E., Craig, J. P. & Kurosky, A. Infect. Immunity 24, 774-779 (1979). Svennerholm, A.-M., Sack, D. A., Bardhan, P. K., Jertborn, M. & Holmgren, J. in Proc.
- 16th US-Japan Cholera Conf. Gifu, 1980 (Osaka University Press, in the pres 66. Sack, D. A., Islam, A., Holmgren, J. & Svennerholm, A.-M. in Proc. 15th Joint US-Japan Cholera Conf. Bethesda, 1979, 423-439 (NIH Publ. 80-2003, 1980)

- 67. Svennerholm, A.-M., Jertborn, M., Gothefors, L., Sack, D. A. & Holmgren, J. (in
- 68. Tavot, J.-L., Holmgren, J., Svennerholm, L., Lindblad, M. & Tardy, J. Eur. J. Biochem. 113, 249-258 (1981)
- 69. Honda, T. & Finkelstein, R. A. Proc. natn. Acad. Sci. U.S.A. 76, 2052-2056 (1979).
- 70. Levine, M. in Nobel Conf. 3 (eds Holme, T., Holmgren, J., Merson, M. & Mölfby, R.) (Elsevier, Amsterdam, in the press)
- Stoll, B. J. et al. Lancet II, 888-891 (1980)
- Pierce, N. F. Levn. Med. 137, 1009-1023 (1973).
- Holmgren, J. Infect. Immunity 8, 851-859 (1973)

- Hirschhorn, N. J. et al. New Engl. J. med. 279, 176-184 (1968).

 Nalin, D. R., Cash, R. A., Islam, R., Molla, M. & Phillips, R. A. Lancet ii, 370-372 (1968).

 Pierce, N. F. et al. Ann. intern. Med. 70, 1173-1183 (1969).

 Schultz, S. G. & Curran, P. F. Curr. Topics Membrane Transport 5, 225-281 (1974).

 Greenough, W. B. Ill & Hendrix, T. R. in Cholera and Related Diarrhoeas: 43rd Nobel Symp., Stockholm 1978, 234-244 (Karger, Basel, 1980).
- Holmgren, J., Lange, S. & Lönnroth, I. Gastroenterology 75, 1103-1108 (1978).
 Lönnroth, I., Lange, S. & Holmgren, J. in Phenothiazines and Structurally Related Drugs Basic and Clinical Studies (eds Usdin, E., Eckert, H. & Forrest, S. J. J.) 303-306 (Elsevier, New York, 1980).
- Turjman, N., Gotterer, G. S. & Hendrix, T. R. J. clin. Invest. 61, 1155-1160 (1978). Finch, A. D. & Katz, R. L. Nature 238, 273-274 (1972).
- Jacoby, H. L. & Marshall, C. H. Nature 235, 163-165 (1972)
- 84. Carpenter, C. C. J., Curlin, G. T. & Greenough, W. B. III J. infect. Dis. 120, 332-338 (1969)
- 85. Taub, M., Bonorris, G., Chung, A., Coyne, M. J. & Schoenfeld, L. J. Gastroenterology 72, 101-105 (1977
- Cassuto, J., Jodal, M., Tuttle, R. & Lundgren, O. Experientia 35, 1467-1468 (1979).
- Lönnroth, I., Andrén, B., Lange, S., Martinsson, K. & Holmgren, J. Infect. Immunity 24, 900-905 (1979).
- 88. Rabbani, G. H., Greenough, W. B. III, Holmgren, J. & Lönnroth, I. Lancet i, 410-412
- 89. Islam, M. R., Sack, D. A., Holmgren, J., Bardhan, P. K. & Rabbani, Lancet (submitted).

- Islam, M. R., Sack, D. A., Houmgren, J., Bardnan, F. K. & Rabbani, Lance
 Lönnroth, I. & Munck, B. G. Acta pharmac. tox. 47, 439–446 (1979).
 Smith, P. L. & Field, M. Gastroenterology 78, 1545–1553 (1980).
 Kakiuchi, S. & Rall, T. W. Molec. Pharmac. 4, 367–378 (1968).
 Wolf, J. & Jones, A. B. Proc. natn. Acad. Sci. U.S.A. 65, 454–459 (1970).
- Cheung, W. Y., Lynch, T. J. & Wallace, R. W. Adv. Cyclic Nucleotide Res. 9, 233-251
- Mata, L., Kronmal, R. A. & Villegas, H. in Cholera and Related Diarrhoeas: 43rd Nobel Symp., Stockholm 1978, 1–14 (Karger, Basel, 1980).
- 96. Barua, D. in Nobel Conf. 3 (eds Holme, T., Holmgren, J., Merson, M. & Möllby, R.) (Elsevier, Amsterdam, in the press).

- (Elsevier, Amsterdain, in the press).
 Sack, R. B. A. Rev. Microbiol. 29, 333-353 (1975).
 Donta, S. T. & Smith, D. M. Infect. Immunity 9, 500-505 (1974).
 Guerrant, R. L., Brunton, L. L., Schnaitman, T. C., Rebhun, L. I. & Gilman, A. G. Infect. Immunity 10, 320-327 (1974).
- Yolken, R., Greenberg, H. B., Merson, M., Sack, R. B. & Kapikian, A. Z. J. clin. Microbiol. 6, 439-444 (1977)
- Svennerholm, A.-M. & Holmgren, J. Curr. Microbiol. 1, 19-27 (1978).
 Sattler, J. et al. Eur. J. Biochem. 57, 309-316 (1975).
- Gill, D. M. Biochemistry 15, 1242-1248 (1976)
- 104. Lai, C.-Y., Mendez, E. & Chang, D. J. infect. Dis. 133, S23-S30 (1976).
- Kurosky, A., Markel, D. E. & Peterson, J. W. J. biol. Chem. 252, 7257-7264 (1977). Lai, C.-Y. J. biol. Chem. 252, 7249-7256 (1977).
- Sigler, P. B., Druyan, M. E., Kiefer, H. C. & Finkelstein, R. A. Science 197, 1277-1279
- 108. Gill, D. M. & Rappaport, R. S. J. infect. Dis. 139, 674-680 (1979)
- 109. Tomasi, M., Battistini, A., Araco, A., Roda, L. G. & D'Agnolo, G. Eur. J. Biochem. 93, 621-627 (1979).
- 110. Ohtomo, N., Muraoka, T., Tashiro, A., Zinnaka, Y. & Amako, K. J. infect. Dis. 133, (Suppl.), S31–S40 (1976).

 111. Fishman, P. J., Moss, J., Richards, R. L., Brady, R. O. & Alving, C. R. Biochemistry 18,
- 112. Staerk, J., Ronneberger, H. J., Wiegandt, H. & Ziegler, W. Eur. J. Biochem. 48, 103-110
- 113. Haksar, A., Maudsley, D. V. & Péron, F. G. Nature 251, 514-515 (1974) 114. Révész, T., Greaves, M. F., Capellaro, D. & Murray, R. K. Br. J. Haemat. 34, 623-630 (1976)
- 115. Holmgren, J. in Bacterial Toxins and Cell Membranes (eds Jeljaszewicz, I. & Wadström, T.) 333-366 (Academic, London, 1978).
- 116. Craig, J. P. Nature 207, 614-616 (1965).
- 117. Sack, R. B. in Nobel Conf. 3 (eds Holme, T., Holmgren, J., Merson, M. & Möllby, R.) (Elsevier, Amsterdam, in the press).
- 118. Powell, D. W. & Field, M. in Secretory Diarrhoea (eds Field, M., Fordtran, J. S. & Schultz, S. G.) 187-209 (American Physiological Society, Maryland, 1980). 119. Jlundain, A. & Naftalin, R. J. *Nature* **279**, 446-448 (1979).

High noble metal concentrations in a late Pliocene sediment

Frank T. Kyte*†, Zhiming Zhou* & John T. Wasson*†‡ *Institute of Geophysics and Planetary Physics, †Department of Earth and Space Sciences, ‡Department of Chemistry, University of California,

Los Angeles, California 90024, USA

A 2.3-Myr-old layer in a sediment from the Antarctic Ocean contains Ir and Au at levels comparable with those at the Cretaceous-Tertiary boundary. A sizable fraction of the noble metals is contained in vesicular, millimetre-sized polymineralic grains that closely resemble ablation debris from chondritic meteorites, and there is little doubt that the noble metals resulted from the accretion of a large extraterrestrial object. No massive extinctions or other evidence of environmental stress seem to be associated with this accretionary event.

THE accretion of kilometre-sized asteroids to the Earth must have a significant impact on the terrestrial ecosystem. Such encounters have been a stochastic source of stress to the biosphere, influencing evolution and extinction rates. Recent discoveries of Ir (ref. 1) and other extraterrestrial siderophiles ¹⁻⁴ in sediments at the 65-Myr-old Cretaceous-Tertiary boundary have supported the hypothesis that encounters of sufficient magnitude can lead to the simultaneous extinction of many species. These discoveries have also illustrated that at least some types of encounters deposit a measurable geochemical signal in the sedimentary record.

If the Earth accreted a massive body at the end of the Cretaceous 65-Myr ago, then other bodies of similar size should have left their signatures elsewhere in the geological record. From relationships given by Grieve and Dence⁵ and an assumed mean impact velocity of 20 km s^{-1} we calculate the relationship $N = 6.7 \times 10^{-7} r^{-2}$ where N is the cumulative number of bodies impacting the Earth each year and r (in km) is the radius of the body. This relationship indicates that ~43 bodies should have impacted the Earth since the Cretaceous. Wetherill⁶ gives a similar estimate. This relationship is based on crater counts, and if preimpact breakup occurs in an appreciable fraction of the cases, the impact rate could be higher⁷.

A logical step in establishing the link between major changes in the terrestrial environment and the accretion of kilometresized bodies is to find and characterize other sediments having enhanced concentrations of noble metals. In this article we report our first success, the discovery a large enhancement of noble metals near the Pliocene-Pleistocene boundary in an Antarctic Ocean deep-sea core. Core USNS Eltanin 13-3 was selected because Crocket and Kuo⁸ found Ir concentrations in one 210-cm long section that were three times higher than those in other sections from the same core. It was also intriguing that the base of this 210-cm unit was associated with radiolarian extinctions, geomagnetic reversals, and climatic change⁹.

Experimental results

Eltanin core 13-3 is a muddy diatomaceous ooze collected in 1964 at a depth of 5,090 m at 57° 00' S and 89° 29' W. It has been heavily sampled and is dried out in some areas. In the region of interest (780-800 cm) it contains ~65% diatoms and radiolaria and 30% clay with ~5% quartz, heavy minerals and trace Mn micronodules. It consists of a pale brown ooze with strings or sedimentary clasts of pale orange diatomaceous ooze. The sedimentation rate as determined by palaeomagnetic stratigraphy is approximately constant at 3.3 $\mu m \ yr^{-1}$ (refs 8, 9). A total of 71 samples, each 3-4 cm in length were taken for a continuous section from 645 to 875 cm, extending from 10 cm above to 10 cm below the interval in which Crocket and Kuo⁸ found the noble-metal enhancement. Two additional control samples were taken from 551-553 and 946-948 cm. Samples of 150-200 mg were irradiated for 60 h at 10¹⁴ neutrons cm⁻² s⁻¹ and analysed by instrumental neutron activation analysis (INAA); results are listed in Table 1. Iridium was the only noble metal that could be determined; the control samples and those in the ranges 645-771 and 797-875 mm were below our detection limit of 0.5 ng per g. On discovery of high Ir (1-9 ng per g) levels in samples from the 771-797 cm level, another 14 samples (600-750 mg) were irradiated for 3 h at a flux at 2×10^{12} neutrons cm⁻² s⁻¹ in the UCLA reactor and Au and Ir were determined by radiochemical neutron activation analysis (RNAA) using the procedure described previously4.

Our RNAA analyses on a second set of samples were carried out to confirm the Ir anomaly and to measure Au concentrations in this horizon (Table 1). We also attempted to analyse for Os, Pt, Pd and Re in two samples, but because of the low neutron flux and low noble metal concentrations we obtained only upper limits with CI-chondrite-normalized element/Ir ratios >2. These are not diagnostic for distinguishing between chondrite groups, but we plan to determine these elements in another experiment. Poor chemical yields caused significant errors on a few Ir data, but in general there was a good correlation between

Au and Ir. Our background Ir values agree well with the value of $0.20~\rm ng$ per g determined by Crocket and Kuo⁸; our background Au value of $\sim 0.32~\rm ng$ per g is about half their value. We cannot readily explain this discrepancy. Our RNAA and INAA Ir values often differed by as much as a factor of 2. As discussed below, these discrepancies probably reflect sample inhomogeneity rather than analytical problems. The band proved wider in the RNAA section than anticipated on the basis of the initial INAA survey. Later, in longer counts an Ir concentration of 2.3 ng per g was found in another sample (35) which may extend the enriched layer another 7 cm above the RNAA data set.

Because the sample sizes were about four times larger we have greater confidence in our RNAA data. Figure 1 shows the RNAA Ir and Au concentrations in the peak and in several background samples. Using a background of 0.20 and 0.32 ng per g for Ir and Au, respectively, and a bulk dry density of the sediment of $2 \, \mathrm{g \, cm^{-3}}$, the net Ir and Au fluences integrated across the horizon are 100 and $63 \, \mathrm{ng \, cm^{-2}}$, respectively. The resulting Au/Ir mass ratio of 0.6 is higher than that in all groups of chondrites except the EH (formerly designated E4 and E5) enstatite chondrites ¹⁰.

By chance, two large (1.5-2.0 mm) grains of Ir-rich material were found in INAA sample 42. Together with a minor amount of adhering clay they weighed 4 mg. Their Ir concentration was 160 ng per g, accounting for $\sim 40\%$ of the total Ir in this sample. In polished section these grains were observed to be highly vesicular ($\sim 50\%$) with a finely microcrystalline texture. This consisted of equant, $\sim 10~\mu \text{m}$ euhedral silicates in a silicate matrix containing a sparse submicrometre opaque phase. Qualitative analysis by electron microprobe indicated that the silicates were zoned Ni-bearing olivine in a Ca, Al, Si-rich matrix.

It seems that these materials correspond to the olivine-magnetite-glass assemblage typical of cosmic spherules and debris produced by the laboratory ablation of chondrites¹¹.

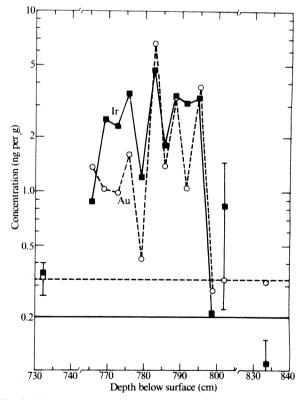


Fig. 1 Concentrations of Ir and Au in Eltanin core 13-3. Background levels (shown by horizontal lines) are 0.20 ng per g Ir and 0.32 ng per g Au; maximum concentrations in the peak in the depth range 765-800 cm are ~25 times the background levels. The integrated flux of Ir is 100 ng cm⁻², of Au 63 ng cm⁻². The observed Au/Ir ratio is similar to values in EH enstatite chondrites. The 70% uncertainty limits are shown for three of the lowest Ir values are examples of the precision at these levels. More exact information regarding the precision is given in Table 1.

Table 1 Concentrations of Ir and Au measured at various depths of Eltanin core 13-3

	9	INAA	RN	IAA
Sample	Depth (cm)	Ir (ng per g)	Ir (ng per g)	Au (ng per g)
1-25	645-731	< 0.5	-	****
27	731-734	≤0.5	0.33+	0.35
28-34	734-757	≤0.5		*****
35	757-760	2.3		*******
36	760-764	≤0.5		-
37	764-767	≤0.5	0.88	1.36
38	767-771	≤0.6	2.5	1.03
39	771-774	1.2	2.3	0.98
40	774-777	2.0	3.5*	1.6
41	777-781	6.7	1.2	0.42
42	781-784	10	4.7	6.6
43	784-787	1.4	1.8	1.39
44	787-790	1.3	3.4*	3.3
45	790-793	1.3	3.1	1.05
46	793-797	1.4	3.3	4.8
47	797-800	≤0.5	0.21*	0.28
48	800-804	≤0.5	0.83‡	0.32
49-57	804-832	≤0.5		
58	832-835	≤0.5	0.11	0.31
59-71	835-875	≤0.5	-	

Relative uncertainty limits are $\pm \le 10\%$ except as indicated.

Their texture, equant olivine grains set in a vesicular matrix, is found in cosmic spherules separated from sea sediments, but is less common than textures such as barred olivine or platy brickwork¹¹. A > 147-µm sieve fraction of another 1g of sample 42 contained a 1-mm particle with similar petrographic properties and four others in the size range 0.2-0.5 mm. The sample heterogeneity mentioned above is probably the result of statistical fluctuations in the number of large grains of chondritic debris through the section.

Only one particle was roughly spherical in outline, the rest having irregular shapes. All particles had severely etched rims, with only their cores retaining an apparently unaltered texture. The obvious dissolution of particle rims may have resulted in a post-depositional redistribution of a fraction of the noble metals in the horizon. Our observed concentrations of ≥ 3 mg cm⁻³ chondritic debris contrasts with typical influxes of ablation debris of 'cosmic spherules' > 0.2 mm of 30 ng cm⁻³ based on a worldwide accretion rate of 90 tons yr⁻¹ (ref. 12) and the reported sedimentation rate.

Discussion

There seems little doubt that the noble metals in our Antarctic Ocean core are of extraterrestrial origin, as indicated by their roughly chondritic Au/Ir ratios, and by the remarkably high concentration of chondritic debris. This debris reaches concentrations about five orders of magnitude higher than expected in sediment having the observed sedimentation rate. The observed particles have petrographic textures of a rare type regularly observed in typical collections of cosmic spherules.

We interpret this horizon as resulting from a single, accretionary event. Presently available information does not allow us to distinguish between oceanic impact and atmospheric ablation as the source of these materials, but similarities of the large 'spherules' to materials produced in artificial ablation experiments¹³ suggests that they were produced by atmospheric ablation of asteroidal or cometary material. Total breakup above the solid or liquid surface of the Earth apparently requires that the material be highly friable as is inferred to be the case for some cometary materials. Microtektites have not been found in sieved samples of our horizon and thus are much less abundant than 'ablation' debris. This probably indicates that at most a minor fraction of the noble metal enhancement can be associated with crater ejecta.

The integrated flux of 80–100 ng Ir cm⁻² is ~0.15 g cm⁻² H₂O-free CI material or an initial horizon ~0.5 mm thick. We suspect that bioturbation has spread out an initially thin, concentrated horizon, although this is difficult to confirm observationally in such an old, heavily sampled core. The 30–40 cm thickness of the enriched layer is similar to thicknesses of microtektite horizons¹⁴ and to bioturbation mixing lengths inferred in core E13-3 in palaeontological studies¹⁵. Rapidly deposited horizons of this type might, in fact, provide interesting markers for estimating effects of bioturbation and of trace-element transport during diagenesis.

element transport during diagenesis.

The 80-100 ng Ir cm⁻² in this horizon is similar to the maximum reported for the Cretaceous-Tertiary boundary at various European sites^{1,2,4}. Thus, if this horizon is worldwide, the mass of accreted chondritic matter is comparable with that of the Cretaceous-Tertiary materials. However, the high abundance of millimetre-sized ablation (or impact) debris suggests that the site of this core is near the location where the meteoroid entered the atmosphere and/or impacted with the surface of the ocean, thus the fluence at distant locations may be significantly lower.

The late Pliocene noble-metal-enrichment is probably not too localized, otherwise it would probably not have been discovered in the few cores analysed by Crocket and Kuo⁸. A major event showering debris over a wide area is a much more likely mechanism for producing discoverable concentrations of this magnitude. Considering that the proposed 0.5 mm-thick horizon was deposited after settling through 5 km of water, and the differential settling times of the particles (~8 h for 2 mm compared with ~11 h for 1 mm diameters) in currents which today are ~0.7 km h⁻¹ (ref. 16) would spread this debris over a very large area even if it originated from a point source. We propose a minimum projectile diameter of 20 m, enough material to produce similar enrichments over only 10 km². We suspect that it must have been significantly larger.

Crocket and Kuo⁸ found no Ir enhancement at the same time range in core Eltanin 17-10 that was collected ~2,800 km away. Their sample containing the corresponding time interval, however, covers a depth range 800-1,400 cm, nearly three times longer than that in 13-3. For this reason their negative result may not be significant. Additionally, palaeomagnetic studies indicate that the horizon would be near the base of the sampled interval and might even overlap into the next interval (1,400-1,585 cm) which has slightly higher Ir (0.42 ng per g).

In contrast to noble metal-enriched sediments at the Cretaceous-Tertiary boundary, there is no major palaeoenvironmental crisis associated with this noble metal horizon. Hayes and Opdyke9 report that radiolarian species vary with depth in core 13-3, but no dramatic change is associated with the peak in Ir concentrations at the 770-800 cm depth. In contrast to the Cretaceous-Tertiary boundary, the nearby Pliocene-Pleistocene boundary does not represent a major biostratigraphic hiatus. Definition of the base of the Pleistocene is now generally accepted as the base of the Calabrian stage in the type section at La Castella, Italy, dated at ~1.8 Myr (refs 17, 18). Earlier attempts to define this boundary based on the onset of glaciation (continental or polar) resulted in proposed boundaries ranging in age from 0.5 to 4 Myr (ref. 18); major cooling occurred as early as 4.0 Myr (ref. 17). Palaeomagnetic evidence indicates that our horizon occurs at ~2.3 Myr. If, in fact, this horizon proves to be worldwide it is an important marker bed for late Pliocene stratigraphy, and might warrant use as the Plio-Pleistocene boundary.

Clearly it is important to extend our studies to sediments of the same age from other localities. Although our best projection based on the available facts is that notice metal concentrations at distant locations will be less than those in core 13-3, we do expect to find that the horizon covers a significant fraction of the Earth. Additionally, the discovery of a second significant horizon during the most recent 65 Myr of Earth history suggests that we can find others of similar magnitude throughout the Cenozoic. If so, this will lead to major improvements both in our knowledge regarding the flux of large bodies to the Earth and in

^{* 70%} confidence limits $\pm 10-19$ %.

^{† 70%} confidence limits $\pm 20-39\%$.

^{‡70%} confidence limits ±40-79%.

our ability to correlate marine sediments from widely separated locations.

If this horizon does prove to be worldwide in extent, the absence of major extinctions associated with this late Pliocene event becomes an important fact bearing on models explaining the massive Cretaceous-Tertiary extinctions in terms of environmental effects associated with a major influx of extraterrestrial matter.

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- Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. Science 208, 1095-1108 (1980).
- Smit, J. & Hertogen, J. Nature 285, 198-200 (1980). Ganapathy, R. Science 209, 921-923 (1980).

- Kyte, F. T., Zhou, Z. & Wasson, J. T. Nature 288, 651-656 (1980). Grieve, R. A. F. & Dence, M. R. Icarus 38, 230-242 (1979).
- Wetherill, G. W. Scient. Am. 240 (3), 54-65 (1979).

 Melosh, H. J. & Passey, Q. Proc. Conf. Multiring Basins (Lunar Planetary Institute, Houston, 1981).
 Crocket, J. H. & Kuo, H. Y. Geochim, cosmochim, Acta 43, 831-842 (1979).
- 9. Hays, J. D. & Opdyke, N. D. Science 158, 1001-1011 (1967)

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- 10. Mason, B. Handbook of Elemental Abundances in Meteorites (Gordon and Breach, New
- 11. Blanchard, M. B., Brownlee, D. E., Bunch, T. E., Hodge, P. W. & Kyte, F. T. Earth planet.
- Murrel, M. T., Davis, P. A. Jr, Nishiizumi, K. & Millard, H. T. Jr Geochim. cosmochim Acta 44, 2067-2074 (1980).

- Kyte, F. T. M. S. thesis, San Jose State Univ. (1977).
 Glass, B. P. & Zwart, M. J. Bull. geol. Soc. Am. 90, 595-602 (1979).
 Hayes, J. D. Bull. geol. Soc. Am. 82, 2433-2447 (1971).
 Goodell, H. G., Meylan, M. A. & Grant, B. AGU Antarctic Res. Ser. 15, 27-92 (1971).
 Berggren, W. A. & Van Couvering, J. A. in Treatise on Invertebrate Paleontology, A (eds. Polyinger, P. & Taichert, C.) 505-542 (Geological Society of America, May, Vant. Robinson, R. A. & Teichert, C.) 505-543 (Geological Society of America, New York,

Controls of RNA splicing and termination in the major late adenovirus transcription unit

Göran Akusjärvi & Håkan Persson

Department of Microbiology, University of Uppsala, The Biomedical Center, Box 581, S-751 23 Uppsala, Sweden

The major late adenovirus promoter is active early after infection, selectively producing messenger RNAs coding for polypeptides with molecular weights of 55,000, 52,000 and 14,000. This selective expression suggests that a differential splicing pattern occurs at the transition from early to late viral gene expression. Activation of the late promoter and splicing of the 55, 52K mRNAs does not require newly synthesized virus polypeptides.

TRANSCRIPTION from the adenovirus genome late in infection initiates predominantly at the promoter located at map coordinate 16.3 (ref. 1) and proceeds in a rightward direction for about 28,000 base pairs (bp)². This long viral transcript is processed into five families of messenger RNAs (mRNAs), each family having co-terminal 3' ends3-5 (L1-L5, Fig. 1A) and each mRNA containing a tripartite leader, encoded at coordinates 16.6, 19.6 and 26, spliced onto the body of the mRNA^{6,7}. The genes for two low-molecular-weight RNAs (VA RNA; and VA RNA_{II}), transcribed by RNA polymerase III, have been located close to coordinate 30 on the viral genome^{8,9}. Three mRNA species, originating from the major late transcription unit, have been mapped by electron microscopy in the vicinity of the VA RNA genes¹⁰ (Fig. 1A).

The lytic cycle of adenovirus infection is divided into two functionally distinct phases, the early phase preceding and the late phase beginning with the onset of virus DNA replication. It has been assumed that the major late transcription unit is activated at the shift from early to late phase. However, a low level of rightward-reading transcripts proximal to the late promoter has been detected in the nuclei of early infected cells11, and truncated cytoplasmic RNAs originating from the late promoter have also been found early in the infectious cycle by electron microscopy^{12,13}. Transcription studies using purified restriction fragments carrying the major late promoter suggest that virus-specific components are not necessary for initiation at this promoter site, at least in vitro 14.15. We now demonstrate that the major late transcription unit is active at early times of infection, producing selected transcripts. This indicates a novel, virus-regulated abundance control of mRNA production.

Viral RNAs from region L1

To study the expression of RNAs from the major late transcription unit, we first determined the structure of the r strandspecific RNAs encoded in region L1. Cytoplasmic RNA prepared late after adenovirus type 2 (Ad2) infection was hybridized to the entire viral DNA and treated with S₁ endonuclease. The S₁-resistant hybrids were separated on an alkaline agarose

gel and subsequently transferred to a nitrocellulose filter. Hybridization of the filter to a 32P-labelled probe specific for region L1 (Fig. 2) shows that five major RNA species (Fig. 1B, lanes a-e) had been present in the cytoplasmic RNA preparation. Species b, c and e have previously been identified by electron microscopy¹⁰. The S₁ analysis revealed two novel species, a and d. The length of species a suggests that it represents a co-linear transcript of the DNA template from the third leader segment up to the poly(A) addition site at position 38.5 (ref. 3). Late viral cytoplasmic RNA was selected by hybridization to a BamHI-HindIII fragment specific for region L1. The selected RNA was then used to determine the structure of region L1 RNAs. The RNA was first hybridized to the HindIII-B fragment¹⁶ of the viral DNA (coordinates 17.0-31.5), ³²P-labelled by nick translation followed by S₁ endonuclease treatment¹⁷ This analysis revealed two major protected species, 520 and 440 nucleotides long (Fig. 2B, lane a). Further mapping of protected fragments showed that VA RNA_I, VA RNA_{II} and a 200nucleotide read through product of the VA RNA_I gene (V₂₀₀)¹⁸ were present in the selected RNA. In the same way, the bands of 73 and 89 nucleotides were shown to correspond to the second and third segments of the common tripartite leader 19,20. The presence of the leader segments in the RNA species which protect fragments of sizes 520 and 440 nucleotides implies that the common tripartite leader is spliced onto their 5' end and that they originate from the major late transcription unit^{6,7}. When the HindIII-B fragment was protected with the selected RNA, digested with S₁, the ³²P-labelled BamHI-HindIII probe hybridized to a 520-nucleotide band, but not to one of 440 nucleotides (data not shown). This demonstrates that the body of the RNA species extends 520 nucleotides into the HindIII-B fragment, and that the 440-nucleotide fragment is encoded elsewhere on the genome (see below).

Recognition signals for splicing coincide with sites for termination of RNA polymerase III

To map the leader-body splice junction at the nucleotide level, we used a modification of the original Berk and Sharp S_I method¹⁷. The ³²P-labelled *HindIII*-B fragment was cleaved with a restriction endonuclease, before hybridization to RNA. If a complementary RNA molecule extends across the restriction enzyme cleavage site, subsequent S₁ analysis will reveal two protected DNA fragments, which will add up to the length of the protected fragment obtained without cleavage.

Cytoplasmic RNA selected by hybridization to the BamHI-HindIII fragment was used for this analysis. Figure 1C shows the cleavage sites for restriction enzymes used to cleave the HindIII-B fragment. Cleavage with endonuclease PvuII resulted in the disappearance of the 520-nucleotide band, and the appearance of two new bands, 275 and 245 nucleotides long (Fig. 2B, lane b). The larger band represents the distance between the PvuII and HindIII site at coordinate 31.5. Therefore, the RNA body extends 245 nucleotides to the left of the PvuII site, positioning the leader-body splice site at coordinate 30.0. Cleavage of the DNA fragment with endonuclease SacII (Fig. 2B, lane c) generated three bands of 125 (coordinates

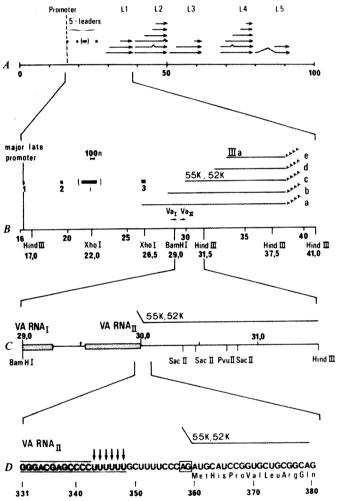


Fig. 1 Organization of the adenovirus 2 genome. A, Schematic diagram of RNAs originating from the major late transcription unit. The 20 identified RNA species are divided into five families (L1-L5), the members of each family having a common position for the poly(A) addition sites. The 5 leaders are spliced onto each of the 20 RNA bodies. The i leader found on a fraction of the RNAs is shown within brackets. B. Enlargement of region 16.0-41.0 encoding the major late promoter site at map coordinate 16.3, the common 5' leaders and the L1 3'-co-terminal family. The position of the VA RNA genes is shown as well as our revised RNA map for region L1. The position of restriction endonuclease cleavage sites relevant to this study are also shown. The mRNAs to which we have assigned translation products are indicated. C, Enlargement of fragment BamHI-HindIII encoding the 5 sequences of the 55, 52K mRNA body. The position of restriction endonuclease cleavage sites used in this study are indicated. D. The leaderbody splice junction of the 55, 52K mRNA. The nucleotide sequence is taken from ref. 9 and the numbering is from the BamHI cleavage site at position 29.0. Arrows indicate the position of the heterogeneous 3' end of VA RNA_{II} and the box shows the A·G dinucleotide invariably found at the 3' side of intervening sequences²¹. The predicted amino-terminal sequence of the 55K and 52K polypeptides is also shown.

Table 1 Early transcription of regions L1, L2 and L3 of the Ad2 genome

Coordinates DNA probe	Region	Length (kilobases)	c.p.m. in hybrid	Molarity
31.5-37.3	L1	2.0	240	1.0
41.0-50.1	L2	3.2	58	0.15
52.6-59.6	L3	1.5	187	1.0

Ad2-infected HeLa cells (500 FFU per cell) were resuspended at 5×107 cells per ml at 4 h.p.i. The cells were labelled with 500 µCi ml-1 of 3H-uridine for 10 min at 37 °C, collected on frozen phosphate-buffered saline and nuclear RNA prepared33. Nuclear RNA from an equivalent amount of cells was hybridized to various Ad2 DNA restriction fragments cloned in the bacterial plasmid pBR322. The cloned fragments were adsorbed to nitrocellulose filters and hybridization was carried out at 65 °C in 6×SSC buffer (1×SSC is 0.15 M NaCl, 15 mM sodium citrate). After hybridization the filters were washed extensively in 2×SSC buffer and treated with pancreatic ribonuclease (20 µg ml-1) for 1 h at 37 °C. Hybridized RNA resistant to the nuclease treatment was measured by liquid scintillation counting. Hybridization to filters containing the pBR322 plasmid were used as background hybridization. This was in all cases fewer than 20 c.p.m. and has been subtracted from each value. The results are the mean from two independent experiments. The length in kilobases was calculated from the size of the Ad2 restriction fragments, assuming that the whole fragments were transcribed. Molarity was calculated by dividing the c.p.m. in hybrid by the length of each fragment and normalizing to 1.0 for the L1 probe (coordinates 31.5-37.3).

30.83-31.5), 235 (30.47-30.83) and 118 nucleotides (splice junction to 30.37), confirming that the splice junction is located at coordinate 30.0. The 5'-end splice site was confirmed by S_1 analysis using a 5'-end-labelled DNA probe (Fig. 2C). The band corresponding to the entire DNA probe is derived from RNA species a and b as they both extend leftward of the BamHI cleavage site at coordinate 29.0.

The genes for the VA RNAs are located close to coordinate 30 on the viral genome^{8,9}. An artefactual cutting site may therefore be created during the S₁ analysis through displacement of the RNA body by VA RNA hybridizing to the same DNA template. To exclude this possibility, RNA was selected on fragment *HindIII-J* (coordinates 37.3–41.0), which is located outside the genes for the VA RNAs. S₁ analysis of this RNA generated the same pattern of bands as did RNA selected on fragment *BamHI-HindIII*, but the bands corresponding to the VA RNAs are missing (Fig. 2B, lane f). This demonstrates that the splice site is not due to VA RNAs hybridizing to the DNA sequences representing the splice junction.

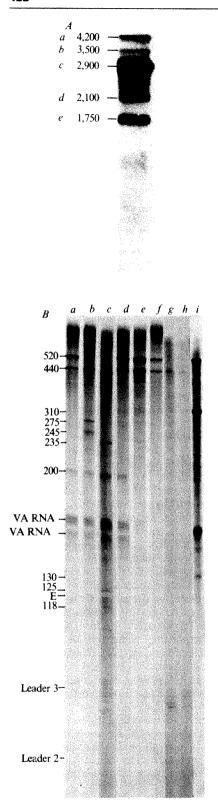
Because almost all known splice sites follow the G·T-A·G rule²¹, and because the nucleotide sequence in this region only contains one A·G dinucleotide, the site at which the third leader segment is spliced on to the RNA body is likely to be located at nucleotide 361 in Fig. 1D. The splice site for this RNA species is thus separated from the termination signal for VA RNA transcription⁹ by only 11 nucleotides (Fig. 1D). This compressed organization of processing signals suggests that the T-cluster, terminating transcription of VA RNA_{II}, also serves as a recognition signal for the enzyme(s) that cleave and ligate the tripartite leader to the RNA body.

Using blot hybridization to analyse DNA protected by BamHI-HindIII-selected RNA from S_1 digestion, the total size of the RNA body was estimated to be 2,900 nucleotides (not shown). From its size and genomic location we conclude that this RNA corresponds to RNA species c (coordinates 30.0-38.5) from region L1 (Fig. 1B).

Identification of an additional leader segment on region L1 RNAs

Electron microscopy of RNA-DNA hybrids has shown that a fraction of late cytoplasmic RNAs from the L1 co-termination family contains an extra leader segment from coordinates 22.0 to 23.2. The 440-nucleotide band observed by S₁ analysis may correspond to this extra leader segment.

The origin of this band was studied by the S₁ endonuclease assay using RNAs selected by hybridization to the BamHI-HindIII fragment. The ³²P-labelled HindIII-B fragment was cleaved with endonuclease XhoI before hybridization to the RNA and subsequent S₁ endonuclease treatment. The 440-nucleotide band was absent after this treatment and instead two



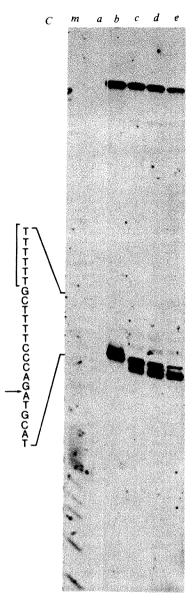


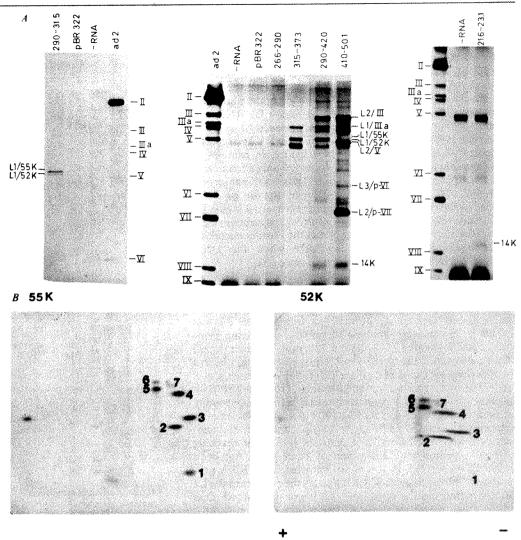
Fig. 2 S₁ endonuclease mapping of late adenovirus cytoplasmic RNA. A, 10 µg of late cytoplasmic RNA, 10 µg of tRNA and 2 µg of Ad2 DNA were mixed precipitated and dissolved in $10 \mu l$ of 50 mM PIPES pH6.4, 0.4 M NaCl, 1 mM EDTA and 80% formamide (buffer A). The DNA template was denatured by incubating the mixture at 70 °C for 10 min, followed by a hybridization for 3 h at 57 °C. The hybridization was terminated by the addition of 400 µl of an ice-cold buffer containing 30 mM NaAc pH 4.4, 4 mM ZnSO4 0.28 M NaCl, 5 µg of single-stranded calf thymus DNA and 5% glycerol. Then 225 units of endonuclease S₁ (Sigma) were added and the reaction mixture further incubated for 20 min at 37 °C. The S_1 cleavage was terminated by the addition of 50 µl S₁ stop solution (3 M NaAc, 80 mM EDTA and 10 µg tRNA) and 800 µl ethanol. The precipitated material was collected by centrifugation and dissolved in 20 µl of a buffer containing 50 mM NaOH, 1 mM NaOH, 1 mM EDTA, 2% Ficoli and market dyes, and separated electrophoretically on a 2% alkaline agarose gel34. After electrophoresis, the gel was soaked in 1 M Tris-HCl pH 7.5, 0.6 M NaCl for 10 min at room temperature and the S₁-protected fragments transferred to a nitrocellulose sheet as described by Southern³⁵. The nitrocellulose filter was subsequently hybridized in 6 × SSC, 3 × Denhardt and 0.5% SDS for 12 h at 65 °C with a 32P-labelled recombinant plasmid containing an insert of the HindIII-I fragment (coordinates 31.5-37.3) of Ad2 DNA¹⁶. The filters were washed in 0.2 × SSC, 0.5% SDS at room temperature for several hours, followed by a 15-min incubation at 65 $^{\circ}\mathrm{C}$ and additional washes at room temperature. The blots were analysed by autoradiography at -70 °C using a DuPont intensifying screen. The HindIII fragments of Ad2 DNA run in a parallel slot on the agarose gel were used as size markers. a-e Relates the S1 protected DNA fragments to the L1 mRNAs. B, Cytoplasmic RNA (as specified below) was mixed with 0.04 µg of a recombinant plasmid containing the *Hin*dIII-B fragment (coordinates 17.0-31.5) of Ad2 DNA16, 32P-labelled by nick translation. The mixture was ethanol precipitated and dissolved in 10 µl of buffer A. The subsequent denaturation, hybridization and S₁ endonuclease treatment were as described above. The precipitate was dissolved in 10 µl of a buffer containing 80% formamide, 1 mM EDTA and marker dyes. Electrophoretic separation was on thin 8% polyacrylamide gels containing 7 M urea and the ³²P-labelled bands were visualized by autoradiography. As size markers, ³²P-labelled ΦX174 DNA, cleaved with endonucleases HaelII and HindII, was included in the gel. In lanes b, c, d and i the HindIII-B clone was cleaved with restriction endonucleases before S₁ treatment. The DNA fragments were mixed with RNA and processed as described

above. Lane a, uncleaved DNA; lane b, PvuII; lane c, SacII; lane d, SaII; lane i, XhoI-cleaved DNA. The RNA used was: in lanes a, b, c, d and i, RNA purified by hybridization selection o on fragment o on f

new bands of 310 and 130 nucleotides were detected (Fig. 2B, lane i). A similar analysis using endonuclease SalI (cleavage sites 26.0 and 26.6) did not cleave the 440-nucleotide band (Fig. 2B, lane d). This shows that the 440-nucleotide band is derived from sequences located around the XhoI cleavage site at coordinate 22.0 (Fig. 1B). The size and location of the 440-nucleotide band suggest that it is identical to the extra leader, designated the i leader, previously identified by electron microscopy¹². As the RNAs used in the S_1 analysis were selected on DNA fragments outside the coding region for the i leader, the results suggest that it is covalently attached to the RNA molecules. RNA selected on fragments BamHI-C (coordinates 41.0-

59.0) and BamHI-A (coordinates 59.0–100) also generated the 440-nucleotide band after S_1 analysis (Fig. 2B, lanes g, h). This demonstrates that the i leader is present on at least a fraction of RNAs derived from all regions of the major late transcription unit. Densitometer tracing of the 520- and 440-nucleotide bands (Fig. 2B, lane a) indicates that there are two types of L1 RNAs, differing with respect to their 5'-leader segments. Type I RNAs have the structure 'leader 1, 2, 3 RNA body', and comprise \sim 40% of the total population, while type II mRNAs have the structure 'leader 1, 2, i, 3, RNA body' and comprise \sim 60% of the total. The 520-nucleotide long band and the leader fragments 2 and 3 were all present in equimolar concentrations.

Fig. 3 In vitro translation of hybridization selected mRNAs. A, Late cytoplasmic Ad2 RNA³⁸ was purified by hybridization to restriction fragments of Ad2 DNA on nitrocellulose immobilized filters³⁶. The hybridization-selected RNAs were translated in a reticulocyte cell-free system and the translation products were analysed by SDS-polyacrylamide gel electro-phoresis³⁸ (SDS-PAGE). The gels were analysed by fluorography. The HindIII restriction fragments were cloned in the plasmid pBR32216 and the hybrid plasmids were used for selection. Hybridization to the bacterial plasmid, pBR322; was used as a control for the specificity of selection. The endogenous synthesis in the cell-free system is shown in slots indicated by -RNA. Ad2 denotes here and in sub-sequent figures a ³⁵S-methioninelabelled Ad2 marker virus. Coordinates for restriction fragments used for hybridization selection are shown at the top of the figure. Restriction fragments used were as follows: SacI-M and -N (21.6-23.1), SalI-BamHI (26.6-29.0), BamHI-HindIII (29.0-31.5),HindIII-I (31.5-37.3), BamHI-D HindIII-D (29.0-42.0), *HindIII-D* (41.0-50.1). The left panel represents a 10% polyacrylamide gel and the right panels 13% polyacrylamide gels. B, Tryptic fingerprint analysis of the 55K and 52K polypeptides. Messenger RNA selected hybridization to fragment BamHI-HindIII (coordinates 29.0-31.5) was translated in a reticulocyte cellfree system in the presence of 35Smethionine. translation products were separated on a 10% SDS-polyacrylamide gel and the 55K and 52K polypeptides were



excised from the gel. Eluted polypeptides were oxidized with performic acid and digested with TPCK-treated trypsin³⁹. Tryptic peptides were separated by electrophoresis in the first dimension followed by ascending chromatography in the second dimension. ³⁵S-methionine-labelled tryptic peptides were visualized by autoradiography.

Polypeptides encoded in region L1

To test whether the RNA species encoded in region L1 code for polypeptides, late cytoplasmic RNA selected on the BamHI-HindIII fragment was used to programme an in vitro translation system. The selected RNA directed the synthesis of two polypeptides with molecular weights of 55,000 (55K) and 52,000 (52K). In addition, the third member of the L1 3'-co-terminal family, polypeptide IIIa²², was detected when RNA was selected by hybridization to fragment HindIII-I (coordinates 31.7-37.3) (Fig. 3A). RNA selected on fragment BamHI-D (coordinates 29.0-42.0) directed the synthesis of the 55K, 52K and IIIa polypeptides from region L1 and polypeptide III from region L2 (Fig. 3A), while the L2 polypeptides III, pVII and V were detected with RNA selected on fragment HindIII-D (coordinates 41.0-50.1). RNA selected on fragment SalI-BamHI (coordinates 26.6-29.0) did not yield any polypeptides (Fig. 3A). This region is absent in all the L1 RNA molecules except in species a and b in Fig. 1B. Interestingly, a 14K polypeptide was synthesized from RNAs selected by hybridization to all DNA probes covering regions L2-L5 (Fig. 3A).

Together, these results demonstrate that the 55K and 52K polypeptides are encoded by mRNA species c, mapping between coordinates 30.0 and 38.5 (Fig. 1B), and that polypeptide IIIa is encoded by mRNA species d or e (coordinates 33.5-38.5). We tentatively assign polypeptide IIIa to mRNA species e (coordinates 33.5-38.5) based on the enhanced accumulation of this species late in infection (see below). RNA selected on the viral r-strand directed the synthesis of the 55K

and 52K polypeptides, confirming that the mRNA is transcribed in a rightward direction (not shown).

The structural relationship between the 55K and the 52K polypeptides was investigated by tryptic fingerprint analysis. A very similar but not identical set of 35 S-labelled peptides was obtained (Fig. 3B), suggesting that these two polypeptides are structurally related. They may therefore be encoded on the same mRNA and their size difference may reflect a post-translational modification which occurs in vitro. We cannot, however, exclude the possibility that the 52K polypeptide is encoded by a minor fraction of species c which has an internal splice in the mRNA body.

Presence of the *i* leader alters the translation of the mRNA

The 55, 52K mRNA was selected by hybridization to fragments SacI-M and -N (coordinates 21.6–23.1) which cover the region of the genome encoding the i leader (not shown). This result is expected because around 60% of the 55, 52K mRNA has the structure 'leader 1, 2, i, 3, RNA body'. However, cell-free translation with SacI-M- and -N-selected mRNA did not result in the synthesis of the 55K and 52K polypeptides (Fig. 3A), but instead, produced a 14K polypeptide; this suggests that mRNA species containing the i leader do not translate the body of their mRNA sequences. Because eukaryotic ribosomes usually initiate translation at the AUG codon closest to the 5'-end²³ of the mRNA, the results suggest that an AUG triplet is encoded within the i leader itself. Late cytoplasmic RNA selected on

DNA fragments distinct from the i leader but covering regions L2-L5, followed by in vitro translation, also resulted in the synthesis of the 14K polypeptide (Fig. 3A). A fraction of these L2-L5 mRNAs contain the i leader, suggesting that the 14K polypeptide is encoded within the i leader itself. Late cytoplasmic RNA selected on DNA fragments covering region L1 showed very little synthesis of the 14K polypeptide. However, this polypeptide was detected when the region L1 RNA was prepared from cells maintained in the presence of cytosine arabinoside (ara C) (Fig. 5B). Most of the L1 mRNAs prepared from ara C-treated cells contain the i leader, in contrast to mRNAs prepared from untreated cells, where only a fraction of the L1 mRNAs contain this leader¹². Thus, our inability to detect the 14K polypeptide among the translation products specified by late RNA selected on fragments covering region L1 may simply reflect a low quantity of RNA containing the i leader. Rigorous proof for the theory that the i leader encodes a 14K polypeptide will become available when the DNA sequence has been determined; this is in progress.

An abundance control for late mRNA production

The temporal appearance of mRNAs from region L1 was studied by S_1 nuclease analysis of poly(A)-containing cytoplasmic RNAs isolated 7, 16, 20 and 30 h post-infection (h.p.i.) (Fig. 4). At 7 h.p.i., the 55, 52K mRNA (species c, Fig. 1B) was abundant and species d and e barely detectable. Species a, b, d and e began to accumulate at late times (16 and 20 h.p.i.). Species e accumulated progressively during infection, becoming the predominant mRNA from L1 at 30 h.p.i., while the amount of species d increased markedly to 16 h.p.i. and then declined. Species a and b remained at a constant level after reaching maximal accumulation. The origin of band x has not been further characterized, as it was not detected in all RNA preparations. The mechanism which regulates the changes in the L1 mRNA pattern during virus infection is not understood. One

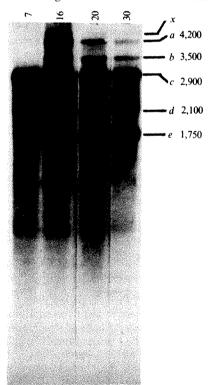


Fig. 4 Demonstration of an abundance control by S_1 endonuclease blot analysis. 2 μg of Ad2 DNA, 5 μg of oligo-d(T)-selected cytoplasmic mRNA isolated at 7, 16, 20 or 24 h.p.i. and 15 μg of tRNA were mixed, precipitated and dissolved in 10 μ l of buffer A. The hybridized RNA was analysed by the S_1 blot assay described for Fig. 2A using a 32 P-labelled recombinant plasmic containing the HindIII-I fragment (coordinates 31.5–37.3) of Ad2 DNA as a probe. a-e Relates the S_1 protected DNA fragments to the L1 mRNAs (Fig. 1B). Numbers at the tops of the slots indicate h.p.i.

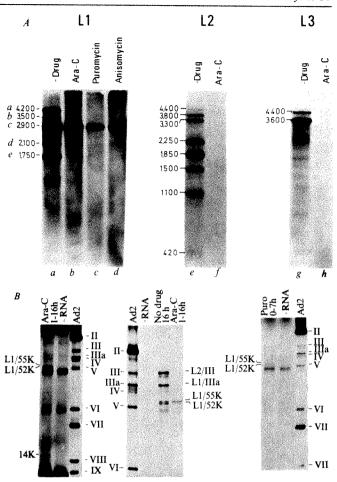


Fig. 5 Analysis of cytoplasmic RNA isolated from cells maintained in the presence of metabolic inhibitors. A, 10 µg of cytoplasmic RNA (specified below), 10 µg of tRNA and 2 µg of Ad2 DNA were mixed, precipitated and analysed by the S₁ blot technique described in Fig. 2 legend. Subsequent hybridization was with ³²P-labelled recombinant plasmids containing inserts of fragment HindIII-I (coordinates 31.5-37.3) (region L1), HindIII-D (coordinates 41.0-50.1) (region L2) and a 680-bp cDNA insert mapping between coordinates 58.8 and 60.7 (region L3). The RNA samples used are: lanes a, e and g, control RNA isolated from cells 16 h.p.i.; lanes b, f and h, RNA prepared from cells maintained in the presence of ara C (25 µg ml⁻¹) from 1 to 16 h.p.i. RNA isolated from cells treated with puromycin (100 μM) or anisomycin (100 µM) from the time of infection to 7 h.p.i. are shown in lanes c and d. The HindIII fragments of Ad2 DNA run in a parallel slot on the agarose gel were used as size markers. a-e Relates the S, protected DNA fragments to the L1 mRNAs (Fig. 1B). B, Cytoplasmic RNA prepared from cells maintained in the presence of ara C (25 µg ml⁻¹) from 1 to 16 h.p.i. or puromycin (100 µM) from the onset of infection to 7 h.p.i. was selected by hybridization to fragment HindIII-J (coordinates 37.3-41.0). The selected mRNAs were translated in a reticulocyte cell-free system and the in vitro synthesized products were analysed by SDS-PAGE. The middle panel represents a 10% polyacrylamide gel, the two other panels are 13% polyacrylamide gels. The gels were analysed by fluorography. The RNA used for hybridization selection is indicated at the top of the figure. -RNA; no RNA added.

possible explanation is a differential splicing of the nuclear precursor transcript at early and late times after infection. Alternatively, the temporal appearance of the mRNAs may be due to virus-regulated changes in mRNA stability during the infectious cycle.

mRNAs from the major late promoter early after adenovirus infection

Detection of the 55, 52K mRNA as early as 7 h.p.i. suggested that the major late promoter is active before the onset of virus DNA replication. To confirm this, we examined RNAs synthesized in the presence of inhibitors which block virus DNA replication. Cytoplasmic RNA, isolated from cells maintained in the presence of ara C, was analysed by the S_1 nuclease assay. Hybridization to a DNA fragment specific for region L1 revealed only species c (Fig. 5A, slot b). Cytoplasmic RNAs

from regions L2 and L3 were not detected in the absence of virus DNA replication (Fig. 5A).

The selective accumulation of mRNA species c early in infection was also demonstrated by in vitro translation of RNA selected on fragment HindIII-J (coordinates 37.3-41.0). RNA isolated from cells treated with ara C yielded the 55K and 52K polypeptides and the 14K polypeptide, whereas cytoplasmic RNA from untreated cells obtained late in infection directed the synthesis of polypeptides III, IIIa, 55K and 52K (Fig. 5B).

Role of newly synthesized viral proteins in major late adenovirus promoter activation

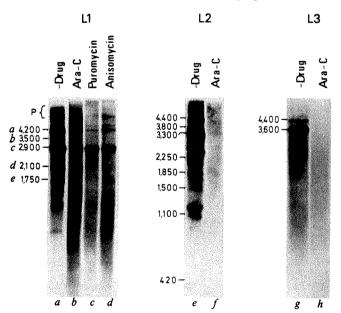
To determine whether virus-specified early proteins are necessary for activation of the major late promoter, we analysed RNA samples prepared from cells maintained in conditions of stringent inhibition of protein synthesis²⁴. Treatment with puromycin or anisomycin from the start of the infection still resulted in selective accumulation of the 55, 52K mRNA (Fig. 5A, slots c, d). Furthermore, the 55, 52K mRNA synthesized in the absence of virus protein synthesis was functionally active when assayed in an *in vitro* translation system (Fig. 5B).

The selective expression of the 55, 52K mRNA from the major late transcription unit, in the absence of protein synthesis, suggests that the activation of the major late promoter, and the splicing and polyadenylation of the 55, 52K mRNA, do not require newly synthesized viral polypeptides. After completion of this study, Lewis and Mathews²⁵ reported similar results concerning the synthesis of the 55K and 52K polypeptides.

Truncated nuclear RNAs accumulate in the absence of virus DNA replication

When virus DNA replication is blocked, cytoplasmic RNAs from region L2 and L3 are undetectable. To determine if the nuclear precursors of these RNAs were present we examined nuclear RNA preparations.

On S₁ analysis of nuclear RNA prepared from cells 18 h.p.i. and not treated with metabolic inhibitors, all cytoplasmic RNA species specific for regions L1, L2 and L3 were detected in nuclear RNA preparations, although the relative abundances in the nucleus differed from those in the cytoplasm. Nuclear



accumulation seems to be greatest for the longest RNAs from each 3'-co-terminal family. The most prominent nuclear RNAs accumulated from region L1 were species a and c (Fig. 6, slot a), but a cluster of long RNAs, ranging in size from 6,000 to 8,000 nucleotides, was also detected, probably representing precursor molecules originating from the major late transcription unit²⁶. Large nuclear RNA species also accumulated from region L2 (bands of 4,400, 3,800 and 3,300 nucleotides, Fig. 6, slot e). Region L3 accumulated nuclear RNAs corresponding to the 4,400- and 3,600-nucleotide long pVI and hexon mRNAs²⁷ (Fig. 6, slot g). The 4,400-nucleotide band seen with the L2 and L3 probes corresponds to the same RNA species, as the pVI mRNA from region L3 extends ~300 nucleotides into the HindIII-D fragment²⁷.

Analysis of nuclear RNAs, prepared from cells where virus DNA replication was blocked, showed a selective accumulation of RNA species a and c from region L1 (Fig. 6, slots b-d). We also detected RNA molecules co-linear with the DNA template from the promoter site at coordinate 16.3 up to the poly(A) addition site at coordinate 38.5. Similar results were obtained when protein synthesis was inhibited with puromycin or anisomycin from the start of virus infection.

Nuclear RNA species from region L2 were barely detectable in RNA preparations from cells treated with ara C (Fig. 6, slot f) and only the 3,300-nucleotide long RNA species was discerned. Nuclear RNA from region L3 was not detected (Fig. 6, slot h), demonstrating that, in the absence of virus DNA replication, RNA beyond coordinate 59 does not accumulate.

To determine the length of the transcript from the major late promoter at early times after infection, cells were pulse-labelled for 10 min at 4 h.p.i. with ³H-uridine and nuclear RNA was prepared. The nuclear RNA was hybridized to cloned DNA fragments covering different regions of the major late transcription unit. Table 1 shows that transcripts from region L1 were 5-10-fold more abundant than from region L2 early in infection. However, there was marked hybridization to the probe from region L3, probably reflecting the fact that the leftward transcript for early region 2 terminates within the double-stranded DNA probe used for the L3 region (cited in ref. 28). The low hybridization to region L2 compared with region L1 together with the S₁ analysis of nuclear RNA suggest that transcription terminates within the L2 region at early times of infection. Similar results were obtained with pulse-labelled RNA from cells treated with anisomycin from the start of virus infection (not shown). The length of the transcript from the major late promoter early in infection was independently determined by Shaw and Ziff²⁹.

Conclusions

We have analysed RNAs originating from the major late transcription units of adenovirus type 2, at different times after infection. The results suggest that the shift from the early to the late phase of virus replication does not involve activation of the major late promoter at map coordinate 16.3. Rather, this promoter is already active during the early phase. Furthermore, the synthesis of viral polypeptides is not required for its activation, as mRNA from the major late promoter is produced under stringent inhibition of protein synthesis24. A preferential synthesis of mRNA species c from region L1 (Fig. 1B), which encodes the two structurally related polypeptides of molecular weight 55,000 and 52,000, was observed at early times. The lack of detectable amounts of L2 and L3 mRNAs may be explained by the fact that nuclear transcripts produced at early times of infection seem to terminate within the L2 region. Therefore, the shift from the early to the late phase of virus replication may involve a mechanism which allows the production of larger transcripts from the major late transcription unit. This mechanism could be mediated by virus-coded factors which associate with the RNA polymerase, by a change in the nucleoprotein structure of the DNA template, or by DNA replication providing single strands of virus DNA which may serve as a more efficient template for late transcription.

The selective accumulation of the 55, 52K mRNA early in infection may be explained by a virus-regulated abundance control of late mRNA production. At different times after infection the individual mRNAs from region L1 (Fig. 1B, species a-e) are differentially expressed. The first mRNA to accumulate in the cytoplasm is the 55, 52K mRNA, whereas at late times of infection, species e, encoding virion polypeptide IIIa, is the most abundant. Thus, the selective appearance of the 55, 52K mRNA early in infection may reflect a regulation of the abundance of region L1 mRNAs. This regulation is probably accomplished by virus-regulated changes in the splicing pattern of the transcript but may also depend on virus-regulated changes in cytoplasmic mRNA stabilities. However, the latter alternative is unlikely because the mRNA stability seems to be enhanced at late times of infection30

As RNA polymerase III transcription normally terminates within a stretch of U residues³⁴, and the termination signal for VA RNA_{II} transcription is separated by only 11 nucleotides from the splice junction of the 55, 52K mRNA (Fig. 1D), the

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- Ziff, E. B. & Evans, R. M. Cell 15, 1463-1475 (1978)
- Evans, R. M. et al. Cell 12, 733-739 (1976). Evans, R. M. et al. Cell 12, 733-739 (1977). Nevins, J. R. & Darnell, J. E. J. Virol. 25, 811-823 (1978). Ziff, E. B. & Fraser, N. J. Virol. 25, 897-906 (1978).

- McGrogan, M. & Raskas, H-J. Proc. natn. Acad. Sci. U.S.A. 75, 625-629 (1978). Berget, S. M., Moore, C. & Sharp, P. A. Proc. natn. Acad. Sci. U.S.A. 74, 3171-3175

- (1977).
 Chow, L. T., Gelinas, R., Broker, T. & Roberts, R. J. Cell 12, 1-8 (1977).
 Mathews, M. B. & Pettersson, U. J. molec. Biol. 119, 293-328 (1978).
 Akusjärvi, G., Mathews, M. B., Andersson, P., Vennström, B. & Pettersson, U. Proc. natn. Acad. Sci. U.S.A. 77, 2424-2428 (1989).
- Chow, L. T. & Broker, T. Cell 15, 497–510 (1978).
 Fraser, N. W., Sehgal, P. B. & Darnell, J. E. Proc. natn. Acad. Sci. U.S.A. 76, 2571–2575 H. Praser, N. W., Sengar, F. B. & Darnen, J. E. Proc. nam. Acad. Sci. U.S.A. 16, (1979).
 Chow, L. T., Broker, T. R. & Lewis, J. B J. molec. Biol. 134, 265–303 (1979).
 Kitchingman, G. R. & Westphal, H. J. molec. Biol. 137, 23–48 (1980).

- Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. Cell 18, 469-484 (1979).
 Wasylyk, B., Kedinger, C., Corden, J., Brison, O. & Chambon, P. Nature 285, 367-372
- Stenlund, A., Perricaudet, M., Tiollais, P. & Pettersson, U. Gene 10, 47-52 (1980).
- Berk, A. J. & Sharp, P. A. Cell 12, 45-56 (1977)
- Weinmann, R., Brendler, T. G., Raskas, H. J. & Roeder, R. G. Cell 7, 557-566 (1976).
- 19. Akusjärvi, G. & Pettersson, U. J. molec. Biol. 134, 143-158 (1979).

acceptor site of the 55, 52K mRNA has a U-rich sequence directly upstream from its splice junction. This sequence arrangement resembles splice sites found in several eukaryotic genes³². If the abundance control of late mRNA production involves virus-controlled splicing events, the appearance of the 55, 52K mRNA at early times of infection may reflect a preference for the acceptor site of the 55, 52K mRNA.

The 55, 52K mRNA exists at about equal frequency in two forms which differ by the presence of the i leader. In the RNA species which have an insertion of the i leader within the tripartite leader, the expression of the polypeptides from the mRNA body seems to be blocked. This suggests that insertion of the i leader, by splicing, controls gene expression at the level of translation.

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- 20. Zain, S., Gingeras, T. R., Bullock, P., Wong, G. & Gelinas, R. E. J. molec. Biol. 135,
- Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. Proc. natn. Acad. Sci. U.S.A. 75, 4853–4857 (1978).
- Lewis, J. B., Anderson, C. W. & Atkins, J. F. Cell 12, 37-44 (1977). Kozak, M. Cell 15, 1109-1123 (1978).
- Persson, H., Monstein, H.J., Akusjärvi, G. & Philipson, L. Cell 23, 485-496 (1981). Lewis, J. B. & Mathews, M. B. Cell 21, 303-313 (1980).
- Berget, S. M. & Sharp, P. A. J. molec. Biol. 129, 547-565 (1979).
 Akusjärvi, G. & Persson, H. J. Virol. 38, 469-482 (1981).
- Nevins, J. R. et al. J. Virol. 32, 727-733 (1979) Shaw, A. R. & Ziff, E. B. Cell 22, 905-916 (1980)
- Wilson, M. W., Nevins, J. & Darnell, J. E. Cold Spring Harb. Symp. quant. Biol. 44, 447-455
- Korn, L. I. & Brown, D. D. Cell 15, 1145-1156 (1978)
- Benoist, C., O'Hare, K., Breathnach, R. & Chambon, P. Nucleic Acids Res. 8, 127-142 (1980)
- Smith, M. M., Reeve, A. E. & Huang, R. C. C. Cell 15, 615-626 (1978).
- Favaloro, J., Treisman, R. & Kamen, R. Meth. Enzym. (in the press) Southern, E. M. J. molec. Biol. 98, 503-518 (1975).
- McGrogan, M., Spector, D. J., Goldenberg, C. J., Halbert, D. & Raskas, H. J. Nucleic Acids Res. 6, 593-607 (1979).
- Weaver, R. S. & Weismann, C. W. Nucleic Acids Res. 7, 1175-1193 (1979).
- Persson, H., Pettersson, U. & Mathews, M. B. Virology 90, 67-79 (1978).
 Persson, H., Jansson, M. & Philipson, L. J. molec. Biol. 137, 375-394 (1980)

Diversity of germ-line immunoglobulin V_H genes

David Givol, Rina Zakut, Kim Effron, Gideon Rechavi, Daniela Ram & Justus B. Cohen

Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

The sequences of four embryonic mouse immunoglobulin V_H genes have been compared. All genes end at codon 98 and code for a hydrophobic signal peptide of 19 residues interrupted at codon -4 by an intron of 83 base pairs. Substitutions occur in all gene segments but at a significantly higher frequency in the hypervariable regions. The data suggest an evolutionary basis for the diversity of immunoglobulin genes. Divergence resulted also in a termination codon in two of the genes, suggesting that part of the V gene repertoire cannot be expressed unless some correction mechanism is available.

IMMUNOGLOBULINS are composed of heavy and light chains. Their variable region (V_L and V_H) is confined to the amino-terminal domain of the molecule and comprises the antibody-combining site. The combining site is formed by three hypervariable segments of the V region which contain the complementarity-determining residues (CDR1-3) for antigen^{1,2}. The variable region of the light chain is encoded in the germ line by three separate gene segments: the leader (L), coding for a hydrophobic signal peptide which is not present in the mature protein, the variable segment (V_L) and the joining segment (J_L)³⁻⁵. The variable region of the heavy chain is encoded by four gene segments: the leader (L), the variable segment (V_H), the 'diversity' segment (D) and the joining segment (J_H). The D and J_H segments contain the codons for CDR3 whereas the V_H segment contains the codons for CDR1 and CDR2 (refs 6, 7). It has been shown that there are only four J_H gene segments⁷. Amino acid sequence data indicated that the genes for the variable regions are organized in subgroups which evolved by gene duplication^{8,9}. Expansion and contraction of these subgroups and evolutionary accumulation of mutations contribute to the diversity of these genes^{8,9}. Recent analysis at the DNA level demonstrated multiple genes in subgroups of both $V_{\rm L}^{\ 10}$ and $V_{\rm H}^{\ 11}.$

A basic problem in immunology is the extent of inherited diversity compared with the diversity generated somatically in the V genes. The V_H segment is present in two different DNA environments during the life of the antibody-forming cells, the embryonic and the mature environments. During lymphocyte differentiation DNA rearrangement joins V with D and J (V-D-J joining) to form the active V_H gene⁶. As evidence for recombination of V_H with each of the four J_H segments has been found^{6,7} and the joining is flexible, part of the V-region diversity in CDR3 is generated somatically by this recombination. These recombination events involve the cutting and joining of DNA

Table 1 Per cent substitution in V_H-gene segments

		Non-coding	segments				Coding se	gments	
		Tron tooning		Signal 1	peptide	F	R	CI	OR
Clone	5' region	Intron	3' region	R	s	R	S	R	S
pCh108B pCh104 pCh111	15 23.8 23.8	15.7 25.3 21.7	20.0 37.9 37.9	7.0 12.3 10.5	1.8 1.8 5.3	4 5.34 6.2	4 4.4 5.8	11.6 30.4 27.5	0 1.5 4.3

The per cent substitution was calculated as compared with the pCh108A sequence. In the coding region substitutions are divided into replacement (R) and silent (S) substitutions. The 3' region was calculated beyond the recombination signals. FR, framework regions of V_H . CDR, complementarity-determining residue regions of V_H .

segments and may be accompanied by other enzymatic activities, such as error-prone repair 12,13 , which result in further substitutions along the $V_{\rm H}$ segment. The question arises as to whether the hypervariability found in CDR1 and -2 pre-exists in the germ line or is generated in the new DNA environment only after somatic rearrangement.

To analyse this question we isolated and sequenced four mouse $V_{\rm H}$ genes isolated from a BALB/c mouse embryo DNA library ¹⁴. These genes belong to the $V_{\rm H}$ II subgroup. Their nucleotide sequence shows 80–90% homology with the cDNA clone of mouse myeloma MPC11 heavy chain ¹⁵ that was used as a probe in isolating these genes. The data indicate that the hypervariability in the regions corresponding to CDR1 and -2 is already present in the embryonic non-rearranged $V_{\rm H}$ genes, suggesting that the substitution in hypervariable regions is a result of an evolutionary process. The sequence also shows that two of the $V_{\rm H}$ genes contain termination codons within their coding region and may be considered pseudogenes. Perhaps this is the price paid for keeping a multiple gene family like the immunoglobulin V genes.

Characterization of V_H-containing clones

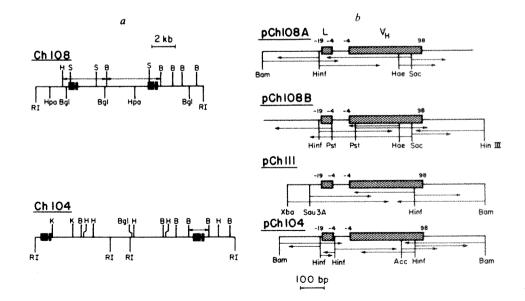
We isolated eight cross-hybridizing clones by screening our mouse embryo DNA library with plasmid $pV(11)^2$ which contains V_H cDNA derived from myeloma MPC11 (ref. 15). Three of these clones that showed strong cross-hybridization were selected for sequencing. One of them (Ch111) contained only one V_H gene; the others contained two V_H genes. In clone Ch108 the two V_H genes are located on a single EcoRI fragment with a spacing of ~ 7 kilobases (kb) (Fig. 1a) suggesting that V_H genes which belong to the same subgroup are close to one another in the genome. Clone Ch104 contained two V_H genes on

separate EcoRI fragments with a spacing of ~ 12 kb. The appropriate segments containing the V_H genes were subcloned in pBR322 and subjected to DNA sequencing, according to the strategy shown in Fig. 1b. The sequence of ~ 800 base pairs (bp) was determined for each of the genes, and compared with that of MPC11 cDNA to locate the coding region. The overall structure of all four genes demonstrates their germ-line nature as the coding sequence ends prematurely at codon 98 and contains neither D nor J segments. The codon corresponding to amino acid 1 is preceded by a sequence that codes for a signal peptide 16.17 of 19 amino acids which is interrupted at codon ~ 4 by an intron of 83 bp.

Sequence of the 5'-noncoding and leader region

Figure 2 depicts the sequence of the four mouse embryonic V_H genes upstream from codon 1 of the mature protein, in comparison with MPC11 cDNA sequence 15. It is clear that the V_H genes, although homologous, are variable in the region corresponding in part to the 5'-untranslated region of the mRNA. Hence, the variability is not confined to the V region, but extends into the 5'-untranslated region. Thus heavy-chain mRNA has a variable 5'-untranslated region while the 3'untranslated region is constant¹⁸. The sequence coding for the 19 residues which compose the hydrophobic signal polypeptide of the precursor heavy chain is separated at residue -4 (Gly) by an intron of 83 nucleotides which starts with GT and ends with AG. The size of this intron in all four genes is identical and differs by 2 nucleotides from the size of another mouse V_H intron⁷ and by 21 nucleotides from a human V_H intron which is 104 bp long¹⁶. It is significant that the location of this intron at codon –4 is identical in mouse $V_{\kappa}^{5,20}$, V_{λ}^{21} and $V_{H}^{6,7}$, as well as in

Fig. 1 Restriction enzyme maps of the mouse DNA inserts in clones Ch108 and Ch104 (a), and strategy used in sequencing of the subclones pCh108A, pCh108B, pCh111 and pCh104 (b). Cleavage sites were determined by single and double digestion of DNA with various restriction enzymes and by the endlabelling method of Smith and Birnstiel³³. The DNA segments between arrows in a were subcloned in pBR322. Sequence determination was by the chemical degradation method³⁴ of end-labelled fragments. End-labelling was done at the 3' end by site filling of 5'-protruding ends with reverse transcriptase. The reaction mixture (20 µl) contained 1-5 μ g of DNA fragments, 20 μ Ci of one or two $[\alpha^{-32}P]$ deoxynucleoside triphosphates and 5 units of reverse transcriptase, and was incubated at 37°C for 30 min. Blunt-ended fragments were labelled at the 3' end



using DNA polymerase (n.Klenow) and the appropriate $[\alpha^{-32}P]$ dNTP. 3'-protruding ends were labelled with $[\alpha^{-32}P]$ cordycepin triphosphate and terminal deoxynucleotidyl transferase³⁵. Enzymes used: RI = EcoRI; B = BamHI; H = HindIII; Bgl = BglII; K = KpnI; S = SacI, Hpa = HpaII; Hae = HaeIII; Acc = AccI; Hinf = HinfI; Pst = PstI; Sau = Sau3A. In b the numbers above the schemes represent codons for amino acids.

		pCh 108A	TGCTGCTTGA	CCTATGAACC TITTAAGTCC TTCCTCTCCA
		pCh 108B		C-A-TAC
		pCh 104)G-TGCCG GTT
		pCh 111		G-TCTG(
		•		5 1
108A	TCTATTCTCC ATTTAGATTG GTTATTATAT	ACAAAGTCCC	CTGCTCATGA	ATATGCAAAT TACCTAAGTC TATGGTAGTT
1088	CCGGC-A -A-CG-		******	
104	TCAGGAC-ATCC-			
111				CGCTC T
	,	5. 7G-A-		
108A	AAAAACAGGG ATATCAACAC GCTGAAAACA	ACATATGTCC	AATGTCCTCT	CCACAGACAC TGAACACACT CACTCTAACC
108B	C			
104	TTGC C			
111	TAGCT T			
	-19 -15	-10		-4
	-19 -15 Met Gly Trp Ser Trp Ile Phe Leu F		Ser Gly Thr	
108A	Met Gly Trp Ser Trp Ile Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T	he Leu Leu	,	Ala G
108A 108B	Met Gly Trp Ser Trp IIe Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T IIe Lys Ser	TC CTC CTG	TCA GGA ACT	Ala G GCA G/GTAAGGGGC TCACCATTTC
108B	Met Gly Trp Ser Trp Ile Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T Ile Lys Ser A AA CT CGlu Cys Val	he Leu Leu TC CTC CTG	TCA GGA ACT	Ala G GCA G/GTAAGGGGC TCACCATTTCA-G
	Met Gly Trp Ser Trp Ile Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T Ile Lys Ser A AAA	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT	Ala G GCA G/GTAAGGGGC TCACCATTTCA-G
108B	Met Gly Trp Ser Trp Ile Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T Ile Lys Ser A AA CT CGlu Cys Val	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT- Val	Ala G GCA G/GTAAGGGGC TCACCATTTC
108B 104 111	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	TC CTC CTG	TCA GGA ACT Leu TT Val Val	A1a G GCA G/GTAAGGGGC TCACCATTTC GCACATTTC GCA
108B 104	Met Gly Trp Ser Trp Ile Phe Leu F ATG GGA TGG AGC TGG ATC TTT CTC T Ile Lys Ser A AA	TC CTC CTG	TCA GGA ACT Leu TT Val Val	A1a G GCA G/GTAAGGGGC TCACCATTTC GCACATTTC GCA
108B 104 111 M 11	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu Val TT- Val TT- Val	Ala G GCA G/GTAAGGGGC TCACCATTTC AA-G C ly Val His Ser
108B 104 111 M 11 108A	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu Leu TT- Val T Val T TCCACTCTGT (Ala G GCA G/GTAAGGGGC TCACCATTTC AAG C
108B 104 111 M 11	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT Val T Val T TCCACTCTGT (Ala G GCA G/GTAAGGGGC TCACCATTTC AA-G AG AG Iy Val His Ser TTTTCTCTCC TCAG/GC GTC CAC TCT TAT Lle Cos
108B 104 111 M 11 108A	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT Val T Val T TCCACTCTGT (Ala G GCA G/GTAAGGGGC TCACCATTTC AAG
108B 104 1111 M 11 108A 108B	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT- T- Val TCCACTCTGT (Ala G GCA G/GTAAGGGGC TCACCATTTC
108B 104 111 M 11 108A 108B	Met Gly Trp Ser Trp Ile Phe Leu P. ATG GGA TGG AGC TGG ATC TTT CTC TT Ile Lys Ser	he Leu Leu TC CTC CTG	TCA GGA ACT Leu TT- T- Val TCCACTCTGT (Ala G GCA G/GTAAGGGGC TCACCATTTC

Fig. 2 Sequence of the 5' segment of four embryonic V_H genes including the codons for the signal peptide. Identical nucleotides are represented by dashes, insertions are introduced above the line and an empty space indicates a deletion. The region between parentheses was not determined (ND). Amino acid residues predicted by the nucleotide sequence are given above the line of the codons. The insert of MPC11 (M11) cDNA clone 15 begins at codon - 14 and the coding sequence of the signal peptide was determined in this cDNA clone (unpublished work).

the human V_H gene¹⁹. The homology at the two ends of the introns (first 30 bp and last 25 bp) in our genes is much greater than that in the middle part. The sequence of another reported V_H intron⁷ is significantly different (50% substitution) from the four sequences shown here (18-25% substitution) and indicates that V_H genes of different subgroups have diverged markedly from one another. The coding sequence for the signal peptide is not identical within the subgroup and the per cent substitution is similar to that of the V_H-coding region. However, comparison with sequences coding for signal peptides in V_H genes of other subgroups^{6,7,22} shows a greater divergence from our genes. The accumulation of mutations is proportional to the divergence time of the genes, and our comparison indicates that the leader sequence is part of the V_H duplication unit. Note that the middle highly hydrophobic region of this predicted amino acid sequence (residues -15 to -3) is very similar in all four genes and changes are more significant at the two ends of the sequence coding for the signal peptide.

The data also show that clone pCh108B has a substitution which changes the first Met (ATG) into Ile (ATA). Unless there is some correction mechanism, the mRNA of this gene may not be translated into an active protein. The validity of this substitution was checked in the original Ch108 clone.

Sequence of V_H -coding region and the recombination signals

Figure 3 shows the sequence of the V_H-coding region and the sequence downstream (3') from it. Comparison with the MPC11 sequence15 and with known JH segments7 demonstrates that all four genes end prematurely at Arg 98 which is exactly the end of framework III segment (no. 94 in the numbering system of Kabat et al.²³). Hence the D segment in the MPC11 V region starts at the beginning of CDR3 and contains eight amino acids (Gly-Ile-Tyr-Tyr-Asn-Ser-Ser-Pro). The V_H-coding sequence clearly demonstrates that substitutions occur preferentially at CDR regions (see later for comparison). When substitutions at the framework regions do occur, they are usually confined to the same position in all four genes. Most of these positions, such as codons 13, 16, 40, 41, 43 and 74, code for amino acid residues in which there is little structural constraint on replacements in the folded V domain. Comparison of these positions with homologous residues in three-dimensional models of V regions demonstrates that these residues are present in positions which

are completely exposed to solvent. It was shown at the protein level that amino acids which are buried in the domain are more conserved than those that are exposed to solvent 24,25 . Many of the substitutions in the framework result in two alternative codons causing conservative changes of amino acids. In several places the extent of diversity is not clear from this limited analysis. For example, codons 81-85 may be analogous to the fourth hypervariable region 26 of human V_H and the limited variations shown here may indicate the presence of this region in mouse embryonic genes.

At the 3' end of these V_H genes there are two recombination signals separated by 23 or 21 (pCH111) bp. The first signal

CACAGTG starts immediately after the codon for Arg 98 whereas in other cases there are 2 or 3 bp between $V_{\rm H}$ and this signal. This sequence is similar to that found in mouse $V_{\rm H}$ and $V_{\rm L}$ and in human $V_{\rm H}$ genes^{3,5-7,19} regardless of subgroups, and it is inversely complementary to a heptanucleotide present just 5' to the $J_{\rm H}$ region. As also noted by others, the second signal nonanucleotide is dA rich and contains more substitutions than the first signal. The sequence between the two signal oligonucleotides is highly homologous within this subgroup while the sequence of the 3' region beyond the second signal diverged more than other segments in these genes.

The immune V-gene repertoire may be rich in pseudogenes

Two of the genes, clones pCh104 and pCh111, contain termination codons at positions 39 and 94 respectively. Because there are some 40 substitutions in the V_H-coding region (294 bp long), a high proportion of these genes may have one substitution leading to a terminator. This is perhaps the price paid for keeping a multiple gene family (like immunoglobulin V genes) in evolution. The survival of the animal does not depend on any individual member of the V-gene family and selection operates on the entire V-gene repertoire to increase the number and diversity of V genes. The evolutionary accumulation of substitutions in V genes is essential for the generation of antibody diversity, and it is unavoidable that some of these genes will also acquire termination codons. The V genes which contain terminators may be pseudogenes²⁷. However, they differ from other pseudogenes reported. Recently Bentley and Rabbitts²⁸ described a human V_s pseudogene containing terminators,

deletions and insertions which result in changes of reading frame. Other pseudogenes also follow this pattern of changes^{29,30}. In contrast, pCh104 and pCh111 described here have one termination codon but otherwise conform to the sequence of a normal V_H gene. We suggest that these genes may be used if there is some correction mechanism. Such a mechanism may be the presence of suppressor tRNAs in lymphocytes or some other mechanism which allows reading through these terminators. Another possibility is that recombination between non-allelic V genes occurs at a high frequency^{10,31} so that their coding potential can be used to form active genes. It is of interest that the presence of pseudogenes in other gene families was recently explained as the consequence of gene duplication and divergence²⁹.

Evolutionary diversity of V_H genes

We analysed by DNA sequencing four cross-hybridizing embryonic mouse V_H genes which belong to subgroup II. The data indicate that these genes accumulated mutations in all their segments (5' region, leader, intron, V_H segment and 3' region), but differences were found in the extent of these substitutions in the various segments. We calculated the substitutions in the different parts of these genes as shown in Table 1. The per cent substitutions in noncoding regions (5', 3' and the intron) is higher than in the coding regions. However, the homology within

	C1	tra t	C1m	1	Cln.	C1+	Sar	Clv	Pro	GIn	Leu	Va1	I va	Pro	GIV	Ala	Ser	Val	Ivs	20 11e	
108A	GAG	GTC	CAG	CTT	CAG	CAG	TCA	GGA	CCT	GAG	CTG	GTG	AAA	CCT	GGG	SCC	TCA	GTG	AAG	ATA	
1088				~=G		***				~ • •											
104	Gln						7						Arg ~GG			Thr A-T					
104	Gln								Ala				Arg			Thr				Lys	
111				G			T		G~-		•••	4-4	~GG		A	A-T				-AG	
Mll	Gin C			~~G			T		G			A	-G-			A+T				G	
	•																				
		Cur.	1		5	C1.	Ture	The	Dha	30	Asp	Tvr	Sen	Met	His	Trn	Val	Lvs	Gin	40 Ser	
108A	TCC	TEC	AAG	GCT	TCT	GGA	TAC	ACA	TTC	ACT	GAC	TAC	AAC	ATG	CAC	TGG	STG	AAG	CAG	AGC	
	Thr					Asp		Ser			Gly -G-		He		AST						
1088										Leu	Thr		Tro		ASTI				Ter	Met	
104						C	T	€		CTC	AC-		TGG		A				T	-TG	
										Ala	Asn A		Trp	He	Gly		A			Arg	
111					Ala			(.		0	Asn		Trp	He	Gly				Glu	Arg	
M11		•••	***		G						Ā				GGT				G+-	G	
										50			CDR	1						60	
	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Tyr	lle	Тут	Pro	Tyr	Asn	Gly	Gly	Thr	Gly	Tyr	
108A	CAT	GGA	AAG	AGC	CTT	GAG	TGG	ATT	GGA	TAT	ATT	TAT	CCT	TAC	AAT	GGT	GGT	ACT	GGC	TAC	
1088						A				Gla G-A	~~~	Asn A									
	Pro		Gln	Glv						Ala		Phe		Ala	Gly		Ser		Asn		
104	~Ç~	***	C	G Gly						GCG		- T -		GUA	GG-		Val		Asn		
111	-C-		C-T	G						G		C		GGA	G-C		-T-		AA-		
	Pra		Hite	GIV						ASD				GIV	GIV		7756		Asn		
M11	-6-		C-1	6											DR 2		.,-		747-		
										70	· · ·	11-1				C	e	The	41.0	80	
108A	Asn	Gln	Lys	Phe	Lys	Ser	Lys	GCC	ACA	TTG	ACT	GTA	GAC	AAT	TCC	TCC	AGC	ACA	GCC	TAC	
toox						GIV								Inr							
1088						G								-CA							
104	T		Met -T-			G								-CA	~						
		C1				C1.						4.1 a		1.28							
111	7	610	Aşr			GIN				C		Ala		Thr			~			,	
M11	~-T	G	7			G-				C		-C-		-CA			~				
							•			90											
	Met	Glu	Let	Sex	Sex	Les	Thi	Ser	Glu	Ast	Ser	Ala	Va 1	Tyr	Tyr	Cys	Ala	Arg			
108A	ATO	GAG	CTO	CAGO	: AGG	: CTG	AC#	TC1	GAC	GAC	TCT	GCA	. GTC	TAT	TAC	TGT	GCA	AG#	CAC	AGTG	TTA
1088				Hi: CA:								Leu								0	G
															12 h e						
104		C		٠	Arı	:						6		Ter	-1-						
111				r														***		C	G
		Gli	3										116	(H15				GIY	ATT	Tyr Tyr TAC TAC
M11		Ç																		r	
																					, •
108A	CAA	ACAC	ATC	CTGA	GTGT	GT C	AGAA	ACCC	T GA	GGTG	CAGC	AAG	CTTC	CTT	GGGA	CTGA	CA A	GACT	TAGA	G AAT	AGTEGET
1088																					TA
104	T	C								AGA-	G		G-	AC-			G- T	CA	G-A-	T-	- AA-
111																					-AA
108A	700	AGAT	יצנידי:	CTTA	GATO	CA C	TYCAT	TTGA	A TO	GTTC		GTG									
1088																					
		- 01							- "												
104	-	m		6		TT		70	. 47		A	A-T									
104 111											A										

Fig. 3 Sequence of V_H segment and 3'-flanking region of the four embryonic genes and MPC11 cDNA. CDR regions are underlined. Recombination recognition signals^{6,7} at the 3' end of the coding segment are boxed. Amino acid residues deduced from the DNA sequence are given above the line of the codons. Ter indicates termination codons. Codon 98 corresponds to residue 94 in the numbering system of Kabat et al.²³ and it is the last residue of FRIII.

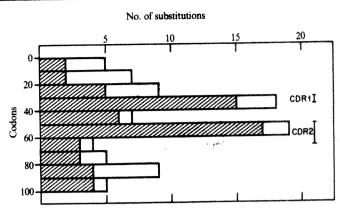


Fig. 4 Distribution of nucleotide substitutions along the $V_{\rm H^-}$ coding segments in all four genes sequenced. Bars represent total substitutions in blocks of 10 codons. Hatched areas represent total replacement substitutions.

noncoding segments of these genes is greater than the homology between these genes and genes of other subgroups. Moreover, the homology between neighbouring genes (pCh108A and pCh108B) is greater than the homology between them and the two other genes sequenced (pCh104 and pCh111). This suggests that V-gene duplication and diversity is a continuous evolutionary process, and the longer the divergence time, the more the mutations accumulate.

An important question is the extent of diversity in CDR regions of the germ-line V_H segment and whether this diversity occurs throughout evolution or after gene rearrangement. The data (Table 1 and Fig. 4) clearly indicate that the per cent substitutions in CDR is greater than in framework regions. Moreover, the per cent of replacement (non-silent) substitutions in CDR is significantly higher than that in the framework. Often more than one nucleotide in a codon is substituted, resulting in non-conservative replacement of amino acids. There are also invariable codons in CDR (like codons 32 in CDR1 and codons 51, 53, 56, 58, 60 and 61 of CDR2) which code for structural residues in CDR segments as suggested previously from the amino acid sequence 32. The data in Table 1 clearly show that the diversity in CDR1 and -2 is present in the germ line, and is generated throughout evolution. Evidence for additional somatic mutations in the hypervariable regions of V_A, V_K or V_H genes has previously been reported^{3,5,7,20}. We have not yet sequenced the embryonic gene that is expressed in myeloma MPC11. However, the sequence of the germ-line J region can be compared with its expressed counterpart. As there are only four germ-line JH-gene's egments, it is easy to locate somatic mutations in this region. A comparison of embryonic J_{H2}⁷ with that expressed in myeloma MPC11 shows a change in the fourth codon, from TAC(Tyr) to TCC(Ser).

This change is clearly an example of somatic mutation in CDR3, and is not merely a result of modulation of the joining site itself.

Conclusion

The DNA region containing the immunoglobulin V genes in a species is composed of several related families (subgroups) of genes. These undergo duplication and accumulate substitutions in all their segments in a process which is similar to the gene diversification that occurs among different species throughout evolution. The duplication unit of the V gene includes the sequence coding for the signal peptide. The entire V-gene

repertoire must be degenerate and contains nonsense mutations and pseudogenes. We may speculate that some correction processes, not yet found, have developed in lymphocytes to enable them to use the genetic potential encoded in such genes. The CDR regions, which are most essential for the function of the antibody, are hypervariable in the germ-line genes and after

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- Wu, T. T. & Kabat, E. A. J. exp. Med. 132, 211-250 (1970).
- Kabat, E. A. & Wu, T. T. Ann. N.Y. Acad. Sci. 190, 382-391 (1971).
- Bernard, O., Hozumi, N. & Tonegawa, S. Cell 15, 1133-1144 (1978).
 Brack, C., Hirama, M., Lenhard-Schuller, R. & Tonegawa, S. Cell 15, 1-14 (1978)
- Max, E. E., Seidman, J. G. & Leder, P. Proc. nath. Acad. Sci. U.S.A. 76, 3450-3454 (1979). Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. Cell 19, 981-992 (1980).
- Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. Nature 286, 676-683
- Milstein, C. & Pink, J. R. L. Prog. Biophys. molec. Biol. 21, 211–263 (1970). Smith, G. P., Hood, L. & Fitch, W. M. A. Rev. Biochem. 40, 969–1012 (1971)
- Seidman, J. G., Leder, A., Nau, M., Norman, B. & Leder, P. Science 202, 11-17 (1978). Rabbitts, T. H., Matthyssens, G. & Hamlyn, P. H. Nature 284, 238-243 (1980).
- Brenner, S. & Milstein, C. *Nature* 211, 242-243 (1966). Baltimore, D. *Nature* 248, 409-411 (1974).

- Zakut, R., Givol, D. & Mory, Y. Nucleic Acids Res. 8, 453-465 (1980). Zakut, R., Cohen, J. B. & Givol, D. Nucleic Acids Res. 8, 3591-3601, 4840 (1980).
- Blobel, G. & Dobberstein, B. J. Cell Biol. 67, 835-851 (1975)
- Milstein, C., Brownlee, G. G., Harrison, T. M. & Matthews, M. B. Nature new Biol. 239, 117-120 (1972)
- Tucker, P. W., Marcu, K. B., Slightom, J. L. & Blattner, F. R. Science 206, 1299-1303

gene rearrangement additional somatic mutations may occur. Both germ-line substitutions and somatic mutations occur preferentially in the regions coding for CDR.

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- 19. Matthyssens, G. & Rabbitts, T. H. Proc. natn. Acad. Sci. U.S.A. 77, 6561-6565 (1980).
- Matthyssens, G. & Radonits, L. H. Froc. nath. Acad. Sci. U.S.A. 17, 0301-0302 (1960).
 Sakano, H., Hüppi, K., Heinrich, G. & Tonegawa, S. Nature 280, 288-294 (1979).
 Tonegawa, S., Maxam, A. M., Tizard, R., Bernard, O. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 75, 1485-1489 (1978).
- 22. Auffray, C., Nageotte, R., Chambraud, B. & Rougeon, F. Nucleic Acids Res. 8, 1231-1241
- 23. Kabat, E. A., Wu, T. T. & Bilofsky, H. Sequences of Immunoglobulin Chains (NIH publication no. 80-2008
- Padlan, E. A., Davies, D. R., Pecht, I., Givol, D. & Wright, C. Cold Spring Harb. Symp. quant. Biol. 41, 627-639 (1977).
- Padlan, E. A. Q. Rev. Biophys. 10, 35-65 (1977).

 Capra, J. D. & Kehoe, J. M. Proc. natn. Acad. Sci. U.S.A. 71, 845-848 (1974).

 Proudfoot, N. J. Nature 286, 840-841 (1980).

 Bentley, D. L. & Rabbitts, T. H. Nature 288, 730-733 (1980).

- Proudfoot, N. J. & Maniatis, T. Cell 21, 545–553 (1980). Nishioka, Y., Leder, A. & Leder, P. Proc. natn. Acad. Sci. U.S.A. 77, 2806–2809 (1980).
- 31.
- Galley, J. A. & Edelman, G. M. Nature 227, 341–348 (1970).

 Kabat, E. A., Wu, T. T. & Bilofsky, H. J. biol. Chem. 252, 6609–6616 (1977).
- Smith, H. O. & Birnstiel, M. L. Nucleic Acids Res. 3, 2387–2398 (1976).

 Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560–564 (1977).
- 35. Tu, C.-P. D. & Coehn, S. N. Gene 10, 177-183 (1980).

γ Rays from the cosmic ray irradiation of local molecular clouds

M. R. Issa & A. W. Wolfendale

Physics Department, University of Durham, Durham DH1 3LE, UK

An analysis is given here of the flux of cosmic γ rays of energy above 100 MeV from the directions of 13 local molecular clouds. We show that only in a few clouds does the cosmic ray intensity need to exceed the value near the Earth to explain the γ-ray fluxes. Possible reasons for the few excesses are considered. Our conclusion is important because some of the enigmatic γ -ray 'sources' are identified; furthermore, γ -ray data can be coupled with knowledge about the distribution of gas in the Galaxy to throw light on the problem 'where do cosmic rays come from?'.

y rays are produced when cosmic ray electrons, protons and heavier nuclei collide with atomic nuclei in the interstellar medium (ISM). Insofar as the ISM is not uniformly distributed but contains regions ('clouds') of rather high gas density the γ -ray sky is correspondingly uneven. Of particular interest are those clouds in which the gas, largely molecular hydrogen, is so massive that an excess γ -ray flux can be seen.

Ideally, such clouds could be used to map the distribution of cosmic rays (CR) in the Galaxy, if certain conditions were met: the clouds were of accurately known mass, those clouds selected were 'cosmic ray inert' (that is, generated γ rays only through the interactions of ambient CR and did not contain significant sources of cosmic ray particles) and if the corresponding y-ray fluxes could be accurately measured. If then clouds which were not 'CR inert' were considered, information could be gained about y-ray emission from specific sources and also about particular particle acceleration mechanisms.

Clearly, none of these provisos are met to anywhere near the extent necessary for an accurate analysis but an attempt is desirable because of previous conflicting results (for example, for the cloud associated with ρ -Ophiuci; see ref. 1). A serious objection to previous studies was that the γ -ray sources were taken as the starting point; here we start more rigorously with a set of molecular clouds and then search the γ-ray data for excesses (or deficits) of flux. Hopefully if a large enough sample is taken and the uncertainties in mass and γ -flux are random then the average behaviour will be correct. Unfortunately, there are too few clouds for which data are available to select 'CR inert' clouds. Instead, we must take all clouds for which reasonable mass and distance estimates have been made. The expected γ -ray flux from a cloud of mass M at distance d is estimated from the relation $\Gamma_{\gamma, exp} = (q/4\pi)(M/d^2)$ where q is the emissivity appropriate to the cosmic ray intensity near the Earth (given by Issa et al.) and to compare this with the observed y-ray flux. $\Gamma_{\gamma, {
m obs}}$. Clearly $\Gamma_{\gamma, {
m obs}}/\Gamma_{\gamma, {
m exp}}=I_{
m cloud}/I_0$ where $I_{
m cloud}$ and I_0 are the cosmic ray intensities in the cloud and near the Earth, respectively.

The questions to be asked are: (1) Is the average cosmic ray intensity in the whole sample of local molecular clouds significantly higher than the intensity near the Earth, I_0 ? (Is the average enhancement factor, $F = I_{cloud}/I_0$, greater than unity?) (2) If there is evidence for F > 1 in some clouds and not in others, do they correlate with any known astronomical parameter, such as number of flare-stars, or OB-association?

There is no homogeneous catalogue of masses of molecular clouds determined by the use of one, generally accepted, technique. However, a series of observations by Blitz² give data on seven cloud complexes and these form over half the clouds examined here. The technique was to measure the column density of ¹²CO and ¹³CO lines and convert to H₂ density using the technique described by Blitz and Shu³ and elsewhere (which uses the ratio of $N(H_2)/N(^{13}CO) = 5 \times 10^5$ of Dickman⁴). Important points are the necessity for an upward correction for the gas associated with each cloud which has too low a density to be recorded directly (usually in the 'halo' around the cloud), and for the effect of channel dilution and non-linearity. Furthermore, the possibility of non-thermodynamic equilibrium in local high density regions is important. Blitz favours upward corrections of between 2 and 3. Here we usually adopt a factor 2 to give our estimated upper limit to the mass (the 'Blitz factor') and take the uncorrected mass as a lower limit. Figure 1 gives the location of the selected clouds and Table 1 the estimated masses and distances.

The local thermodynamic equilibrium (LTE) masses are those given by Blitz. Allowance has been made for excesses of atomic hydrogen and ionized gas as well as molecular hydrogen where data are available. Also given are the approximate areas included in our analysis of the clouds and some astronomical information.

Table 1 Estimated masses and distance	s o	of selecte	d clouds
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Object	Distance (pc)	LTE mass (M _☉)	Mass range (M_{\odot})	Area (deg ²)	Comments	Ref.
Cas OB6	2,000	1×10^5	$1-2\times10^5$	4	W3 & W3N, well known H ₂ regions, best known examples of OB star formation. Strong ionization fronts	
Mon OB1	800	1×10^5	$1-2 \times 10^{5}$	8	Much recent star formation: T-Tauri	2
Mon OB2	1,600	1×10^5 (1 × 10 ⁵ for H ₂	$3-5\times10^5$	4	Rich grouping of early type stars: O stars	2
C.M. OD1	1,100	2×10^{5} for H 1×10^{5}		4	OB and R associations	2
C Ma OB1 Orion OB1	450	2×10^{5}	$2-4 \times 10^{5}$	20	Many flare stars; T-Tauri common; rich in O-associations	2 2
Per OB2	350	4×10^4	$4-8\times10^{4}$	24	Some star formation, T-Tauri and O-type	2
Cygnus	1.700	7×10^{5}	$7-21 \times 10^{5}$	12	Active star formation—OB associations	2
Gum	400	1.2×10^5	$0.7-3\times10^5$	400	Argument about origin of this nebula; considerable ionized gas from supergiants plus stellar winds	28, 29
Cor Aust	150	6.9×10^3	$0.7 - 1.4 \times 10^4$	8	Contains young stellar objects but efficiency of star forma- tion is very low	15, 30
Taurus	140	1.7×10^4	$1.5 - 3 \times 10^4$	100	T-Tauri stars distributed throughout the cloud	6, 7
M17	2,300	$>3\times10^5$	$3-10\times10^{5}$	4	Cluster of O stars has formed at the edge of the molecular cloud	9
Car Neb	2,700		$1.5-3\times10^5\dagger$	5	An unusual object. Star in nebula is one of most luminous in Galaxy. Flared during last century	31
ρ-Oph	160	_	$4.5 - 9 \times 10^3 \dagger$	16	Cloud very dense; comparatively inert but some star formation	32, 33

[†] See text.

There is much argument about the origin of the Gum nebula but agreement that there is considerable ionized gas. In view of the proximity of the Vela pulsar and its spillover (in γ rays) we take only the region $b < -6^{\circ}$. We estimate that $\sim 60\%$ of the gas is in this region (following the H α photograph of Sivan⁵, which suggests that there is more material below the galactic plane than above). The range of masses indicated, which refers to the restricted region, endeavours to bracket the quoted values.

Corona Australis is a useful cloud in that it mirrors ρ -Oph in position and would be expected to have the same cosmic ray intensity in it. Densities in the cores of the sub-clouds are very high. The Blitz factor of 2 possible enhancement in mass has been used but the mass quoted is probably an underestimate.

Taurus 'may be the nearest major aggregate of dust and gas in which star formation is in progress'⁶. It has many T Tauri stars spread throughout the complex. A mass of $1.1 \times 10^4 M_{\odot}$ (within the 0.2 K contour) is quoted by Baud and Wouterloot' for a distance of 113 pc, from observations of OH. For our adopted distance of 140 pc (ref. 6) the corresponding mass is $1.7 \times 10^4 M_{\odot}$ (this is probably an underestimate by ~25% because all the cloud was not surveyed). Another estimate is $2 \times 10^4 M_{\odot}$ using recent ¹²CO data of G. P. Baran (personal communication) for the Taurus and surrounding regions calibrated with the LTE mass obtained by Blitz for the nearby Per OB2 cloud. Bearing in mind the 'Blitz factor' we adopt $1.5-3 \times 10^4 M_{\odot}$ as the likely range for the total mass.

M17 is a bright H II region located on the edge of a large molecular cloud complex which 'may be one of the most massive objects in the Galaxy'⁸. Elmegreen *et al.*⁹ quote a lower limit to the mass of $3.2 \times 10^5 M_{\odot}$, and Elmegreen and Lada⁸ suggest $10^6 M_{\odot}$ for the whole complex. Thus, $3-10 \times 10^5 M_{\odot}$ seems likely.

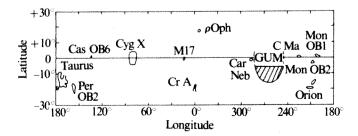


Fig. 1 Positions and approximate shapes of the molecular cloud complexes.

The star associated with the Carina Nebula (η Carinae) is the most luminous in the Galaxy in the IR ($L \sim 6 \times 10^6 L_{\odot}$). There is strong evidence for an ionization front generated by stars in the Trumpler 14 and 16 clusters, and associated compressed H and H₂ regions¹⁰. The mass is very uncertain because of the lack of CO observations. The estimate of gas in ionized form comes from Dickel's analysis: $1-2\times10^5 M_{\odot}$. In view of the inevitable contribution from H and H₂ and the Blitz factor we adopt $1.5-3\times10^5 M_{\odot}$ as the likely range.

We previously favoured ρ -Ophiuchi because of its being comparatively inactive: astronomically^{1,11} there are no associated O-stars or bright H II regions ¹² although there are several small compact H II regions ¹³. There is uncertainty in the mass (see refs 11, 14 for conflicting estimates). Here we consider the following: Myers et al. ¹² quote $1.8 \times 10^3 M_{\odot}$ (assuming LTE) out to a radial distance of 2.1 pc for H + H₂; Grasdalen et al. ¹³ give $4 \times 10^3 M_{\odot}$ for a somewhat smaller region, Vrba ¹⁵ quotes $6.1 \times 10^3 M_{\odot}$ in total. Our previous estimate overall from CO data was $\sim 9 \times 10^3 M_{\odot}$, inclusive of ionized gas (Baart et al. ¹⁶ find that such gas is common) but our analysis of stellar extinction led to values of about half this. Thus, we adopt $4.5-9 \times 10^3 M_{\odot}$ in the present case.

The γ -ray results will now be considered. The data come from the SAS 2 and COS B experiments. The SAS 2 data comprise the comprehensive tables of Fichtel $et~al.^{17}$ for the two energy ranges 35–100 MeV and $E_{\gamma} > 100$ MeV, and the contours for l 150°–240° and |b| < 25° given by Thompson $et~al.^{18}$. COS B results have not yet been given in tabular form and we have used other sources: the contours for $E_{\gamma} > 70$ MeV of Wills $et~al.^{19}$ which relate to all longitudes and largely to |b| < 15°, the cut along b=0° given by the same author and sections for 70–150 MeV, |b| < 7°; 150–300 MeV, |b| < 5° and 300–5,000 MeV, |b| < 4° given by Mayer-Hasselwander $et~al.^{20}$. The 2CG catalogue of COS B sources of Hermsen²¹ has also been used.

For each cloud we delineate an area surrounding it some $3-4^{\circ}$ wider than the cloud (the exact area depending on γ -ray energy and the source of data: tabulated fluxes, contours and flux versus longitude plots for fixed latitude bins) and compare the integrated γ -ray flux in this region with its surroundings, usually by way of adjacent longitude bins for the same latitude range. The adopted flux is the flux in the region less the average of its surroundings. The greater coverage than the 'source' area is to allow for at least some of the γ rays which fall outside the area because of errors of γ -ray direction (the 'point spread function'); a correction is applied for the effect of residual loss, that is, net loss even out of the enlarged area. The excess flux which can be

Table 2 Observed and expected γ-ray fluxes and the enhancement factor

		Fluxes (10 ⁻⁶ cm ⁻² s ⁻¹	$(E_{\gamma} > 100 \text{ MeV})$		
Object	SAS 2	COS B	Best flux estimate	Expected flux	F needed to give measured γ-flux
Cas OB6	0.5	1.0	1.0	0.07-0.14	7-14
		(if 2CG135+01)		0.07 0.14	/-14
Mon OB1	< 0.3	0.5	0.3	0.4-0.9	0200
Mon OB2	< 0.2	< 0.5	< 0.4	0.3-0.5	0.3-0.8
CMaOB1	< 0.5	0.5	0.5	0.23-0.5	<1.3
Orion OB1	1.0	1.4	1.4	2.8-5.6	1-2 0.25-0.5
		(ref. 22)		2.0 5.0	0.25-0.5
Per OB2	< 0.8		< 0.8	0.9-1.8	< 0.9
Cygnus	4.0	3.8	3.8	0.7-2.1)	
		(if 2CG075-00		2.7*	1.8-5.4
		and $2CG078+01$)		,	
Gum	4.2	******	4.2	1.2-5.3	0.8-3.5
Cor Aust	0.4	< 0.4	0.4	0.9-1.8	0.2-0.4
Taurus	0.5	-	0.5	2.1-4.2	0.12-0.24
M17	< 0.6	1.0	1.0	0.16-0.53	1.9-6.3
		(if 2CG13+00)			21,5 0.2
Car Neb	<2.2	1.6	1.6	0.06-0.12	13-27
		(if 2CG288-00)			
$ ho ext{-Oph}$	< 0.7	1.1	1.1	0.5-1.0	1.1-2.2
		(if 2CG353+16)			*** ****

^{*} This value is from the analysis of Protheroe et al. 34 using column densities but converting to $q/4\pi = 2.2 \times 10^{-26} \text{ s}^{-1} \text{ sr}^{-1}$ as adopted here.

attributed to the cloud is estimated using the above sources of data. Where the flux is negative, upper limits at the 1σ level are derived.

This cannot be an accurate procedure as there are many problems associated with inhomogeneous backgrounds and poor statistics; however, it seems to be objective and we can see no reason for the eventual average (or better, the median) value of the flux enhancement factor, F, being either underestimated or overestimated.

To improve accuracy, the data are combined to give an estimate of the flux above one energy level: $100 \, \text{MeV}$. To this end, the SAS 2 fluxes for $35:100 \, \text{MeV}$ are multiplied by 0.68 to give the equivalent flux above $100 \, \text{MeV}$.

Table 2 gives the observed and our estimated γ -ray fluxes and the enhancement factor, F. The 2CG notation refers to the COS B source catalogue of Hermsen²¹ and these fluxes are taken where the 'sources' are in such positions as to be not inconsistent with the clouds. Positive identification is not possible, however, and there are doubts in some cases. The COS B flux value for Orion comes from Caraveo et al.²² which relates to $E_{\gamma} > 70 \text{ MeV}$ ($\approx 2 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$); conversion by the spectral shape factor yields $\approx 1.4 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$ above 100 MeV. This value is close to the $1.0 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$ derived from analysis of SAS 2 data¹¹. (Note that Caraveo et al. derive $F \approx 1$ by adopting a mass of $1.2 \times 10^{5} M_{\odot}$, derived from Galaxy count studies.) No value is given for the flux for those regions where no data have been reported (or at least there are no data over a wide enough $\Delta l \times \Delta b$ range to allow flux estimates to be made).

Figure 2 shows the values of F listed in Table 2 plotted against the distance from the Earth, d. Ideally F should be plotted against distance from the axis of the nearest spiral arm (in an effort to search for an arm-interarm contrast in cosmic ray intensity) but the positions of the axes are not sufficiently well known. Although not necessarily expecting a dependence of F on d over the range indicated we do expect a loss of reliability at large d because of the possibility of confusion from other clouds and this will be particularly severe when looking roughly along the axis of a spiral arm (specifically for Car Neb and, to a lesser extent, Cygnus). In fact, the 2CG COS B source catalogue lists 2CG288-00 (the Car Neb source) and 2CG284-00 nearby as 'could be an extended feature'.

Taking the data in Fig. 2, the average value of F is ~ 1.3 , weighting according to the errors indicated. Although this estimate is not very precise in view of there being errors in γ -ray

flux, which have not been included, the extra errors should be symmetrical and the estimate should not be too far out. It seems likely, then, that most of the clouds do not have much enhancement $(F \le 2)$ of cosmic ray intensity within them. In this sense the results are consistent with a previous analysis²³ in

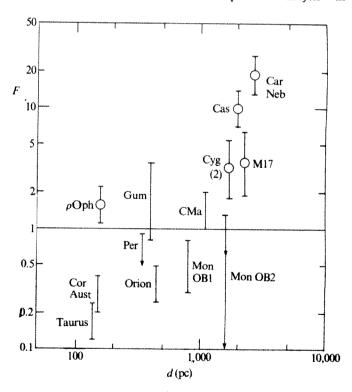


Fig. 2 F-values for the molecular clouds indicated. The ranges shown come from uncertainty in cloud mass; no uncertainty in γ -ray flux has been allowed for. The flux errors are expected to be roughly symmetrical so that the best-estimate F-values will not change. The abscissa is the distance from the Earth. The circles on some of the error bars indicate clouds which may be identified with γ -ray sources in the 2CG catalogue; the 2CG γ -ray fluxes have been adopted in the calculations (the (2) against Cygnus denotes two COS B sources). The astronomical data of Table 1 are probably best for Orion OB1, Per OB2 and Mon OB2 (J. Meaburn, personal communication).

which it is claimed that over half of the 2CG sources could be explained as being due to CRI molecular clouds, the analysis being made on a statistical basis.

Despite the large uncertainties there are some features which are worthy of further attention.

(1) The cloud complexes within 1 kpc of the Earth mainly have F < 1. Some of these have many T-Tauri stars and this indicates that the stellar winds from T-Tauri stars are not the seat of much cosmic ray acceleration or, at least, if they do accelerate particles then they escape rapidly from the environment of the stars (the likelihood of CR acceleration by stellar winds was first pointed out by Cassé and Paul²⁴). It is possible, but not certain, that some of the low values of F relate to the position of the clouds—they may be preferentially in interarm regions. Beyond 1 kpc (where T-Tauri stars cannot be identified) F-values are generally higher and there seem to be frequent OB-associations. The significance of this is not clear because of selection phenomena; the relevance of OB-associations to y-ray sources has already been discussed by Montmerle25 who required nearby supernovae ('SNOB'S') to augment I_{cloud} . We do not feel much increase in flux is needed.

(2) The only clouds which seem to need F-values significantly greater than unity are Car Neb and Cas OB6, the differences from unity for the others can be attributed in part to errors of γ-ray flux, and of cloud mass and to the likelihood of small variations of F from place to place on a large scale.

The excess in Car Neb may be genuine, with particle acceleration occurring in a manner such as described by Montmerle26. However, several features need stressing. The first concerns the fact that the direction of Car Neb is congested in the sense that it is along a spiral arm and other clouds may also be contributing within the angular resolution of the instrument. The mass of cloud needed (at the distance of Car Neb) is $=3\times10^6 M_{\odot}$; although large, this is not an impossibly large mass for a single giant molecular cloud. The inclusion of other clouds would relax the mass requirement considerably. Also the identification of 2CG288-00 with Car Neb may be erroneous. The source could be, for example, a pulsar in this direction; pulsars do emit γ rays.

There is the same possibility of mis-identification with Cas OB6. Coincidence of more than one cloud is unlikely, however, because the direction is not along an arm. What is interesting is the fact that Cas OB6 is in a well developed arm—the Perseus arm-and the possibility of some general CR intensity enhancement arises.

Although all the data—both γ-ray and astronomical—are poor such evidence as there is does not call for much CR enhancement in most of the molecular clouds within 1 or 2 kpc of the Earth $(\langle F \rangle = 1.3)$ for all the clouds in Fig. 2 and even nearer unity for d < 2 kpc). Unidentified molecular clouds irradiated by CR and situated beyond 2 kpc are likely contenders for the so-called y-ray sources and, indeed, there are indications²³ that ~60% of the COS B sources can be explained in this way.

Thus the studies of the large scale distribution of γ -ray flux, in which unresolved discrete sources have been neglected and it has been concluded that there are large scale cosmic ray gradients in the Galaxy, should be valid (see, for example, ref.

We do not suggest that the sources of some of the low energy cosmic rays are not in molecular clouds. They could well be, but diffusion will probably distribute the particles so rapidly that those interacting in a particular cloud are mainly from the general ambient cosmic radiation.

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Sivan, J. P. Astr. Astrophys. Suppl. 16, 163 (1974).
 Elias, J. H. Astrophys. J. 224, 857 (1978).

Ellias, J. H. Astrophys. J. G. Astr. Astrophys. 90, 297 (1980). Blunegreen, B. G. & Lada, C. J. Astr. J. 81, 1089 (1976).

Elemgreen, B. G., Lada, C. J. & Dickinson, D. F. Astrophys. J. 230, 415 (1979).

Dickel, H. R. Astr. Astrophys. 31, 11 (1974).

- Wolfendale, A. W. IUPAP/IAU Symp. No. 94, 309. Myers, P. C., Ho, P. T. P. & Schneps, M. H. Astrophys. J. 220, 864 (1978). Grasdalen, G. L., Strom, K. M. & Strom, S. E. Astrophys. J. Lett. 184, L53 (1973).
- Paul, J. A., Cassé, M. & Montmerle, T. IUPAP/IAU Symp. No. 94, 325

Vrba, F. J. Astr. J. 82, 198 (1977).

Baart, E. E., de Jager, G. & Mountfort, P. I. (in the press). Fichtel, C. E. et al. NASA Tech. Mem. 79656 (GSFC, Gre

Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. NASA Tech. Mem. X-662-76-198 (GSFC, Greenbelt, 1976).

Wills, R. D. et al. Non-solar Gamma Rays (COSPAR) (eds Cowsik, R. & Wills, R. D.) 43 (Pergamon, Oxford, 1980).

Mayer-Hasselwander, H. A. et al. Ann. N.Y. Acad. Sci. 336, 211 (1980). Hermsen, W. thesis, Univ. Leiden (1980).

Caraveo, P. A. et al. Astr. Astrophys. 91, L3 (1980). Li, T. P. & Wolfendale, A. W. Astr. Astrophys. (in the press).

Cassé, M. & Paul, J. A. Astrophys. J. 237, 236 (1980). Montmerle, T. Astrophys. J. 231, 95 (1979).

Montmerle, T. Phil. Trans. R. Soc. (in the press).
 Issa, M. R., Riley, P. L., Strong, A. W. & Wolfendale, A. W. Nature 287, 810 (1980).

Reynolds, R. J. Astrophys. J. 203, 151 (1976).

29. Brandt, J. C., Stecher, T. P., Crawford, D. L. & Maran, S. P. Astrophys. J. Lett. 163, L99

30 Rossano, G. S. Astr. J. 83, 234 (1978).

Turner, D. G. & Moffat, A. F. J. Mon. Not. R. astr. Soc. 192, 283 (1980).
 Bertiaud, F. C. Astrophys. J. 128, 533 (1958).

Whittet, D. C. B. Mon. Not. R. astr. Soc. 168, 371 (1974).
 Protheroe, R. J., Strong, A. W. & Wolfendale, A. W. Mon. Not. R. astr. Soc. 188, 863

Collisional amplification of density fluctuations in Saturn's rings

J. Lukkari

Department of Astronomy, University of Oulu, Oulu, Finland

The encounter of Voyager 1 with Saturn in November 1980 revealed the ringlet structure of its rings. As the theoretical examination of the collisional evolution of keplerian systems1,2 had predicted such a structure in dense matter as a consequence of amplified fluctuations in density, some new computer simulations have now been carried out to check this effect. These simulations actually led to a strong, irreversible growth in the density maximum.

Let a keplerian system consist of frictionless, spherical and identical particles revolving around the central body along elliptic orbits and having the radius σ . Let a denote the semimajor axis, ε the eccentricity, i the inclination, γ Newton's constant and m the mass of the central body. The local value of optical thickness is τ .

Solid materials have a restitution coefficient, α , which decreases with the velocity of impact, v. If α_0 denotes the particular value of α which corresponds to the root-meansquare velocity of impacts, the system adjusts itself to a state in which2

$$\frac{9\pi^2(19\alpha_0 - 13)}{64(1 - \alpha_0^2)(3 - \alpha_0)^3} = g^2\tau^2, \qquad \alpha_0 = \alpha(\sqrt{\overline{v^2}})$$
 (1)

The weight function g is a correction for the finite volume of the particles; if the space density is low, we have g = 1. As the space density depends on the geometric thickness of the system, its optical thickness is not restricted by the value of g. If equation (1) is valid, the radial component of the two-dimensional flux vector which represents the transport of particles on the equatorial plane of the system can be calculated from

$$F^{a} = -\frac{8}{9\pi^{2}\sigma^{2}\sqrt{\gamma ma}}\frac{\partial}{\partial a}\left[g\tau^{2}a^{2}T(1-\alpha)\sqrt{(1+\alpha)(3-\alpha)}\right]$$
 (2)

The temperature-like quantity T is approximately equal to $3\overline{v^2}/4$. According to equation (1), $\sqrt{v^2}$ is a decreasing function of

^{1.} Issa, M. R., Strong, A. W. & Wolfendale, A. W. J. Phys. G: Nucl. Phys. 7, 565 (1981).

^{2.} Blitz, L. in Giant Molecular Clouds in the Galaxy (eds Solomon, P. M. & Edmunds, M. G.) 1

⁽Pergamon, Oxford, 1980). Blitz, L. & Shu, F. H. Astrophys. J. 238, 148 (1980)

^{4.} Dickman, R. L. Astrophys. J. Suppl. 37, 407 (1978).

the optical thickness, as has also been found by Goldreich and Tremaine³. If τ is high enough, this effect produces a decrease in the function inside the square brackets in equation (2). For example, if $\alpha = (1+v/v_0)^{-1}$ ($v_0 = \text{constant}$) and g = 1, this function has its largest value at $\tau = 0.75$. If τ exceeds it, the flux is directed to the density maximum. If τ is small, the same function increases with τ and the flux is directed away from the maximum density. In particular, as $\tau \to 0$ at the boundaries of the system, these expand with a macroscopic velocity $\pi \sigma^2 F^a / \tau \sim -\partial \tau / \partial a$. As a whole, the system therefore expands. This agrees with Brahic's conclusion. As Brahic derives the expansion from the conservation of the total angular momentum of the whole system and from a simultaneous decrease in its total energy, his result cannot be generalized for parts of a system. Their angular momentum may increase or decrease according to the interaction with adjacent regions. A local contraction of matter is therefore possible.

In the present report we are not studying the general expansion, but are testing the reality of the amplification of fluctuations which is predicted by equation (2) at high values of τ . Because Saturn's rings exist, the time scale of the expansion must be long or some mechanism must prevent it. The formation of ringlets has a shorter time scale. Once the fluctuations have begun to grow, the gradient in equation (2) becomes progressively steeper and the flux increases until the finite size of particles stops the process.

Previous simulations⁵ with a constant coefficient of restitution indicate that the validity of theoretical predictions with g = 1 is restricted by the condition $\sqrt{i^2} \gg 4\sigma/a$. On the other hand, in a ring-shaped system of radial width w, the gradients in density and in the distribution functions are too high unless $w/2 \gg a\sqrt{\varepsilon^2}$, $a\sqrt{i^2}$. This follows from the limits of validity for theoretical expressions^{2,5}. As $\sqrt{i^2/\overline{\epsilon}^2} = 2/3$ in the equilibrium state⁵, we can also write both conditions in the form $w \gg 2a\sqrt{\overline{\epsilon}^2} \gg 12\sigma$. If the sign » is interpreted to mean the ratio 5:1, the above condition can be written $w \ge 300\sigma$. The number of particles in a ring of optical thickness τ is $2aw\tau/\sigma^2$. If $\tau > 0.75$ as in the abovementioned numerical example, and if $\sigma/a = 0.001$, a system of 450,000 particles is needed to satisfy the condition $w \ge 300\sigma$. The large number of particles prevents the direct simulation of dense matter (Saturn's rings). Because the dependence between τ and α is of a statistical nature, one cannot even use a Monte Carlo method, which only increases the number of particles but does not change their statistics. We must therefore use an indirect method.

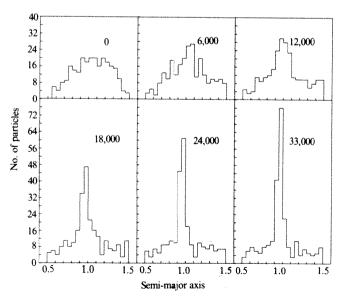


Fig. 1 Radial distribution of particles during one simulation. The number of collisions is given beside each histogram. The corresponding times are 0, 132,000, 247,000, 335,000, 403,000 and 484,000 in units in which $\sigma = 0.001$ and $\gamma m = 1$.

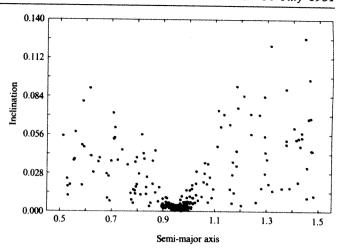


Fig. 2 Inclinations of the orbits as a function of semi-major axis after 30,000 collisions.

In rarefied matter, equation (1) gives the result $\alpha_0 = 13/19$, which has also been confirmed by a computer simulation. Applying this result we can artificially force a rarefied system to behave in the same manner as a dense one. For this purpose it is sufficient to use a coefficient of restitution which depends on both v and τ and produces the result $d\sqrt{v^2}/d\tau < 0$, when the system adjusts itself to $\alpha_0 = 13/19$. This corresponds to equation (1). The function $\alpha(\tau, v)$ can be so chosen that the simulation produces the same flux as equation (2) (but not with the same value of v^2) if the elastic properties of matter are specified. Because we lack this information we can choose $\alpha(\tau, v)$ freely.

In our simulations the restitution coefficient was

$$\alpha = \frac{1}{1 + k(\tau^3 + \tau_0^3)v} \qquad k, \tau_0 = \text{constant}$$
 (3)

In units $\gamma m = 1$, the constants of equation (3) are $k = 1.91 \times 10^{12}$ and $\tau_0 = 7.52 \times 10^{-5}$. The number of particles in zones of width 0.1 was used to calculate the local values of τ . The number of particles is 250 and their radius is 0.001. The initial distribution of τ is proportional to (1.5-a)(a-0.5) while the initial values of $\sqrt{\overline{\varepsilon^2}}$ and $\sqrt{\overline{i^2}}$ are 0.049 and 0.032, respectively. As the number of particles is restricted, we cannot simulate a sequence of adjacent density fluctuations. It is therefore necessary to pick up just one condensation and to follow its evolution. To simulate the pressure of adjacent regions, the particles which were outside the initial zone 0.5 < a < 1.5 after a collision were brought back into it. The percentage of such impacts was usually <1%. A slow expansion of this zone would not change the result. The same process can also be interpreted as replacing the unknown mechanism which prevents the general expansion of boundaries in saturnian rings. If the post-collisional semi-major axis was <0.5, it was replaced by 1-a. If it was >1.5, it was replaced by 3-a. This has no primary effect on the development of the central spike, because the maximum change of semimajor axis in a collision is usually not larger than 2ea. Figure 1 shows the evolution in the radial distribution of the particles in one of the simulations. The increase in density is clearly seen after 6,000 collisions and it continued when the simulation was stopped after 33,000 collisions. Contraction continues although the inclination was at its densest part $\sim 2\sigma/a$, which has previously led to an increasing dispersion of the system⁵. The system thus achieved a state in which α was not able to reach its quasi-equilibrium value 13/19 but remained smaller.

Figure 2 represents the distribution of inclinations as a function of the semi-major axis after 30,000 collisions. As can be seen the large inclinations in rarefied matter between the ringlets may well cause the observed thickness of Saturn's rings, whereas the thickness of the ringlets is much smaller.

Extremely rarefied matter outside Saturn's rings or selfgravitation or some external perturbation is needed to prevent the extreme borders of the whole sequence of ringlets from dispersing into space. If this is assumed, then the simulations show in accordance with the theory 1.2 that an initially almost homogeneous ring of dense matter is spontaneously divided into ringlets.

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- Hämeen-Anttila, K. A. Astrophys. Space Sci. 56, 421-430 (1978). Hämeen-Anttila, K. A. Astrophys. Space Sci. 58, 477-519 (1978). Goldreich, P. & Tremaine, S. Icarus 34, 227-239 (1978).

- Brahic, A. Astr. Astrophys. 54, 895-907 (1977).
- Hämeen-Anttila, K. A. & Lukkari, J. Astrophys. Space Sci. 71, 475-497 (1980).

The ice layer in Uranus and Neptune—diamonds in the sky?

Marvin Ross

University of California, Lawrence Livermore National Laboratory, Livermore, California 94550, USA

Many of the current models of Uranus and Neptune postulate a three-layer structure, consisting of an inner rocky core, a middle 'ice' layer of fluid, H2O, CH4, NH3 and an outer hydrogenhelium layer of solar composition1. The estimated pressures and temperatures of the ice layer ranges from about 6 Mbar and 7.000 K at the inner core-ice boundary, to ~0.2 Mbar and 2,200 K at the outer ice/hydrogen-helium boundary. I point out here that shockwave experiments on these liquids²⁻⁵, as well as theoretical studies⁵⁻⁷, imply that the H₂O and NH₃ in the ice layer are almost totally ionized and the CH4 has been pyrolysed to carbon, possibly in the metallic or diamond form

In recent years shock-wave experiments have been carried out on all of the fluid constituents of the outer planets except helium. In a shock-wave experiment, the change in pressure, density, and energy, on compression are measured, but the temperature must be computed from the equation of state. A series of experiments starting from the same initial conditions (that is, the normal liquid) and spanning a range of final pressures and densities is referred to as a Hugoniot curve. Figure 1, in comparing some of these data for the 'ices' with an isentrope calculated for Uranus (Neptune is very similar) demonstrates the significance of the data to theoretical modelling studies of these planets. For methane the experimental data, only some of which is shown, consists of a principal Hugoniot up to 0.45 Mbar, plus one data point reflected from 0.23 to

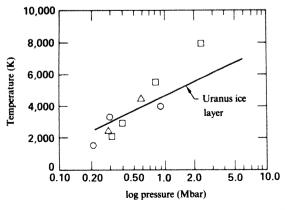


Fig. 1 Plot of some of the experimental shock-wave data for water (\Box) , ammonia (\triangle) and methane (\bigcirc) in the range of temperatures and pressures believed to exist in the Uranus (and Neptune) ice layer.

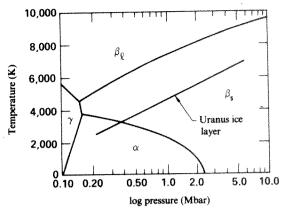


Fig. 2 The Uranus (and Neptune) ice layer (straight line) compared with pressure and temperatures for the diamond phase diagram of Grover⁸. α , Diamond; γ , graphite; β_s , metallic solid; β_1 , metallic liquid. Van Vechten's phase diagram predicts that in the region of the isentrope, above ~0.4 Mbar, carbon will be a liquid metal.

0.91 Mbar. It has recently been shown that an apparent deviation from predicted molecular behaviour could be explained by the tendency for CH₄ to dissociate in these conditions⁶. These conclusions were based on chemical equilibria calculations which predicted that above 0.20 Mbar and 2,000 K, methane is converted into elemental carbon and molecular hydrogen, Recent theoretical calculations on Hugoniots of many hydrocarbons indicate that shock heating induces breaking of the C-H bonds, and the compression encourages condensation of the dissociated carbon atoms into a residue7. If we assume that the hydrocarbons have been completely converted into a mixture of condensed carbon and molecular hydrogen, we can use known equations of state for each of these materials to compute highdensity Hugoniots that are in excellent agreement with the experimental hydrocarbon data. The results of similar calculations for molecular methane⁶ also agree with the experimental data suggesting that the final product is also hydrogen and a carbon residue.

The Uranus isentrope is shown again in Fig. 2, this time plotted with the recently proposed carbon phase diagrams of Grover⁸. Van Vechten⁹ had previously proposed a carbon phase diagram. Although they differ somewhat in detail above 3,000 K and 0.4 Mbar, they do agree in predicting that below these conditions carbon will exist in the well-known 4-coordinated diamond form. Above these conditions Grover predicts that carbon along the planetary isentrope will be in a metallic solid phase whereas Van Vechten predicts a liquid metallic phase. In Van Vechten's theory the close-packed metal becomes liquid at temperatures well below those of the planetary ice layer. Currently, hydrogen is believed to become a metal somewhere between 2.0 and 5.0 Mbar. Consequently, over much of the higher pressure range of the isentrope the hydrogen freed from the molecular methane will also be metallic and may form a metallic carbon hydrogen mixture.

As indicated in Fig. 1, shock-wave data on water and ammonia, including electrical conductivities, have been measured over part of the Uranus (Neptune) pressuretemperature range. The electrical conductivity data shows that above 0.2 Mbar and about 2,000 K the conductivities become constant at ~20-30 S cm⁻³, as if the processes leading to ionization have become saturated. Water has apparently become fully ionized³. For ammonia similar results have been observed³. Consequently we must conclude that in the Uranus and Neptune ice layer H₂O and NH₃ are not molecular but ionic, and that the carbon in methane has been converted to a diamond or metallic phase with molecular or metallic hydrogen. Shock-wave data on diamond and graphite shows that the carbon condensate at a few megabars will be denser than NH₃ or H₂O and, unless it is highly soluble in the fluids, may separate out and sink below to form a denser layer.

The electrical properties of these ices are of great interest because they can lead to an explanation or a prediction of a magnetic field. The motion of charged particles trapped in such magnetic fields generates radio waves. There is one report of possible radio signals from Uranus, but there have been no similar reports from Neptune 10. The significance of a conducting layer of carbon may be very great because it is estimated that the mass of available carbon in Uranus and Neptune is 17% of the total planetary mass1.

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- Hubbard, W. B. & McFarland, J. J. J. geophys. Res. 85, 225 (1980).
 Mitchell, A. C. & Nellis, W. J. in High Pressure Science and Technology Vol. 1 (eds Timmerhans, K. D. & Barber, M. S.) 428 (Plenum, New York, 1980).
 Mitchell, A. C., Kovel, M. I. Nellis, W. J. & Keeler, R. N. in High Pressure Science and
- Technology Vol. 2 (eds Vodar, B. & Marteau, P.) 1048 (Pergamon, Oxford, 1980).

 4. Nellis, W. J., Mitchell, A. C., Ross, M. & van Thiel, M. in High Pressure Science and
- Nellis, W. J., Mitchell, A. C., Ross, M. & van Thiel, M. in High Pressure Science Technology Vol. 2 (eds Vodar, B. & Marteau, P.) 1043 (Pergamon, Oxford, 1980). Nellis, W. J., Ree, F. H., van Thiel, M. & Mitchell, A. C. J. chem. Phys. (submitted). Ross, M. & Ree, F. H. J. chem. Phys. 73, 6146 (1980). Ree, F. H. J. chem. Phys. 70, 974 (1979).

- Grover, R. J. chem. Phys. 71, 3824 (1979). Van Vechten, J. A. Phys. Rev. B7, 1479 (1973)
- 10. Brown, W. L. Astrophys. J. Lett. 207, L209 (1976)

A refined estimate of the fine structure constant

John Ellis & D. V. Nanopoulos

CERN, CH 1211 Geneva 23, Switzerland

Quantum electrodynamics (QED) is an immensely powerful theory whose validity extends over distance scales from 10^{-16} cm to light years. But it is unsatisfactory for many reasons, not least of which is the arbitrariness of the electromagnetic coupling strength, the fine structure constant α , and the incomprehensible commensurability of the electric charges of different particles. When extending QED to distances smaller than 10^{-16} is common to combine it first with the weak interactions 1-3 and subsequently with the strong interactions into a grand unified theory (GUT) of all elementary particle interactions^{4,5}. The incorporation of QED and the other fundamental interactions into a GUT is only possible if these interactions and the particles experiencing them satisfy some non-trivial constraints⁶. It is well-known that the incorporation of QED into a simple unifying group imposes charge quantization 4,5. Stability of baryons in GUTs requires the grand unification energy scale m_x to be \geq 10¹⁴ GeV. Couplings in renormalizable field theories such as QED evolve logarithmically with energy, and for the effective α to be less than unity-necessary if the GUT is to make sensethen >1/25 (ref. 7). We point out here that within the same standard set of grand unifying assumptions used previously, a much more refined estimate of the fine structure constant is possible: $1/120 \ge \alpha \ge 1/170$. We use only the general observations⁶ that light fermions can be grouped into 'families' or 'generations' of 15 helicity states, that the strong interactions become strong of a scale of the order of 1 GeV, and that any grand unification energy scale must lie between 1014 (for baryons to be stable enough) and 10¹⁹ GeV (the energy at which gravity becomes of unit strength). We know of no other theoretical framework⁸ for particle interactions which can provide such convincing and restrictive constraints on α , and we find it hard to believe that the experimental value of $\alpha = 1/137$ is a coincidence.

We now recall some basic features of the GUTs before deriving our upper and lower limits of α (for more complete descriptions see refs 6, 7, 9). The strong interactions are described by a non-Abelian SU(3) gauge group, while the weak and electromagnetic interactions are described by a theory

based on the group $SU(2) \times U(1)$. The ratio of the gauge coupling constants g_2 and g_1 in this theory is arbitrary, and is parametrized by $\sin^2 \theta_W$, and the fine structure constant $\alpha = \alpha_2$ $\sin^2 \theta_{\rm W}$ where $\alpha_2 = g_2^2/4\pi$. The strong, weak and electromagnetic interactions act on fundamental fermions, the quarks and leptons, which seem to be arranged^{4,5} in sets of 15 helicity states called 'families' or 'generations'. GUTs combine the different SU(3), SU(2) and U(1) gauge interactions into a simple group G, and assign the fermions to 15 dimensional representations of G. It has been emphasized⁶ that it is not trivial that the observed fermions fall into representations of 'low energy' symmetries which can be grand unified.

Grand unification introduces new gauge interactions between the fermions which violate baryon and lepton number conservation. They are mediated by heavy gauge bosons of masses m_x heavier than 1014 GeV if protons and bound nucleons are not to have lifetimes shorter than the present experimental lower limit¹⁰ of 10³⁰ yr. In their present formulations, GUTs do not include gravitation^{11,12}. As quantum gravity effects (such as one-graviton exchange) become important at energies comparable with the Planck mass of 1.2×10^{19} GeV, it follows that the grand unification mass scale must be $<10^{19}$ GeV if the GUT concept is to make sense.

The grand unification mass scale can be estimated¹³ using the renormalization group 14,15, the idea being that while the SU(3), SU(2) and U(1) coupling constants are equal above this scale, below it they have different logarithmic dependences on the effective energy scale at which they are measured. This is how13 the SU(3) interactions can become strong at energies of the order of 1 GeV, while the $SU(2) \times U(1)$ weak interactions can be weak at low energies. For the GUT philosophy to be viable, the 'low-energy' weak interactions must be neither too strong-in which case grand unification would occur at too low a mass scale and baryons would decay too fast—nor too weak—in which case grand unification would not occur before the Planck mass and it would have been wrong not to have included gravity.

The fine structure constant $\alpha = \alpha_2 \sin^2 \theta_W$, where $\sin^2 \theta_W$ is determined by GUT to be 3/8 in the symmetry limit above the grand unification scale^{4,5,13}. (The variation of $\sin^2 \theta_W$ below this scale has been computed^{13,16,17}, and the value of 0.21–0.22 found at low energies lies within 1 s.d. of the present experimental value (I. Liede and M. Roos, personal communication).) Knowing the symmetry value of $\sin^2 \theta_w$ we can translate the 'grand unifiability' restriction on the weakness of the weak interactions into upper and lower limits on α itself. In the leading order approximation, the renormalization group equations at energy scales Q between 100 GeV ($\approx m_{W\pm}$, m_{Z0}) and the grand unification scale m_X tell us that

$$\frac{1}{\alpha(Q)} \approx \frac{11}{\pi} \ln \left(m_{\rm X}/Q \right) + \frac{8}{3} \frac{1}{\alpha_3(Q)} \tag{1}$$

if we only include GUT representations of fermions and gauge bosons. From renormalization, if $\alpha_3(Q)$ is of the order of 1 on a typical hadronic energy scale then it should be of the order of 0.1-0.2 when $Q \sim 100$ GeV (refs 18-21). There is also a change in the effective $\alpha(Q)$ between its value at the Thompson limit Q = 0, which is where we define and measure α , and its value at $Q \approx 100 \,\text{GeV}$:

$$\frac{1}{\alpha} \approx \frac{1}{\alpha (100 \,\text{GeV})} + 9 \tag{2}$$

If we use these low energy renormalizations of $\alpha_3(Q)$ and $\alpha(Q)$ and require 10^{14} GeV $< m_X < 10^{19}$ GeV in equation (1) we find $1/119 < \alpha < 1/174$ which we have rounded off to the values quoted earlier. Including a possible light Higgs boson into the leading order renormalization group equations changes these bounds by $\sim 1\%$.

Any theorist working on grand unification would have known

$$\frac{m_{\rm X}}{\Lambda_{\rm QCD}} = \exp\left\{\frac{0(1)}{\alpha} + 0(1) \times \ln \alpha + 0(1) \times \alpha^0 + \ldots\right\}$$
(3)

and put in the known value of α to determine m_X in minimal GUTs within a factor of 10 or less²². However, to our knowledge no-one has inverted the procedure and pointed out how tightly constrained a grand unfiable α must be. Indeed the previous upper limit on α from GUTs was much weaker than the present one. The most precise estimates²² of m_X have gone beyond our leading order formula (1) to include all the lnc and oterms of equation (3). As we intend to convey a qualitative message, we will not repeat all those calculations here. However, we emphasize that they should enable one to make an even more refined estimate of α if baryon decay is ever observed. This is because the baryon lifetime²² is very sensitive to the value of m_x :

$$\tau_{\text{baryon}} \approx 8 \times 10^{30 \pm 1} \,\text{yr} \times \left(\frac{m_{\text{X}}}{6 \times 10^{14} \,\text{GeV}}\right)^4$$
 (4)

Hence a determination of $\tau_{\rm baryon}$ should enable us to fix $m_{\rm X}$ within a factor of 1.5 or so. This 10-fold improvement in bounding m_X would in turn enable us to estimate α^{-1} an order of magnitude more precisely say to ± 3 . The fact that α lies closer to the upper limit we have established gives us hope that m_X is closer to 10^{14} GeV than to 10^{19} GeV, and more detailed estimates in popular GUTs strongly suggest²² that the baryon lifetime is sufficiently short to be detectable in the forthcoming generation of experiments.

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- Glashow, S. L. Nucl. Phys. 22, 579 (1961). Weinberg, S. Phys. Rev. Lett. 19, 1264 (1967)
- Salam A. Proc. 8th Nobel Symp. Stockholm (ed. Svartholm N.) 367 (Almqvist and Wiksells, Stockholm, 1968).
- Georgi H. & Glashow, S. L. Phys. Rev. Lett. 32, 236 (1974). Pati, J. C. & Salam, A. Phys. Rev. Lett. 31, 661 (1973); Phys. Rev. D8 1240 (1973); D10, 275 (1974).
- Georgi, H. Nature 288, 649 (1980).
- Glashow, S. L. & Nanopoulos, D. V. *Nature* **281**, 464 (1979). Farhi, E. & Susskind, L. Preprint TH. 2975 CERN (1980).
- Ellis J. Preprint TH. 2942 CERN (1980).
- 10. Learned, J., Reines F. & Soni, A. Phys. Rev. Lett. 43, 907 (1979).
- 11. Ellis, J. Gaillard, M. K., Maiani, L. & Zumino, B. Unification of the Fundamental Particle Interactions (eds Ferrara, S., Ellis, J. & van Nieuwenhuizen, P.) 69 (Plenum, New York 1980).
- 12. Ellis J., Gaillard M. K. & Zumino, B. Phys. Lett. 94B, 343 (1980).
- Georgi, H., Quinn, H., R. & S. Weinberg, Phys. Rev. Lett. 33, 451 (1974).
 Stueckelberg E. C. G. & Peterman, A. Helv. phys. Acta 26, 499 (1953).

- Gell-Mann, M. & Low, F. E. Phys. Rev. 95, 1300 (1954).
 Buras, A. J., Ellis, J., Gaillard, M. K. & Nanopoulos, D. V. Nucl. Phys. B135, 66 (1978).
 Marciano, W. J. & Sirlin A. Phys. Rev. Lett. 46, 163 (1981).
 Barber, D. P. et al. Phys. Lett. 89B, 139 (1979).

- Bartel, W. et al. Phys. Lett. 91B, 142 (1980).
 Brandelik, R. et al. Phys. Lett. 94B, 437 (1980).

- Berger, C. et al. Phys. Lett. 97B, 459 (1980).
 Ellis, J., Gaillard, M. K., Nanopoulos, D. V. & Rudaz, S. Nucl. Phys. B176, 61 (1980).

Dynamical symmetry in the quadratic Zeeman effect

C. W. Clark & K. T. Taylor

Science Research Council, Daresbury Laboratory, Daresbury, Warrington WA4 4AD, UK

Zimmerman, Kash and Kleppner¹ have recently posulated the existence of a 'hidden' symmetry in atomic hydrogen in a uniform magnetic field. We have drawn similar conclusions from independent work2. Here, we show that the quasi-constant of the motion in this system can be related in a simple way to the asymptotic form of the quantum mechanical wavefunction. This development is consistent with the broad outline drawn by Fano^{3,4} who has emphasized the role of this particular problem as a member of a large and important class of dynamical systems characterized by the existence of motion along a 'ridge' of a potential surface.

Such motions are intrinsically unstable, because a fall off the ridge into a region of lesser potential is always possible. Nevertheless, it is frequently the case that important observable quantities are determined solely by these unstable motions. This has long been known^{5,6} to be so for the most prominent other member of this class, the system of two electrons escaping simultaneously from the field of an ion, which occurs in problems of electron impact ionization and multiple photoionization of atoms. Thus, a thorough understanding of the quadratic Zeeman effect, necessary in itself for the interpretation of current experiments, may also be relevant to more general questions of atomic dynamics. We draw special attention here to the analogy provided by the problem of two-electron escape, in which competing modes of partial escape

$$e + e + A^{2+} \rightarrow e + (A^{+})^{*}$$

give rise to the Wannier threshold law^{5,6}.

We focus our discussion on the interpretation of Fig. 1, the bottom frame of which shows the oscillator strength for dipole transitions from the ground state of hydrogen to excited states with a single quantum of angular momentum in the direction of the magnetic field. These 'experimental' data were obtained by diagonalizing the hamiltonian within a large basis (\sim 1,500) of sturmian functions, using a set of banded matrix routines developed at the National Physical Laboratory which have been implemented on the CRAY-1 computer at Daresbury. The field strength is 4.7 T. Transitions to these states would result from absorption of light with circular polarization about the axis of the magnetic field. The lowest cluster of lines shown corresponds to the perturbed manifold of hydrogenic states with odd parity and principal quantum number n = 23. As the energy of the final

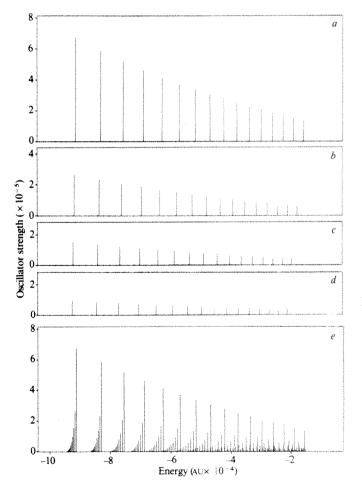


Fig. 1 The oscillator strength for absorption of circularly polarized light by atomic hydrogen in the ground state, for photon energies below the ionization limit. a, The principal series; b-d, second, third and fourth series; e, all lines present in this energy range. The abscissa is the total energy minus the linear Zeeman shift.

state increases the line clusters interpenetrate, with a surprising lack of mutual perturbation among lines of comparable oscillator strength with nearly equal energy. We are thus led to view this spectrum as a superposition of many essentially independent line series. The first four such series, in order of spectroscopic prominence, are drawn out in the upper frames of Fig. 1 (the 16 lowest members of each series only are displayed in these frames). Complementary evidence for this classification scheme can be inferred from the independent work of Zimmerman et al.¹, who have shown the dependence of lower energy levels on magnetic field strength at stronger fields. Their plots exhibit many near crossings of energy levels, which would be forbidden on general grounds⁷ except in the presence of an additional constant of the motion.

Corroborative information is obtained by examining the appropriate solutions to the quantum mechanical equations of motion. The hamiltonian for this system, if the centre-of-mass motion and relativistic effects are disregarded, is the same as that for a hydrogen atom in the absence of a field, with an additional term describing the interaction of the electron with the field. This term contains a part that is linear in the field strength and depends only on the projection of the electron's angular momentum on the field direction; this gives rise to the familiar linear Zeeman effect, which consists of a constant energy shift of all lines in the spectrum of Fig. 1. The remainder of the magnetic interaction is quadratic in the field strength, and results in the Schrödinger equation (in atomic units)

$$\left(-\frac{1}{2}\frac{\partial^2}{\partial r^2} + \frac{\vec{\mathbf{l}}^2}{2r^2} - \frac{1}{r} + \frac{1}{2}\boldsymbol{\beta}^2 r^2 \sin^2\theta\right) \psi = \varepsilon \psi \tag{1}$$

where $\tilde{\mathbf{I}}$ is the electron angular momentum operator, β one-half the magnetic cyclotron frequency, θ the angle between the magnetic field $\vec{B} = B\hat{z}$ and the electron position vector \vec{r} , and ε is the total energy minus the linear Zeeman shift. The last term on the left-hand side of equation (1) is a repulsive potential which assumes its maximum value in the plane z = 0. Because of the very small value of β ($\beta = 10^{-5}$ for a 4.7 T field), this term has a significant effect on the motion only at large distances r, where the potential surface takes the form indicated in Fig. 2. The equipotential surfaces are essentially spherical at small r, and cylindrical at large r. Thus, in photoabsorption the initial state of the electron, being tightly bound to the nucleus, is essentially spherical; the final state, which has significant amplitude at large distances, must eventually conform to the cylindrical geometry of the potential surface there. Understanding this transition between disparate geometries has constituted a major difficulty for the theory. In the problem of double photoionization also, the initial state has a more or less spherical symmetry, and the two electrons are not too strongly correlated. Dynamical analysis shows, however, that the only final state configurations which

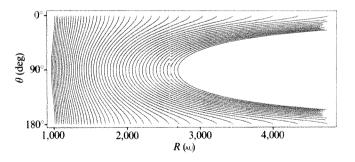


Fig. 2 The equipotential lines for electron motion in the field of a proton and a 4.7 T magnetic field. The ordinate is the angle θ subtended by the position vector and the magnetic field, the abscissa the electron-proton distance, R, in units of Bohr radius. The rightmost equipotential line corresponds to the energy $\varepsilon = 0$; the energy spacing between successive lines is one-fifth of the magnetic cyclotron frequency. A potential ridge is apparent at the angle $\theta = 90^\circ$.

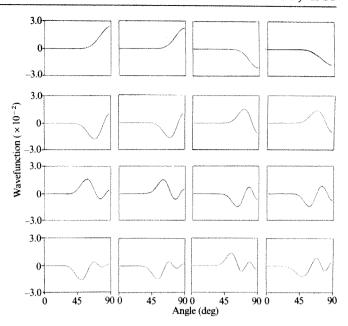


Fig. 3 Wavefunction versus angle along the appropriate turning line for selected states of the first four series. First row: the 1st, 5th, 10th and 16th states of the principal series as indicated in Fig. 1; second and third rows: the corresponding states of the second and third series; fourth row: the 1st, 5th, 10th and 15th states of the fourth series, the 16th state being strongly perturbed. All wavefunctions are symmetric about 90°.

lead to double ionization are those in which the two electrons lie at equal and opposite distances from the ion. Such configurations are unstable and can be considered as located on a ridge of the potential describing the interaction among the three particles of the system. A fall off the ridge results in capture of one of the electrons into a high Rydberg state. In our Zeeman problem the quadratic potential displays a ridge in the plane z=0. We shall show that a large fraction of the oscillator strength is associated with motion of the electron along this ridge, as has been suggested by early semi-classical treatments of the problem. In this case the fall off the ridge leads to escape of the electron along the magnetic field.

We consider first the principal series of Fig. 1, the members of which are shown in the top frame. We have computed accurate wavefunctions for the states of this series using the method indicated above, which has been described in more detail elsewhere². Briefly, each wavefunction is represented by expansion in terms of products of spherical harmonics and sturmian functions, the coefficients of which are determined by the diagonalization of the hamiltonian matrix. In the absence of a magnetic field these states would reduce to hydrogen wavefunctions with angular momentum l=1, and would carry all the oscillator strength. If the effect of the field is regarded as perturbative, which is appropriate for the lowest members, some higher angular momenta are mixed in with a resulting diminution in the oscillator strength. The higher members of this series are not characterized by the predominance of any one value of the angular momentum at small r. If, however, we look at the wavefunction at large r a simple consistent pattern emerges. Figure 3 shows the variation of the wavefunctions for some of these states along their classical turning lines, that is, those lines of Fig. 2 for which the equipotential energy is equal to the energy of the states. The wavefunctions are evaluated simply by summing the weighted individual values of the sturmian and spherical harmonic functions, computed by recursion formulae over the locus of the equation $\frac{1}{2}\beta^2 r^2 \sin^2 \theta - 1/r = \varepsilon$. It is seen that the states of the principal series may be characterized by having no nodes along their turning lines, and have a significant concentration of amplitude on the ridge. If, on the other hand, one examines the wavefunctions for these states in the z = 0plane, it will be seen that each successive state has an additional node along the ridge. This is the source of the rather close agreement of the energies of these levels with that predicted by the simple two-dimensional WKB theory^{2,8}. The subsequent series admit a similar classification. The states of the second series exhibit two nodes along the turning line, those of the third exhibit four, and those of the fourth, six-the number of nodes increases by two between series because all these states are necessarily even under reflection in the plane. The states with odd numbers of nodes, not shown in Fig. 1, have wavefunctions which vanish on the ridge and have much smaller oscillator strengths; these would be obtained in absorption of light polarized in the direction of the magnetic field.

Thus, the magnitude of the oscillator strength associated with a given state is directly correlated with the localization of its wavefunction on the ridge at large distances. Moreover, the spread of the wavefunction off the ridge occurs in a regular manner for the states of greatest spectral prominence. The members of a given series differ by their degree of excitation along the rising ridge of the potential; the separate series are distinguished by the degree of excitation of the unstable motion across the ridge. This picture of two nearly uncoupled modes of oscillation, which holds at least throughout the energy range of Fig. 1, explains the observed weakness of interseries perturbations. Note that this classification is an approximate one, and that some states, for example the 15th member of the third series and the 16th member of the fourth, show marked departures from the regular scheme. However, these isolated irregularities occur, in every case examined so far, when lines from separate series coalesce. Although one cannot extrapolate these results to higher energies or other atoms with complete confidence, nevertheless, it seems probable that the major structures seen in photoabsorption cross-sections at energies above the ionization limit are due to resonance states which can be placed in a principal series. The secondary features which are seen would then be associated with the higher series, and should be expected to be broadened more rapidly with increasing energy due to the greater probability of escape off the ridge.

Localization of a quantum mechanical wavefunction in a region of maximum potential, although at first seemingly contradictory, is probably a phenomenon of general importance. For example, the occurrence of barrier top resonances in heavy ion direct reactions have been analysed by Friedman and Goebel¹⁰. Some qualitative remarks are suggested by the form of the line clusters in the low energy region of Fig. 1e. If viewed in the framework of degenerate perturbation theory, the energy levels within a cluster are isomorphic to the eigenfrequencies of a chain of oscillators with nearest-neighbour coupling¹¹. If the spring constants along such a chain were uniform, a sinusoidal distribution of energy levels would be obtained and the eigenmodes of oscillation would be standing waves. This occurs, for instance, in the simple Hückel model for a π electron system of conjugated double bonds, resulting in electron wavefunctions which are entirely delocalized. If, however, the coupling along the chain is non-uniform-corresponding to the insertion of a substitutional impurity in a Hückel molecule—the eigenmodes of oscillation must become partially localized. In our problem the analogous nearestneighbour coupling is non-uniform, and gives rise to a linear distribution of energy levels at the extremities of each cluster. The wavefunctions of states at the top and the bottom of a cluster are then necessarily localized, respectively, in the regions of maximum and minimum potential.

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- Zimmerman, M. L., Kash, M. M. & Kleppner, D. Phys. Rev. Lett. 45, 1092-1094 (1980).
 Clark, C. W. & Taylor, K. T. J. Phys. B13, L737-L743 (1980).
 Fano, U. Phys. Rev. A22, 2660 (1980).

- Fano, U. J. Phys. B13, L519 (1980). Wannier, G. H. Phys. Rev. 90, 817-825 (1953).
- Rau, A. R. P. Phys. Rev. A4, 207-220 (1971)
- Von Neumann, J. & Wigner, E. Phys. Z. 30, 467 (1929). Starace, A. F. J. Phys. B6, 585-590 (1973).
- Lu, K. T., Tomkins, F. S. & Garton, W. R. S. Proc. R. Soc. A362, 421-424 (1978).
- 10. Friedman, W. A. & Goebel, C. J. Ann. Phys. 104, 145-183 (1977).
 11. Clark, C. W. Phys. Rev. A24 (in the press).

Failure of Arrhenius equation for hydroxyl radicalbicarbonate ion reaction above 100 °C

David Ritchie McCracken*

CEGB, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire GL13 9PB, UK

George V. Buxton

The University of Leeds, Cookridge Radiation Research Centre, Cookridge Hospital, Leeds LS16 6QB, UK

Although the chemical kinetics of reactive species in water at temperatures above 100 °C has received very little attention, the radiation chemistry of aqueous coolant at the high pressures (150 bar) and temperatures (330 °C) of pressurized and boilingwater nuclear reactors is of obvious interest to reactor chemists, engineers and designers1. There is a wealth of data2 on rate constants for reactions of the radiolytically important radicals e_{aq}, H, OH, O⁻, HO₂ and O₂ at room temperature, but only a few rates have been measured up to 90 °C and only one up to 200 °C. It has thus become common⁵⁻⁹ to calculate the rate constants required for modelling water radiolysis in reactor conditions by extrapolating room temperature data using the Arrhenius equation $k = A \exp(-E/RT)$ where E, the assumed activation energy, has a value in the range 12-20 kJ mol⁻¹. We report here the failure of this method to predict the rate of reaction of hydroxyl radicals with bicarbonate at temperatures up to 200 °C.

We are making a systematic study of the reaction kinetics of the primary radicals and their secondary products, formed in the radiolysis of water, using pulse radiolysis-kinetic spectrophotometry over the temperature range 25-200 °C. Simultaneously, measurements are being made of the effect of temperature on yields of the primary radicals, as this is another area where data are sparse and conflicting 7,9-12

We began by studying the hydroxyl radical reaction with bicarbonate ion for two reasons: first, this reaction has already been investigated at room temperature¹³, and second, it will occur when alkaline water is exposed to air and radiation, as happens in nuclear power operations.

The rate of reaction (1) was measured by observing the formation of the carbonate radical ion CO₃ which has an optical adsorption band with a maximum at 600 nm, in nitrous oxidesaturated solutions of sodium bicarbonate at natural pH.

$$OH + HCO_3^- \rightarrow CO_3^- + H_2O \tag{1}$$

$$e_{aq}^{-} + N_2O \xrightarrow{H_2O} N_2 + OH + OH^{-}$$
 (2)

$$CO_3^- + CO_3^- \to CO_2 + CO_4^{2-}$$
 (3)

Conditions were chosen to ensure that reaction (2) was too fast, and reaction (3) too slow, to interfere in the measurement of k_1 .

The solution was contained in a conventional Pyrex-quartz flow system inside a pressure vessel and subjected to an overpressure of helium at 20 bar to prevent boiling. The temperature of the solution was measured with a thermocouple in a quartz sleeve which protruded into the radiolysis cell. The thermocouple was checked by observing the boiling point of water as a function of pressure and this showed that the error in the temperature readings was ≤2 °C. The radiation source was a Van de Graaff accelerator producing 0.6-µs pulses of 3-MeV

^{*} Present address: Atomic Energy of Canada, Chalk River Nuclear Laboratories, Ontario,

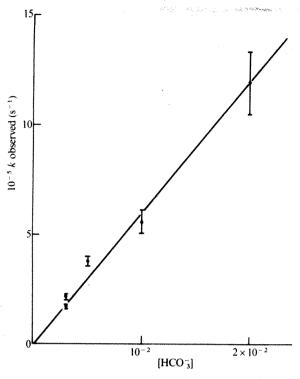


Fig. 1 Observed first-order rate constant for build up of CO₃ absorption at 308 K.

electrons. The dose per pulse was ~ 1 krad and five pulses could be given to each sample of solution without producing a detectable change in the results. The sample was renewed at each temperature.

There was no change in either the shape or the intensity of the spectrum of CO_3^- with temperature. As $G(CO_3^-) = G(e_{aq}^-) + G(OH)$, this indicates that there is little change in the yield of these primary species up to 200 °C.

The dependence of the observed pseudo-first-order rate constant on $[HCO_3^-]$ is shown in Figs 1 and 2 for 35 and 196 °C respectively. Second-order rate constants are plotted according to the Arrhenius equation in Fig. 3. Here the data have been corrected for changes in activity resulting from changes in the density of water ¹⁴ and the equilibrium constants ¹⁵ K_4 and K_5 .

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$
 (4)

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$
 (5)

Activity coefficients were calculated using extended Debye-Hückel theory 16 with appropriate constants 17 for the temperature range 25-200 °C. The largest correction (for $3\times$

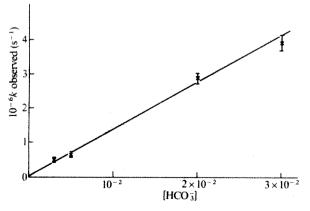


Fig. 2 Observed first-order rate constant for build up of CO_3^- absorption at 469 K not corrected for changing density or activity, with temperature. True $k = k_{\text{Obs}}/0.84$.

 10^{-3} mol dm⁻³ bicarbonate at 200 °C) was only 5%, and the most important factor was the density of water, for which the largest correction was 14%. In all cases $[CO_3^2^-] \le 0.01 \, [HCO_3^-]$ and we estimate the contribution of reaction (6) to the observed rate of formation of CO_3^- to be $(10\pm1)\%$ over the whole range of temperature and $[HCO_3^-]$ (based on our unpublished measurements of $k(OH+CO_3^{2-})$ in the range $25-200\,^{\circ}C$). Accordingly, the data in Fig. 3 have been reduced by 10%, although such a correction does not alter the shape of the Arrhenius plot and is less than the spread of the data $(\pm20\%)$ about the solid curve.

$$OH + CO_3^{2-} \rightarrow CO_3^{-} + OH^{-}$$
 (6)

There is no evidence for reaction of OH with carbonic acid and we assume that it can be neglected.

Figure 3 clearly shows that the data for k_1 do not obey the Arrhenius equation, but the deviation is not obvious below $100\,^{\circ}$ C. The low temperature data extrapolate to $k_1 \approx 10^9\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ at $330\,^{\circ}$ C with an apparent activation energy $\sim 14\,\mathrm{kJ\,mol^{-1}}$, whereas the high temperature results give $k_1 \approx 2 \times 10^8\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ and show scarcely any apparent activation energy. A reaction scheme which is consistent with the data is

$$OH + HCO_3^- \xrightarrow{k_a} X \xrightarrow{k_c} H_2O + CO_3^-$$

where X is an adduct of OH and HCO3. The adduct could be a

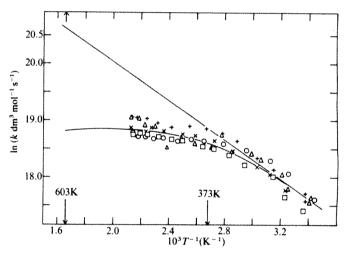


Fig. 3 In (corrected observed second-order rate constant) as a function of reciprocal temperature (K^{-1}) . The solid curve is a fit of the data according to the mechanism

OH+HCO₃
$$\stackrel{k_a}{\rightleftharpoons}$$
 X $\stackrel{k_c}{\rightleftharpoons}$ CO₃+H₂O.
 \square , 3×10^{-2} ; ×, 2×10^{-2} ; +, 1×10^{-2} ; \bigcirc , 5×10^{-3} ; \triangle , 3×10^{-3} mol dm⁻³ HCO₃.

carbon-centred radical, which rearranges to an oxygen-centred one and then splits off water to leave CO_3^- .

Then,

$$k_{\text{obs}} = \frac{k_{\text{a}}k_{\text{c}}}{k_{\text{b}} + k_{\text{c}}} = \frac{A_{\text{a}} \exp(-E_{\text{a}}/RT)}{1 + (A_{\text{b}}/A_{\text{c}}) \exp(E_{\text{c}} - E_{\text{b}})/RT)}$$

The solid curve in Fig. 3 has been calculated with $A_a = 1.3 \times 10^{11} \, \mathrm{dm^3 \, mol^{-1} \, s^{-1}};$ $A_b/A_c = 1.2 \times 10^3;$ $E_a = 18.9 \, \mathrm{kJ \, mol^{-1}};$ $E_b - E_c = 21.3 \, \mathrm{kJ \, mol^{-1}}.$ Unfortunately, the data

have not been obtained over a sufficiently large temperature range to fix these parameters to better than 50% for Ea and $E_b - E_c$ or within an order of magnitude for A_a and A_b/A_c . Experiments are planned which will extend the temperature range to ≥330 °C.

In spite of the present limitations in the data, they demonstrate quite clearly that the extrapolation of linear Arrhenius plots of rate constants measured at low temperatures (<100 °C) to obtain their high temperature values could be quite misleading, and that experimental measurements at temperatures pertaining to nuclear reactor operations need to be made if water radiolysis is to be modelled correctly in these conditions.

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- 1. Cohen, P. Water Coolant Technology of Power Reactors (Gordon and Breach, Edinburgh
- 2. Selected Specific Rates of Reactions of Transients from Water in Aqueous Solution (NSRDS-NBS 43 (1973); 51 (1975); 59 (1977). Schmidt, K. H. J. phys. Chem. 81, 1257 (1977)
- Christensen, H. & Schested, K. Radiat. phys. Chem. 16, 183 (1980).
 Jenks, G. H. ORNL-3848 (1965).

- Turner, S. E. Design Construct. Pract. 12, 66 (1968). Michael, B. D., Hart, E. J. & Schmidt, K. H. J. phys. Chem. 75, 2798 (1971).
- Burns, W. G. & Moore, P. B. Radiat. Eff. 30, 233 (1976). Burns, W. G. & Marsh, W. R. JCS Faraday 1 77, 197 (1981)
- Jha, K. N., Ryan, T. G. & Freeman, G. R. J. phys. Chem. 79, 868 (1975). Kalecinski, J. Bull. Acad. Pol. Sci. 21, 209 (1973).
- Burns, W. G., Marsh, W. R. & Barton, R. A. Nature 241, 86 (1973). Buxton, G. V. Trans. Faraday Soc. 65, 2150 (1969).
- Engineering Sciences Data Unit, No. 68010 (1978).
- Chem. Soc. Spec. Publ. No. 25 (1971).
- Moore, W. J. Physical Chemistry (Longmans, New York, 1962). Eisenberg, D. & Kauzmann, W. The Structure and Properties of Water (Oxford University

Radio-echo layering in polar ice sheets and past volcanic activity

D. H. M. Millar

Scott Polar Research Institute, University of Cambridge, Lensfield Road, Cambridge CB2 1ER, UK

A recent study has shown how acidity profiles along dated Greenland ice cores can reveal large volcanic eruptions in the Northern Hemisphere during the past 10,000 yr. It has been suggested that these layers of acidic ice in the polar ice sheets may also be detected by airborne radio-echo sounding (RES) techniques as stratification echoes²⁻⁴. Explosive volcanic eruptions eject large amounts of SO2 into the stratosphere5 where it forms an H₂SO₄ aerosol⁶. Studies of Antarctic snow⁷ and Greenland ice cores² show that this material can be deposited in layers of large areal extent on polar ice sheets. Calculations based on observations of these slightly acidic layers show that they should give rise to radar reflections of similar magnitude to those observed for stratification echoes. New RES data from the Antarctic enables the present comparison to be made between observed layer power reflection coefficients (PRCs), and calculated values for reflections from acidic ice layers and from layers of ice of changed density. A gap in layering has been identified which seems to coincide with a similar gap reported in Greenland at Crête⁸; profiles of layer PRC against age show a common pattern for many sites on the Antarctic ice sheet. This PRC/age profile may provide a record of explosive volcanic activity for the Southern Hemisphere over the past 150,000 yr.

During a joint Antarctic RES Programme conducted by the NSF, the Scott Polar Research Institute and the Electromagnetics Institute of the Technical University of Denmark (TUD), calibrated A-scope radar displays (showing returned/transmitted power versus range, Fig. 1) from the 60 MHz TUD ice-sounding radar were recorded, from which PRCs for internal layers were obtained after allowing for geometrical

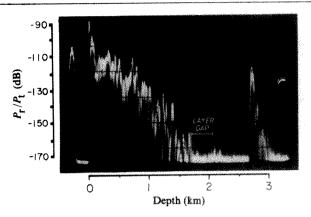


Fig. 1 Calibrated A-scope record from near Crête, Greenland. Note gap in layering at 1,700-2,100-m depth.

spreading and absorption losses to give profiles of layer PRC against depth (see Fig. 2a). Errors are estimated to be ± 10 m for depths and ±2 dB for PRCs; layer roughness causes the echo strength to fluctuate rapidly over a 10 dB range as the aircraft moves so that repeated, closely-spaced measurements are necessary to average out this effect.

Fluctuations in ice density have been suggested 9-11 as a cause of layer echoes in ice sheets, and stratigraphic analysis 12 of ice cores indicates that clear ice layers (millimetres thick) and similar features do occur in the upper parts. A simple expression for the maximum density contrast between clear ice and 'bubbly' polar ice as a function of depth9 (which compares well with estimates from the density profile of Byrd Station, Antarctica) has been used to compute the upper limit of PRCs from this mechanism (Fig. 2a, solid line). At Byrd observed PRCs are ~10-20 dB weaker than this limit, but decrease with depth in the same way; this is consistent with the observation of occasional density fluctuations in the core 10

At Byrd the ice is completely bubble-free below 1,100 m (ref. 13) and so this mechanism cannot apply to the deeper echoes seen. Paren and Robin¹⁴ suggested that deeper layer echoes are caused by layers of ice of changed loss tangent, due to changed impurity levels, and Hammer² thought that acids of volcanic origin may provide the impurity. It is suggested here that varying concentrations of such acids are the only plausible cause for changes in loss tangent detectable by RES. The highest acidity ice layers observed in the 400 m core from Crête are believed to coincide in depth with layer echoes, however, no comparison of reflection coefficients was attempted. In the absence of experimental measurements of the loss tangent of acidic polar ice, approximate estimates of the PRC for a layer of ice containing an elevated H⁺ concentration are made from laboratory measurements¹⁵ of the high frequency (h.f.) conductivity of HF-doped ice. The difference in loss tangent, $\Delta(\tan \delta)$, between pure ice and ice doped with acid is in theory16 related to the associated change in h.f. conductivity, $\Delta \sigma_{\infty}$:

$$\Delta(\tan \delta) = \Delta \sigma_{\infty} / 2\pi f \varepsilon' \varepsilon_0 \tag{1}$$

where ε' is the permittivity of polar ice, ε_0 the permittivity of

Table 1 Observed concentrations of acids in polar ice sheets following known volcanic eruptions, and estimated high frequency conductivities and power reflection coefficients

Eruption	Peak[H ⁺] (mmol m ⁻³)	Peak h.f. conductivity (Sm ⁻¹ ×10 ⁻⁶)	R, (dB)
Thera (1390 BC)	4.5	7.0	-83
Eldgia (AD 934)	20.0	30.0	-64
Hekla (1104)	5.5	9.0	-79
Laki (1783)	21.0	30.0	-64
Tambora (1815)	8.0	15.0	-72
Katmai (1912)	4.5	7.0	-83

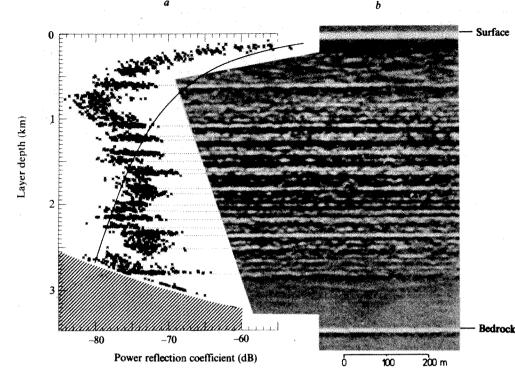


Fig. 2 a, PRC/depth profile (at site ~100 km north-west of Dome C) from many power measurements obtained with the TUD 60 MHz radar, using a 250-ns pulse length, 4-MHz receiver bandwidth and ~10-kW peak power. Solid line shows maximum PRC expected from density fluctuations, and shaded area is that beyond the theoretical detection limit. b, Corresponding section of Z-scope film (a differentiated, intensity-modulated version of the power return against time along flight-track); dotted lines connect groups of PRC measurements with lavers.

free space and f the radar carrier frequency. The high mobility of the H^+ ion is thought to dominate the h.f. dielectric properties of acid-doped ice, and so the nature of the anion will be expected to be of secondary importance. Impurities other than H^+ do not alter the loss tangent sufficiently (except in very high concentrations) to give detectable echoes. PRCs for a single interface (R_*) and a thin layer (R) of ice of changed loss tangent may then be obtained from the relations given by Paren and Robin:

$$R = 4\sin^2(2\pi l/\lambda)R_s \tag{2}$$

$$R_s = 1/16[\Delta(\tan \delta)]^2 \tag{3}$$

where l is the layer thickness and λ the radar wavelength in ice. Table 1 shows measured H⁺ concentrations associated with several large historical eruptions³, and estimated h.f. conductivities and PRCs; errors in estimating R_s are thought to be <5 dB. The estimated values are similar to those observed in the deeper ice below the region where density effects dominate. Little temperature dependence of PRC on H⁺ concentration is estimated at temperatures above -40 °C, so any variations of PRC with depth are expected to reflect past volcanic acid input to the ice sheet. PRC/layer age profiles from widely spaced Antarctic sites show many common features (Fig. 3), and this pattern may thus be interpreted as a record of the varying amounts of volcanic acid deposited on the ice sheet at different times.

Dated Z-scope layering from several Antarctic sites also reveals a common gap at ~15-20 kyr BP (this is not obvious on individual Z-scope records due to the close spacing of layers in the Antarctic), which seems to be simultaneous with a similar gap in layering at 1,900-2,300 m depth at Crête⁸ and dated¹⁷ at ~13-23 kyr BP. The uniqueness and coincidence of these gaps suggest a common cause: one possibility is chemical neutralization of volcanic acids by calcareous dust blown on to the ice sheets at the end of the last glaciation, due to exposure of former shallow water areas. Large increases in ice calcium levels are observed at Camp Century (Greenland) and Byrd at this time¹ and at Camp Century the ice is known to be slightly alkaline during this period. Such neutralization could affect the PRC/age profiles at other times, but this is thought to be unlikely because of the steadier Ca levels18 before 20 kyr BP, the probable link between Ca dust production and the end of the glaciation, and the lack of any other gaps in Z-scope layering. The PRC/age

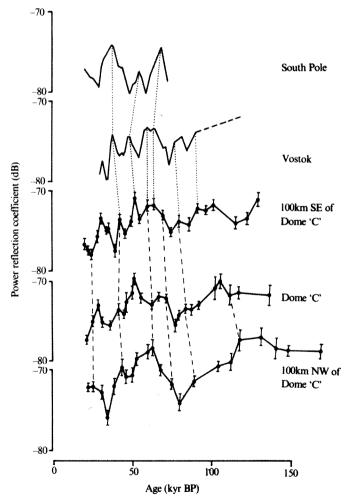


Fig. 3 PRC/age profiles for layers observed at South Pole, Vostok, Dome C and two sites $(1) \sim 100$ km south-east and $(2) \sim 100$ km north-west of Dome C. Bars indicate standard errors in power reflection coefficients and dashed lines join layers followed between sites on the Z-scope record. Dotted lines indicate suggested common features (data do not exist to enable layers to be followed on the Z-scope record).

pattern may therefore provide a useful indication of past volcanic acid input to the ice sheets, reflecting explosive volcanic activity in the Southern Hemisphere.

Conventional airborne echo sounding has already proved valuable in revealing the flow structure of the ice. The extension of the technique, using purpose-built radars (with better resolution) to the field of ice chemistry offers advantages of speed, economy, and the ability to sample the ice over representative areas, rather than at isolated points (with inherent uncertainties due to accumulation variability) plus deep penetration and consequently a long time scale. Such a technique should be complementary to drilling as core results are necessary to 'calibrate' the radar results.

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- Hammer, C. U., Clausen, H. B. & Dansgaard, W. Nature 288, 230-235 (1980).
- Hammer, C. U. Nature 270, 482-486 (1977). Hammer, C. U. J. Glaciol 25, 359-372 (1980)
- Gudmandsen, P. & Overgaard, S. Technical University of Denmark, Electromagnetics Institute, Rep. P312 (1978).

- Institute, Rep. P512 (1978). Lazrus, A. L. et al. J. geophys. Res. 84, 7869-7875 (1979). Arnold, F. & Fabian, R. Nature 283, 55-57 (1980). Delmas, R. & Boutron, C. J. geophys. Res. 85, 5645-5649 (1980).
- Gudmandsen, P. J. Glaciol. 15, 95-101 (1975)

- Gudmandsen, P. J. Glaciol. 15, 95-101 (1975).
 Robin, G. de Q., Evans, S. & Bailey, J. T. Phil. Trans. R. Soc. A265, 437-505 (1969).
 Clough, J. W. J. Glaciol. 18, 3-14 (1977).
 Ackley, S. F. & Keliher, T. E. J. Geophys. Res. 84, 5675-5680 (1979).
 Langway, C. C. Jr Geol. Soc. Am. Spec. Pap. 125 (1970).
 Gow, A. J. Int. Symp. Antarctic Glaciological Exploration, Hanover 78-90 (1970).
 Gaw, A. J. G. & Robin, G. de Q. J. Glaciol. 14, 251-259 (1975).
 Camplin, G. C., Glen, J. W. & Paren, J. G. J. Glaciol. 21, 123-142 (1978).
 Hobbs, P. V. Ice Physics (Clarendon, Oxford, 1974).
 Hammer, C. U. et al. J. Glaciol. 20, 3-26 (1978).
 Chemia, J. H. Hersen, M. M. Lannway, C. C. & Klouda, G. Proc. Palar Oceans Company of the Control of the Contr

- 18. Cragin, J. H., Herron, M. M., Langway, C. C. & Klouda, G. Proc. Polar Oceans Conf Montreal, 617-631 (1977).

Intermediate depth seismicity in the western Mediterranean unrelated to subduction of oceanic lithosphere

D. Hatzfeld

Laboratoire de Géophysique Interne, IRIGM, Université Scientifique et Médicale de Grenoble, BP 53X, 38041 Grenoble Cédex, France

M. Frogneux

Service de Physique du Globe, Institut Scientifique, av. Ibn Batouta, Rabat, Morocco

There are three zones in the Gibraltar area where intermediate depth earthquakes occur down to 150 km. Two of these, the Gulf of Cadiz and the western Alboran Sea, are oceanic areas which could be associated with the subduction of oceanic lithosphere. Our present measurements of arrival times of P and S waves show, however, that the third zone beneath the High Atlas is a typical intracontinental chain, where it is not likely that oceanic lithosphere has been subducted during the past 20 million years.

In the oceanic domain, West of 15 °W, the Azores-Gibraltar seismicity clearly outlines the Eurasian-African plate boundary. Towards the East, however, where the continental domain begins, previous studies have shown the presence of widely scattered epicentres¹⁻³ that have led to postulations of an Alboran buffer sub-plate. Precise relocations reduced the scatter and the seismicity can rather be interpretated as a reactivation of old tectonic faults^{4,5}.

Table 1 Velocity structure for HYP071

Mean continental		Oc	eanic	Thick continental		
z(km)	$v(\text{km s}^{-1})$	z(km)	$v(\text{km s}^{-1})$	z(km)	v(km s ⁻¹)	
0	6.10	0	6.10	0	6.10	
15	6.7	15	7.9	15	6.2	
30	8.0	30	8.0	45	8.0	
100	8.2	100	8.2	100	8.2	
200	8.3	200	8.3	200	8.3	
300	8.58	300	8.58	300	8.58	

z is the depth, v the velocity.

One major problem remains: the depth of the foci. Two deep earthquakes (h = 640 km) which do not fit easily in the active tectonics schemes, occurred beneath Granada, in southern Spain (29 March 1954 and 30 January 1973). Moreover, Munuera⁶ listed some intermediate depth earthquakes, but as the seismic network in Morocco and the location procedure were less developed, his locations are not very well established.

Arrival times, of both P and S waves, given in bulletins for stations in Spain, Algeria and Portugal are used here with our data in Morocco for the period 1972-78. The velocity structure used in the HYP071 routine (Table 1) represents an average continental structure as is the case in the Moroccan Meseta7 From more than 1,000 earthquakes of magnitude >3 detected in this area, we selected 401 events recorded in 5-25 stations (depending on the magnitude of the earthquake), with a residual smaller than 2 s. Among these we selected 247 earthquakes for which the standard deviation in location or depth, determined by HYP071, was <20 km (Fig. 1).

However, HYP071 error determination is a statistical evaluation of the error based on the dispersion of arrival times but does not take into account systematic bias due to the use of a wrong velocity structure or V_P/V_S ratio. We carried out a series of tests, on a few sample events in different regions (Alboran Sea, Gulf of Cadiz, High Atlas, Betic Cordillera, Rif) located with 5-15 arrival times. Starting with a synthetic zero-residual solution, we re-ran the location program, altering one parameter each time. The difference from the initial result gives us an estimate of the error associated with each parameter.

The first test examined the effects of errors in readings. Assuming that misreadings are <1 s for P waves and <2 s for S waves, random noise was added to synthetic travel times. The variations in computed location and depth were respectively <10 km and <20 km in all cases.

The effect of the V_P/V_S ratio was also investigated. Again synthetic travel times were used and locations recomputed using V_P/V_S ranging from 1.70 to 1.80. The resulting variations in locations and depth were similar to those obtained in the previous test.

The influence of the velocity structure was checked by calculating locations using the two extreme models given Table 1. These models correspond to a thin crust overlying an anomalously low upper mantle as in the Alboran Sea^{5,8} and to a thick crust as in the Betic Cordillera 10 or the Rif zone 5. Between these models the epicentres differ by 2 km and the depth by 13 km. HYP071 does not take into account lateral variations of velocity but it is unlikely that locations will be much altered by such variations.

One further question is related to the use of a flat layer velocity model, which could not be appropriate over an area ~1,000 km wide, because of the sphericity of the Earth. Chatelain et al. have shown that the resulting error for a 300-km wide network is less than 1 km. We assume the error to be of the same order in our case.

Figure 2a shows an east-west cross-section of the Alboran Sea. Most of the earthquakes are deeper than 50 km. One of them (7 August 1975) was recorded in 198 stations; its depth computed by the ISC is 94±2 km. Observations of pP and sP waves confirm this result⁵. Our determination using 13 stations is 105±4 km. The seismic zone lies between the peridotite

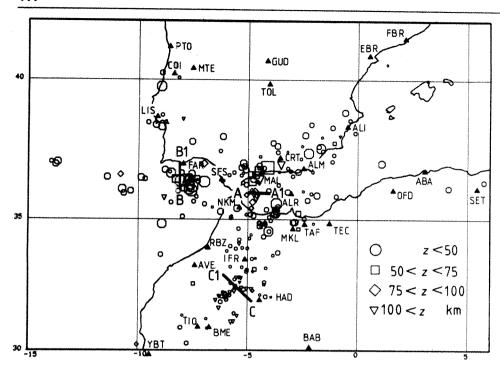


Fig. 1 Seismicity map of the Gibraltar area for the period 1972–78. Errors in location and depth (given by HYP071) are smaller than 20 km. Different symbols are functions of the depth of the focus; the size of the symbol is function of the number of stations which recorded the earthquake. A, Stations. The location of the cross-sections are shown.

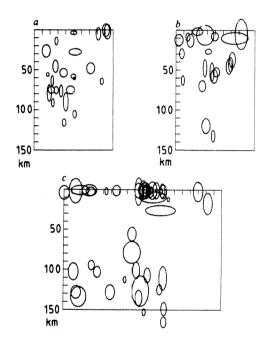


Fig. 2 Cross-sections for the three zones with intermediate earthquakes. a, The Western Alboran Sea; b, the Gulf of Cadiz; c, the High Atlas. The ellipses represent the error in location and depth given by HYP071.

massifs of Ronda (Spain) and Beni Bouchera (Morocco). A refraction profile in this area shows an anomalously high upper mantle velocity⁵ and that relative residuals at nearby stations are large¹¹, corroborating the existence of an anomaly in the velocity structure of this area.

Figure 2b shows a north-south cross-section of the Gulf of Cadiz. Most earthquakes there are shallower than 70 km, but three are deeper than 100 km. Commenting on the seismic intensity map of the 15 March 1964 earthquake, Udias and Lopez-Arroyo¹² suggest that its depth should be greater than the 27 km value inferred by the ISC.

In the High Atlas region we recorded 17 earthquakes deeper than 30 km in 7 yr. Because of their small magnitude (<3.5) these earthquakes are usually clearly recorded by only five

Moroccan stations. Thus it is important to have some confidence in the S arrival times. Figure 3 shows typical seismograms for an event at 125 km depth. Because of the significance of intermediate earthquakes in this region, the tests described earlier where applied individually to each event. Figure 2c shows a cross-section striking NW-SE across the High Atlas. Of the 17 events, 14 are deeper than 100 km, and there is a gap in activity between 30 and 55 km. The only three events in the 20-90 km range are on the north-east border of the seismic zone.

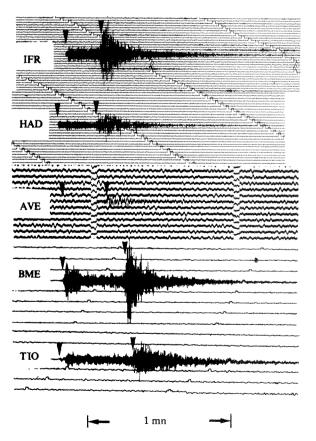


Fig. 3 Seismograms of the 1 August 1977 earthquake located at 125 km depth beneath the High Atlas. Notice the quality of P and S arrival times. Stations names refer to Fig. 1.

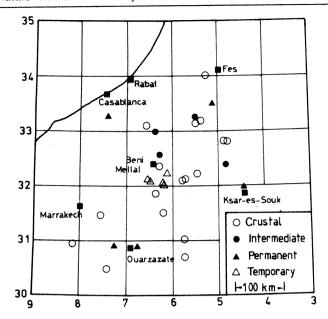


Fig. 4 Epicentres of a 15-day microearthquake survey in the High Atlas. Only events with a difference in arrival of P and S >5 s and error in depth <25 km are shown. ○●, Earthquakes; △▲, seismological stations.

To check the intermediate seismicity beneath the High Atlas, we carried out a microearthquakes survey for 15 days in 1978¹³. The network of six stations had an aperture of 30 km. During this period, 99 events were recorded in at least four stations; 43 of them have a travel time difference between P and S of <5 s and are shallower than 20 km. Among the 56 others, 51 are shallower than 20 km, one is 27 km deep and the last four are deeper than 110 km (Fig. 4). Their magnitude ranges between 1.7 and 2.5 and the closest station is at a mean distance of 60 km. The tests described above applied to these four events show a variation of 25 km in depth and 30 km in epicentre.

Two of the three zones where there are reliable intermediate earthquakes are close to the oceanic boundary between Africa and Europa and could be related to subduction (Gulf of Cadiz and Alboran Sea). On the other hand, the High Atlas whose basement and surroundings are continental is considered a typical intracontinental chain¹⁴. The existence of basaltic intrusions of Jurassic age¹⁵ is probably related to an extension episode into an entirely continental surrounding^{14,16}

Other zones, with intermediate depth earthquakes, are known in the Alpine-Himalayan belt such as Romania, Hindu-Kush, Tibet and Burma. In the Hindu-Kush and Romania a gap in seismicity is observed between 20 and 70 km and the activity decreases at 150 km and disappears at 200 km. This is similar to the results we observe in the High Atlas and differs from that in our two oceanic areas. Although we cannot exclude the possibility that deep seismicity represents the remains of subducting oceanic crust in all of these regions, it is open to question. In the High Atlas particularly, the possibility of a subduction zone in the past 20 Myr seems impossible.

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- McKenzie, D. P. Geophys, J. R. astr. Soc. 30, 109 (1972).
- Udias, A. & Lopez-Arroyo, A. Tectonophysics 31, 259 (1976).
- Udias, A. Rock Mechan. Suppl. 9, 75 (1980). Hatzfeld, D. Ann. Géophys. 32, 71 (1976).
- Hatzfeld, D. Ann. Geophys. 32, 71 (1970).
 Hatzfeld, D. thesis, Univ. Grenoble (1978).
 Munuera, J. M. El Mapa de Zonas Sismicas Generalizadas de la Peninsula Iberica (Instituto Geografico y Catastral, Madrid, 1969).
 Hatzfeld, D. & Bensari, D. Bull. Soc. geol. Fp. 7, 749 (1977).

- Hatzfeld, D. C. r. hebd. Séanc. Acad. Sci., Paris 283, 1021 (1976).
 Chatelain, J. L., Roeckers, S. W., Hatzfeld, D. & Molnar, P. J. geophys. Res. 85, 1365
- 10. Banda, E. & Ansorge, J. Geophys. J. R. astr. Soc. 63, 515 (1980).
- Goula, X., DEA, Univ. Grenoble (1976).
- 12. Udias, A. & Lopez-Arroyo, A. Tectonophysics 9, 323 (1970).
- 13. Frogneaux, M. thesis, Univ. Grenoble (1980)
- Mattauer, M., Tapponnier, P. & Proust, F. Bull. Soc. géol. Fr. 7, 521 (1977).
 Hailwood, E. A. & Mitchell, J. G. Geophys. J. astr. Soc. 24, 351 (1971).
- 16. Michard, A. Notes et Mémoires du Service Géologique, Rabat, 252 (1976)

Palaeomagnetism of the Ibero-Armorican arc and the Hercynian orogeny in Western Europe

H. Perroud & N. Bonhommet

Laboratoire de Géophysique Interne-Centre Armoricain d'Etude Structurale des Socles, (CNRS), Université de Rennes I, Campus de Beaulieu, 35042 Rennes Cédex, France

Van der Voo1 has pointed out that further advances in the understanding of the Hercynian orogeny require palaeomagnetic data from well studied orogenic areas. We report here palaeomagnetic results of Palaeozoic rocks taken in Brittany, Portugal and Spain in the Ibero-Armorican arc (IAA), a large geological feature considered to be a key area for the Hercynian orogeny in Western Europe. They show evidence that the IAA formed in two stages, extending results obtained in Spain by Ries et al.². Giving precise constraints for the former shape of the arc we can use pre-Hercynian palaeomagnetic directions found in Spain and Portugal to follow its evolution during Palaeozoic times. This megastructure linking Brittany to Iberia. squeezed between Gondwana and the northern continents during the Carboniferous, did break away from the former block during Ordovician times.

Geological^{3,4} and geophysical⁵ evidence suggests that the IAA is formed of large juxtaposed curved units lying on the two sides of the Bay of Biscay. Stratigraphic studies of Palaeozoic formations from the arc^{6,7}, performed at the Rennes CNRS centre, suggest that the shape of this structure could have been quite different before the Hercynian deformation. In particular, the very strong correlations between the stratigraphy and palaeontology of the Crozon area (Brittany) and the Buçaco syncline (Portugal) for Ordovician rocks imply that these two regions belonged to the same sedimentary basin in the Lower Palaeozoic, and thus palaeogeographic reconstructions' tend to place these two regions next to each other. Moreover, the question of whether the arc is of primary or secondary origin is crucial for several of the models proposed for the Hercynian orogeny. A primary origin implies that the arc always had the same curvature whereas a secondary origin implies oroclinal bending following Carey's model⁸. Ries et al.² recently showed that the Iberian arc, the inner part of the IAA, had a predominant secondary origin. The absence of definitive evidence that the two structures did evolve simultaneously led us to extend the sampling to the whole IAA and to different stages in

Samples were from: (1) Brittany: Upper Ordovician dolerites of Crozon (14 sites, 100 samples); (2) Spain: Ordovician volcanics of Cabo de Penas (2 sites, 31 samples), Upper Devonian red sandstones of Candas (3 sites, 32 samples), Lower Carboniferous red grits, Alba formation from Candas (3 sites, 23 samples) and San Emiliano (6 sites, 42 samples); (3) Portugal: Cambro-Ordovician redbeds (7 sites, 60 samples) and Upper Ordovician dolerites (4 sites, 34 samples) from Buçaco (Fig. 1). Natural remanent magnetization (NRM) was measured for these samples and for most of them a viscosity test⁹ was also performed; a.c. field, thermal and chemical demagnetizations were carried out (all samples being treated by at least one of these methods, mostly by two). In addition, rock magnetic experiments such as coercivity spectrum analysis to of pairs of

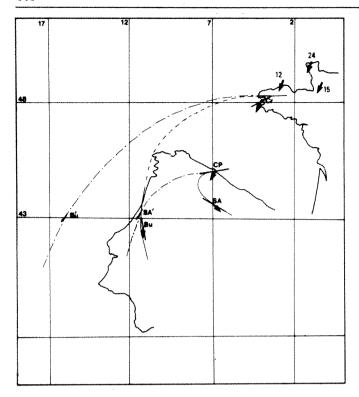


Fig. 1 Palaeomagnetic Carboniferous directions (thick arrows) found on the Ibero-Armorican arc with sampling locations (CR, Crozon; CP, Cabo de Penas and Candas; SA, San Emiliano; BU, Buçaco) after closing of the Bay of Biscay (fitted by Lefort²⁴). Values for additional data in Brittany correspond to the references given in the text (see also Table 1). The ante-tectonic shape of the arc deduced from the data is also given with the corrected Carboniferous directions (small arrows).

leached and unleached samples, heating to Curie temperature or cooling to the liquid air temperature of an acquired induced remanent magnetization (IRM), were carried out to support the interpretation of the multivectorial magnetization with physical arguments on the magnetic carriers. The results obtained from

these studies¹¹, which will be published in detail elsewhere, can be summarized as follows.

The magnetic behaviour of the Upper Ordovician dolerites from Buçaco and Crozon is identical. Intensities are low, with a maximum frequency between 10^{-2} and 10^{-3} A m⁻¹ and viscosities are high (>20% for 50% of the samples); the IRM acquisition curves are identical; they display the same magnetic transition at $-160\,^{\circ}$ C, suggesting the presence of magnetite, and current work at the Rennes CNRS centre gives K-Ar ages which range for both regions from 190 to 300 Myr. In accordance with the radio-dating results, the remanent magnetization is interpreted as being secondary with an acquisition period between Carboniferous and Triassic or Jurassic time (Table 1), thus confirming the geological relationships between Crozon and Buçaco.

Buçaco redbeds exhibit magnetization directions interpreted as pre-tectonic and remagnetization directions as being from the Upper Cretaceous and Quaternary¹¹. A characteristic of the pre-tectonic directions is a constant declination of 150° and variable inclination. The same behaviour was found by Duff¹² in an extensive palaeomagnetic survey of redbeds in Brittany with ages extending from Lower Cambrian to Lower Ordovician. From this work two sites at Crozon are well suited for comparison with Bucaco as both areas are thought to be part of the same sedimentary basin⁷, and thus allow comparison with data from contemporaneous redbeds. Components B3 and C found by Duff¹² on 10 specimens of these two sites using acid leaching, thermal and a.f. cleaning, gave a mean direction of 220° after tectonic correction (sites CP, CR in Tables 2 and 3 of ref. 12). The difference in declination between the two areas suggests a partial secondary origin of the IAA if we correct for the rotation of Spain during the Cretaceous and look at the variation of strike of the arc. Again, the palaeomagnetic characteristics of contemporaneous samples from Brittany and Portugal are identical.

At Cabo de Penas, Ordovician volcanics show two well defined pretectonic directions of magnetization (Table 1). One (component A) has a very steep inclination, coercivities over 70 mT and unblocking temperatures over 500 °C, and has been interpreted as Ordovician; the other (component B) has a shallow inclination and is comparable with the direction obtained east of Cabo de Penas for the Upper Devonian and Lower Carboniferous redbeds of Candas (component A); the

Site (reference on Fig. 1)	Rock unit	Component of magnetization	N	D	I	k	α	Š	Age of magnetization	Ref.
South limb										
SA	San Emiliano redgrits		30	102	13	53	2	270°	Lower Carboniferous	14
BU	Buçaco dolerites		20	139	-43	58	4	145	Jurassic ?	This work
20	Buçaco redbeds	Α	7	151	80	83	6	145	Ordovician	This work
	Dujus Junius	В	7	143	61	41	9	145	Between Ordovician and	
		C	6	144	36	45	9	145	Lower Carboniferous ?	This work
		Ď	3	153	5	212	6	145	Lower Carboniferous	
North limb										
CP	Cabo de Penas volcanics	Α	13	208	78	50	6	230	Ordovician	This work
		B)	78	178	19	39	8	230	Lower Carboniferous	This work
	Candas redbeds	A Ì								
		в′	8	185	23	27	10	230	Middle-Upper Carboniferous	This work
CR	Crozon dolerites	Α	19	208	-15	215	2	240	Triassic	This work
		В	38	205	29	33	4	240	Middle Carboniferous	This work
		C	12	350	-11	300	3	240	Upper Carboniferous	This work
	Armorican redbeds	В3	10	216	41	35	10		Devonian to early Carboniferous	12
		C	16	228	63	70	7		Silurian to early Carboniferous	12
15	Montmartin redbeds		186	208	16	20	7	270	Late Devonian-early Carboniferous	15
24	Flamanville granite		8	203	13		14		Carboniferous	22
12	Ploumanach granite (300 Myr)	access.	31	200	9	121	7		Middle Carboniferous	12
	Jersey dolerites	Α		199	16	71	9		Middle-late Carboniferous	16
		В		204	57	59	8		Siluro-Devonian	16
	Jersey volcanics (533 Myr)		66	292	78	8	19		Cambrian	16

Palaeomagnetic component directions found on the Ibero-Armorican arc. N, Number of samples used; D, I, declination, inclination; k, α_{95} Fisher statistical parameters²³; \bar{S} , mean structural direction. Statistics may refer to samples, specimen, directions or sites: see in each case original work given in the reference; only the total number of samples used is given here; all data were cleaned using a.f., acid and thermal cleaning as done by Duff^{12.16}, N.B. et al. 4 and Jones et al. 5 and a.c. field up to 0.2 T (2,000 oe) as in ref. 22.

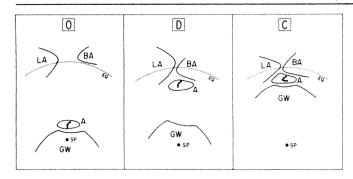


Fig. 2 Scheme of the evolution of the main plates involved in the Hercynian orogeny in Palaeozoic times (O, Ordovician; D, Devonian; C, Carboniferous) with mention of the Ibero-Armorican arc. LA, Laurentia; BA, Baltica; GW, Gondwana; A, Armorica.

eight sites (volcanics and sedimentary) were used to give a mean direction which is pre-tectonic as shown by a fold test. We can do better than the classical tilt correction on this example to calculate the mean direction: if we look at the angle between the strike direction and the magnetization on each site, this angle seems to be very constant $(52.5^{\circ} \pm 5^{\circ})$ regardless of the site and even though strike directions vary on this example between 210° and 244°. This suggests that deformation occurred in two stages, the latter one with a vertical axis correlated with the tectonic closure of the arc13, and that the magnetization was acquired before deformation; taking this result into account and using a regional mean direction for strike of 230°, the mean palaeomagnetic direction of the eight sites in Table 1 has been obtained after correcting each site for strike variation. The six sites of redbeds near Candas also show a soft component obtained on a few samples but scattered among all the sites (Candas redbeds component B, Table 1); the post-tectonic origin of this component is clearly indicated by a negative fold test¹¹. The corresponding inclination is very close to that obtained at Crozon on the dolerites (Crozon dolerites component B, Table 1); this last component has also been interpreted as post-tectonic but with a magnetization predating the closure of the arc, as shown by using a 'strike direction' correction on the 14 sites of Crozon. Magnetization was therefore acquired at Crozon between two main phases of deformation of the IAA.

The direction given in Table 1 for San Emiliano was obtained by N.B. et al. 14 using an unfolding model which took into using an unfolding model which took into account the two phases of deformation in the area; the effect of the model was to increase the precision parameter k (from 14 to 64) in comparison with the usual tilt correction. A pre-tectonic direction was found (Table 1) and a Lower Carboniferous age for the magnetization inferred from the age of the sediments.

Ries et al.² sampled the same Alba formation at Carranques near Candas and at San Emiliano; however, their result cannot be used for comparison because only three samples at Carranques were thermally cleaned and the authors report a direction $D = 211^{\circ}$, $I = 48^{\circ}$ with k = 2 showing large scatter after thermal cleaning; this is a general behaviour of all results obtained after cleaning as reported in that study. At San Emiliano four sites have been studied for the NRM but none appears among the thermal cleaned results given in Table 2 of their study.

Carboniferous data from Brittany obtained by Jones et al. 15 on the syncline of Montmartin and by Duff¹² from the Ploumanach granite, Jersey dolerites and volcanics, together with our own results at Crozon and Candas (CR, CP, Fig. 1) reveal (Table 1) that the corresponding directions are not significantly different for the northern part of the IAA before, (Candas A) and after (Candas B, Crozon B) deformation: most of the rotation seems to have occurred in the southern part of the arc. A new shape of the IAA can then be inferred taking the simplest compatible solution with the palaeomagnetic data. This

indicates that 80° of the curvature of the arc are secondary and that 70° are primary or pre-Carboniferous.

Ries et al. used results based on NRM directions from 29 sites along the Iberian arc to demonstrate convincingly the partially secondary origin of this structure; however, because these authors did not consistently use cleaned results to look for multicomponent magnetization, because they infer a Variscan age for all magnetizations, their data cannot be used for comparison as they give no directions associated with the age of magnetization for the different rock units studied. However, their result, a bending of 110°, is not very different from the 80° reported here.

From the new position of the sampling areas and the palaeomagnetic vectors calculated for the new shape of the arc (that is, after elimination of the Hercynian deformation), we can determine virtual palaeomagnetic poles for our Ordovician sites. The Buçaco (25°N, 335°E) and Cabo de Penas (30°N, 330°E) poles coincide in the new reconstruction, indicating that the shape of the arc we deduced from the Carboniferous data is also compatible with our Ordovician results. We therefore propose that 70° of the arc are effectively primary (presedimentary), inherited from Precambrian times. The mean Carboniferous declination of the north limb of the IAA differs by a few degrees (10°-15°) from that of stable Europe as shown by Jones et al. 15 for Montmartin syncline; it is not clear, however, whether the bending took place entirely in the southern part of the arc with a further rotation of 15° of the whole Ibero-Armorican block or whether it was the closure of the arc that caused the 15° difference with Europe. However, this does not change the former shape of the IAA found from the data. The Crozon B and Candas B components, both post-tectonic but ante-closure of the arc, in agreement with results of Jones et al. at Montmartin, show that at that time the IAA with its primary shape was close to Europe, with a large ocean separating it from Gondwana, a result shown for Brittany by Jones et al. 15 (Fig. 2).

The Ordovician mean palaeomagnetic pole obtained (27°N, 332°E) falls between Cambrian and Siluro-Devonian poles found by Duff¹⁶ for the Armorican massif and this agrees with the Ordovician age assumed for the magnetization. Interestingly, this pole is quite different from conventional Ordovician poles for the British Isles¹⁷ but could more easily be correlated with north Wales data, which have until now been considered as anomalous¹⁸. Recent results by Hagstrum et al. 19 showing that the apparent polar wander curve of the Armorican massif is very similar to the Eo-Cambrian-Cambrian curve for Gondwanaland prompted us to compare the Ordovician palaeolatitude for the IAA (70°) with the African Ordovician pole²⁰; this shows that the IAA should have been very near to Gondwanaland in the early Palaeozoic. These results show, moreover, that the Armorican massif and Iberia Meseta were probably a single unit during the entire Palaeozoic, thus justifying the name of 'Armorica plate' given by Van der Voo²¹ to these regions of Western Europe; the large drift of this plate since the Ordovician shows that collision and subduction tectonic processes did exist during the Hercynian orogeny. Finally, taking into account all these results and using available data given by Van der Voo¹, we propose a scheme of the main plates involved in the Hercynian orogeny with particular reference to the Armorica plate bearing the IAA (Fig. 2).

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- Van der Voo, R. et al. Int. geol. Congr. Colloq. C6, 204-212 (1980). Ries, A. C. et al. Earth planet. Sci. Lett. 50, 301-310 (1980).
- Cogné, J. Histoire Structurale du Golfe de Gascogne (IFP) II, 1, I1-23 (1971). Matte, P. Nova Acta Leopoldina 224, 239-262 (1974).

- Lefort, J. P. Geology 7, 384-388 (1979). Henry, J. L. et al. Commun. Serv. Géol. Port \$7, 303-345 (1974).
- Paris, F. & Robardet, M. Bull. Soc. Géol. Fr. 7, 1121-1126 (1977).
 Carey S. W. The Tectonic Approach to Continental Drift (ed. Carey, S. A.) (University of
- Tasmania, 1958)
- Thellier, E. & Thellier, O. Ann. Geophys. 15, 285-376 (1959).
 Dunlop, D. J. Geophys. J. R. astr. Soc. 27, 37-56 (1972).

- 11. Perroud, H. thesis, Univ. Rennes (1980).
- Duff, B. A. Geophys. J. R. astr. Soc. 59, 345-365 (1979).
 Julivert, M. Histoire Structurale du Golfe de Gascogne (IFP) I2-1, I2-28 (1971).
 Bonhommet N. et al. J. geophys. Res. 86, 1873-1887 (1981).
 Jones, M. et al. Geophys. J. R. astr. Soc. 58, 287-308 (1979).
 Duff, B. A. Geophys. J. R. astr. Soc. 60, 355-375 (1980).
 Piper, J. Earth planet. Sci. Lett. 44, 176-192 (1979).
 Thomes C. R. Paiden, J. C. Marca 186, 282-282.

- Thomas, C. & Briden, J. C. Nature 259, 380-382 (1976).
 Hagstrum, J. T. et al. Geophys. J. R. astr Soc. 61, 489-517 (1980).

- Morel, P. & Irving, E. J. Geol. 86, 535-561 (1978).
 Van der Voo, R. EOS Trans. 66, 241 (1979).
 Van der Voo, R. & Klootwijk, C. T. Geelogie Mijn. 51, 609-617 (1972).
 Fisher, R. A. Proc. R. Soc. A217, 295-305 (1953).
- 24. Lefort, J. P. Mar. Geol. 37, 355-369 (1980).

Dietary carbon sources of mussels and tubeworms from Galápagos hydrothermal vents determined from tissue ¹⁴C activity

P. M. Williams*, K. L. Smith*, E. M. Druffel† & T. W. Linick†

* Scripps Institution of Oceanography and

† Department of Chemistry, University of California, San Diego, La Jolla, California 92093, USA

The large quantities of reduced carbon that are required to support the filter-feeding mytilid mussels (Mytilus sp.), vesicomyid clams (Calyptogena sp.) and various other animals in the Galápagos hydrothermal vent systems are thought to be derived from either the in situ synthesis of particulate organic matter by chemoautotrophic, sulphide-oxidizing bacteria 1,2 or by the advection of sedimentary organic carbon into the vent environment from surrounding areas 3.4. In contrast, the dense populations of vestimentiferan tubeworms (Riftia pachyptila), which lack mouth organs and digestive tracts, apparently utilize organic carbon synthesized by symbiotic chemoautotrophs⁵. We present evidence here, based on ¹⁴C activities and ¹³C/¹²C ratios, that the principal source of dietary carbon for mussels and tubeworms is derived from the dissolved inorganic carbon (DIOC) in the vent effluent waters.

There are two postulated sources of particulate organic carbon (POC) available as dietary carbon to filter-feeding organisms. The first is sedimentary POC derived from DIOC fixed photosynthetically at the ocean's surface which subsequently reaches the ocean floor. Its radiocarbon activity is assumed to be the same as that of the DIOC in surface water $(+20\pm20, \text{ Table 1})$. The δ^{13} C of this POC (-22, Table 1) is a mean value derived from δ^{13} C measurements of POC^{6,7} collected below 500 m. The second source is POC synthesized chemoautotrophically from DIOC in the vent waters. This DIOC originates from both magmatic activity $(\Delta^{14}C = -1,000)$ or 'dead' carbon) and the ambient bottom water ($\Delta^{14}C = -233$, Table 1) which mixes with the high-temperature hydrothermal fluid. As the organic 14 C activities of the organisms grown in the vent systems reflect the Δ^{14} C of these three carbon sources and the 15C/12C ratios reflect the carbon isotope fractionation occurring during synthesis of organic tissues in these organisms, it is possible to calculate the relative contribution of each carbon source using the following equations:

$$\Delta^{14}C_{\text{mussel}} = x(-1,000) + y(-233) + z(20) \tag{1}$$

$$\delta^{13}C_{\text{mussel}} = (x+y)(\delta^{13}C_{\text{vent POC}}) + z(-22)$$
 (2)

where x, y and z are the relative amounts of magmatic DIOC. ambient DIOC and sedimentary POC, respectively. The δ^{13} C of POC in the vent water is unknown. Both of the above equations have to be satisfied to determine the relative amounts of the three possible dietary carbon sources utilized by the filterfeeding organisms.

The quantitative contribution of sedimenting POC to the food supply for filter-feeding vent organisms is questionable considering the flux of POC to the ocean floor and the in situ respiration rates of mussels. The total, mean flux of POC to the sea floor at 2,670 m 0°35.75' N, 86°05.66' W measured 20 and 100 m above the bottom on 4 July 1976 was $2.0 \text{ g C m}^{-2}\text{yr}^{-1}$ (ref. 9). In situ measurements of individual mussel respiration rates by K. Smith (unpublished results) range from 0.30 to 0.81 ml O₂ h⁻¹ in dense mussel beds. Assuming a respiratory quotient of 0.85, then these rates are equivalent to an organic carbon requirement of 1.2-3.3 g C yr⁻¹ (mean = 2.3 g C yr⁻¹). Thus, each mussel would essentially require the total annual POC flux per square metre for maintenance with no growth.

The 14C activity of the mussel tissue could result from a mixture of sedimentary POC and POC of low 14C activity derived from DIOC in the vent waters. The maximum amount of magmatic DIOC in the Galápagos vent waters (T = 17 °C) has been estimated to be 375 μ mol kg⁻¹, a 13% increase above the ambient DIOC concentration of ~2,400 μ mol kg⁻¹ (refs 10, 11). If 13% of the vent water DIOC is magmatic (Δ^{14} C = 1,000) and the remaining 87% is composed of ambient DIOC $(\Delta^{14}C = -233)$ plus sedimentary POC derived from the DIOC in surface water ($\Delta^{14}C = +20$), then the maximum amount of sedimentary POC that could be incorporated into mussel tissue $(\Delta^{14}C = -267)$ is 26% (equation 1). Estimates of mussel density in the 'Musselbed' vent area (W. Smithy, personal communication) are 20 ± 9 specimens per 0.25 m^2 in the immediate vicinity of the vent plume. If the mussels are utilizing the calculated maximum of 26% of sedimentary POC for growth (taken here as equivalent to maintenance), then each vent musselbed would use $\sim 48 \text{ g C m}^{-2}\text{yr}^{-1} (0.26 \times 2.3 \text{ g C yr}^{-1} \text{ per}$ mussel × 80 mussels per m²), or about 35% of the annual primary productivity per square metre in the euphotic zone9 for the growth of mussels alone.

There is a significant difference between the ¹³C/¹²C ratios, as well as ¹⁴C activities, of mussel tissue collected 1 and 8 m from the vent plume (Table 1). If sedimentary POC (δ^{13} C = -22) was incorporated into the tissue of the 8-m mussel, the resulting δ^{13} C value (-32.3) would be less than that observed (-32.8). This calculation assumes that the 14C activity of the 8-m mussel tissue ($\Delta^{14}C = -228$) is derived from a mixture of 14% sedimentary POC (Δ^{14} C = +20) and 86% chemosynthetic POC $(\Delta^{14}C = -267)$ (equation 1).

If the ¹⁴C activity of mussel shell carbonate reflects the ¹⁴C activity of DIOC in the surrounding seawater, then the 21% decrease in Δ^{14} C between the ambient seawater DIOC and the mussel shell carbonate reflects dilution of the ambient DIOC with 3% magmatic DIOC. For the two clam shell carbonates, the 30% decrease in Δ^{14} C would represent dilution of the ambient DIOC with 4% magmatic DIOC, taking the 14C activity of the bottom water at 21° N to be the same as at the Galápagos rift zone. A similar calculation gives 4.4% magmatic DIOC incorporated into cellular carbon synthesized by the chemoautotrophic bacteria and used by the mussels 1 m from the plume (provided the contribution from sedimentary POC is negligible).

The identical 14C activities of the vestimentiferan tubeworm tissue and the mussel tissues collected 1 m from the vents strongly suggest that the tubeworms and mussels are utilizing the same DIOC sources, even though δ^{13} C in the tubeworm tissue is 23% greater than in the mussel tissue (Table 1). These atypical 13 C/ 12 C ratios found in the vent organisms have been discussed by Rau^{12,13}. If bacterial chemosynthesis is the major process providing dietary carbon for the mussels and tubeworms, it is still unclear why carbon isotope fractionation should be different between chemoautotrophic synthesis occurring in the vent waters and internally (symbiotically) in the tubeworms.

An additional carbon source which may contribute to the bacterial synthesis of POC in the vent waters is methane, energetically a more favourable substrate for the growth of heterotrophic bacteria than DIOC. Methane concentrations up to 3.4 µmol kg⁻¹ have been measured in the Galápagos vent

Table 1 Δ^{14} C and δ^{13} C values of Galápagos vent organisms and relevant carbon sources

Sample description	(%) ∆ ¹⁴ C	(%) δ ¹³ C	Ref.
Mussel tissue—dive 880 ('Musselbed', 1 m from vent)	-270 ± 6	-33.9	This work
Mussal tissue—dive 895 ('Mussalbed', 1 m from vent)	-263 ± 8	33.8	This work
Mussel tissue—dive 880 ('Musselbed', 8 m from vent)	-228 ± 12	-32.8	This work
Mussel shell—dive 991 ('Musselbed', 1 m from vent)	-254 ± 6	+2.8	This work
Tubeworm these—dive 993 ('Garden of Eden', in vent plume)	-270 ± 20	-10.9	This work
Clam shell—dive 981 (East Pacific Rise, 21° N)	-263 ± 6	+3.1	This work
Mussel tissue ('Clambake I' specimen, 8 replicates)	_ `	-32.7 to -33.6	12
Tubeworm tissues ('Rose Garden')	-	-10.8 to -11.0	13
Clam shell ('Clambake I')	-263 ± 10	 ,	10
Total DIOC, 2584 m* (Geosecs Station 337)	-233.4		14
Total DIOC, surface sea water†	$+20\pm20$	-1.7 to -3.5	15
Particulate organic detritus > 500 m	_	-20 to -25 ; mean -22	6, 7

The three mussels for tissue analysis were collected 1 and 8 m from the 'Musselbed' vent plume, Alvin dives 880 (21 January 1979) and 895 (20 February 1979), 0°47.89' N, 86°09.21' W at 2,493 and 2,480 m. The mussel for shell analysis was collected 1 m from the 'Musselbed' vent plume, Alvin dive 991 (8 December 1979) at 2,490 m. The tubeworm was collected in the 'Garden of Eden' vent plume, Alvin dive 993 (10 December 1979), 0°47.69' N, 86°07.74' W at 2,518 m. The clam for shell analysis was collected from the East Pacific Rise hydrothermal vent system, Alvin dive 981 (5 November 1979), 20°50' N, 109°06' W at ~2,600 m. The mussel tissue and tubeworm specimens were frozen after collection, and the tissue and shell samples rinsed with $^{14}\text{CO}_2$ -free, organic carbon-free distilled water before drying. To determine $\&\Delta^{14}\text{C}$ and $\&\delta^{13}\text{C}$, all samples but the tubeworm tissue were burned in O_2 or acidified with HCl to give CO_2 which was converted to acetylene, via lithium carbide 13 and then counted for 2 days in each of two stainless-steel 1.0-1 and 0.4-1 gas proportional counters. The worm tissue CO2 was purified by absorption onto CaO, diluted with background gas and counted twice in a 200-ml copper proportional counter for a total of 12 days. All countings were corrected for isotope fractionation (to a standard value of $\delta^{13}C = -25.0\%$ PDB-1) and for decay since the time of formation (to AD 1950). The standard was 95% of the net National Bureau of Standards oxalic acid count rate, corrected to a $\delta^{13}C = -19.0\%$, and the results are reported in terms of $\Delta^{14}C (\pm 1 \sigma \text{ counting error})$ which is the per million (%) deviation from the ¹⁴C activity of nineteenth-century wood ¹⁶. The ¹³C/¹²C ratios are reported as $\delta^{13}C (\pm 0.2\%)$ relative to the PDB-1 standard.

* The Δ^{14} C of the 2,490-m bottom water at the 'Musselbed' was assumed to be identical to the Δ^{14} C in 2,584-m water at Geosecs station 337, 24

May, 1974, 04°50' N, 124°05' W. σ_T at 2,500 m at both locations is 27.76±0.01 (ref. 17). † Δ^{14} C values taken from corals collected from 3-5-m depth at 1.5° S, 90-91° W, assuming the Ca¹⁴CO₃ reflects the ¹⁴C activity of the surface seawater DIOC.

waters (M. D. Lilly, M. A. de Angelis and L. I. Gordon, unpublished results), and it has been calculated that 50 µmol kg⁻¹ of abiogenic CH₄ of magmatic origin could be present in the 'end member' 350 °C hydrothermal fluid at the East Pacific Rise¹⁸. If methane were the sole carbon source for the synthesis of POC utilized by the mussels, then the mussel tissue would contain no measurable radiocarbon activity. Obviously this is not the case. It is possible that the 14C activity of the mussel tissues (-267) is 13% lower than that of the mussel shell carbonate (-254) due to mussels taking up both 'dead' CH₄ and DIOC, whereas the shell is only using 'dead' DIOC.

On the basis of the ¹⁴C activities and ¹³C/¹²C ratios reported here, we conclude that: (1) filter-feeding organisms in the vent system are directly or indirectly incorporating 'dead' carbon of magmatic origin into their tissue; (2) $\sim 25\%$ or less of the dietary carbon available to the mussels is from sedimenting POC fixed photosynthetically at the surface; and (3) mussel tissue is incorporating relatively more 'dead' DIOC than is mussel shell carbonate in specimens collected at the same location near the

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- Jannssch, H. W. & Wirsen, C. O. Bisscience 29, 592-598 (1979).
 Karl, D. M., Wirsen, C. O. & Jannssch, H. W. Science 207, 1345-1347 (1980).
 Lonedele, P. Deep-See Res. 24, 852-863 (1977).
- Enright, J. T., Newman, W. A., Hessier, R. R. & McGowan, J. A. Namer 239, 219–221 (1981).
- 5. Cavanangh, C., Gardner, S. L., Jones, M. L., Jannasch, H. W. & Waterbury, J. B. Solence (in
- the press). Williams, P.
- Williams, P. M. & Gordon, L. L. Desp-See Res. 17, 19–27 (1970).
 Eadle, B. J., Jeffrey, L. M. & Sackett, W. M. Geschies. cosmochis.
- (1978).

 8. Corfins, J. B. *et al. Science* 203, 1073-1083 (1979)

 9. Cobier, R. & Dymond, J. Science 269, 801-803 (1980).

 10. Turskinn, K., Coohran, J. K. & Norski, Y. Neisser 280, 385-387 (1979).

 11. Craig, H. & Weins, R. F. J. geophys Res. 78, 7641-7647 (1970).

 12. Ran, G. H. & Hadges, J. L. Science 203, 648-649 (1979).

 13. Ran, G. H. Soisses (in the press).

- Östhund, H. G., Brescher, R., Olesco, R. & Pergusco, M. J. Univ. Mismi Resential School. Misr. Abrico. Sci. Tritiam Lab. Data Rep. 8, (1979).
 Druffel, E. M. Geephys. Res. Lett. 8, 59–62 (1981).
 Broocker, W. S. & Olson, E. A. Radiocarber 3, 176–204 (1961).
 Wooster, W. S. Preier. Rep. Step-1 Exped. 15 Sept.—14 Dec. 1960, Part I. Phys. chem. Data, 810 pp. 100, 61–9 (1961).
- 8IO ref. no. 61-9 (1961).
- 18. Welhan, J. Trans. Am. geophys. Union (EOS) 61, 992 (1980).

A natural biological control of Dutch elm disease

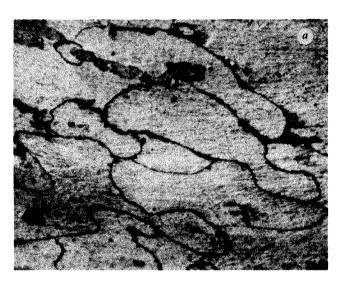
Joan Webber

Department of Botany and Microbiology, University College of Wales, Aberystwyth SY23 3DA, Dyfed, UK

Since its arrival in the late 1960s, the aggressive strain of Ceratocystis ulmi, the fungus that causes Dutch elm disease, has destroyed over 20 million elms in Britain and subsequently inflicted similar heavy losses across much of continental Europe^{1,2}. Successful control of the disease has been achieved only locally, using intensive sanitation and fungicide injection programmes^{3,4}. However, it has recently become apparent that disease spread may also be limited naturally. I present here evidence of a biological control of Dutch elm disease which could be exerting an important effect in some parts of Britain. This control process acts by preventing successful breeding of scolytid beeties which are the vectors of C. ulmi.

The control phenomenon was first encountered during 1976 and 1977 in several stands of trees in mid and south Wales. Each stand contained between 10 and 100 wych elms (Ulmus glabra) and although C. ulmi had killed some trees in all these stands, disease spread to healthy trees was surprisingly slow, suggesting that only small numbers of beetle vectors were present. Investigation of beetle breeding material, that is, the inner bark of dying or recently dead trees, revealed only a limited amount of active breeding. Instead such bark commonly exhibited dark zone lines (Fig. 1a), which on isolation yielded the fungus Phomopsis oblonga. The presence of Phomopsis was also clearly associated with the disruption of beetle breeding and disruption usually took one of two forms. Most commonly, when Phomopsis and a breeding gallery occupied the same area of bark, beetle larvae were retarded in their development and constructed very elongated, contorted galleries. Less commonly observed was the evasive action of larvae on encountering a discrete Phomopsis lesion. In such circumstances larval galleries swerved away from the Phomopsis-colonized area into uncolonized tissue.

To compare effectiveness of scolytid breeding in *Phomopsis*-colonized and uncolonized bark, breeding experiments were done using the two British species of beetle vector, *Scolytus scolytus* and *Scolytus multistriatus*. The breeding material was of two types: logs cut from wych elm with recent, extensive bark colonization by *Phomopsis*, and control logs also from wych elm but with no observable *Phomopsis* colonization. Pairs of logs (one of each type) were presented to beetles in either a 'choice' or a 'forced' breeding situation. In the former, a standing pair of logs was enclosed in a fine net bag and beetles introduced. This allowed beetles to choose as breeding material either the *Phomopsis*-colonized log or the control log. In the forced breeding situation each log was enclosed individually in a bag, so that introduced beetles had no choice of breeding material. The



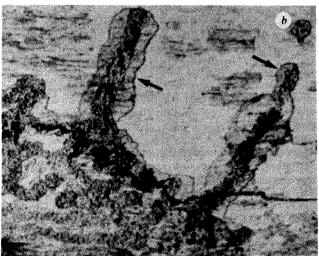


Fig. 1 a, Characteristic zone lines produced by *Phomopsis* colonizing the inner bark of diseased elms. These dark lines are thought to result from antagonistic interactions between different forms of the fungus present in the bark 7 . $\times \sim 2$. b, Modified zone lines (arrowed) formed around the larval galleries of *Scolytus*. Such modified zone lines are probably formed by *Phomopsis* in response to changes in the moisture content of the bark as a result of gallery excavation. $\times \sim 2.4$.

Table 1 Effect of *Phomopsis* colonization on scolytid breeding in elm bark

Breeding situation in log pairs	Maternal galleries per 100 cm ² bark	Total no. of emergent beetle progeny
S. scolytus		
Forced breeding		
Uncolonized control log	1.0	756*
Phomopsis-colonized log	1.3	0*
Choice breeding		
Uncolonized control log	1.8	1,690*
Phomopsis-colonized log	0.7	0*
S. multistriatus		
Forced breeding		
Uncolonized control log	2.5	780
Phomopsis-colonized log	2.3	17*
Choice breeding		
Uncolonized control log	4.3	1,048
Phomopsis-colonized log	1.7	0*

Four pairs of logs were used in all. The mean area of bark on logs presented to *S. scolytus* was 3,286 cm², and on logs presented to *S. multistriatus* was 2,290 cm². The number of maternal galleries per 100 cm² bark is a mean.

numbers of beetles introduced in these conditions depended on the total bark area available and the beetle species used. In the case of S. scolytus, one beetle pair, consisting of male and female, was allotted to each $100 \, \mathrm{cm^2}$ of bark, whereas one pair per $25 \, \mathrm{cm^2}$ of bark was used for the smaller bark beetle, S. multistriatus.

After 6 weeks the beetle breeding cycles were completed and the bark was stripped from the logs and gallery formation compared. It was immediately apparent that the breeding of both Scolytus species was strongly influenced by Phomopsis. When female beetles attempted to cut maternal galleries in a Phomopsis-colonized log many were abandoned at various stages before completion. If galleries were completed and eggs laid, the number of resulting larvae was drastically reduced and few developed into new adult beetles. In contrast, breeding in control logs was successful, and many young adult beetles emerged (Table 1). Figure 2b clearly shows the attempts at maternal gallery excavation in bark colonized by Phomopsis. Abnormal larval galleries are evident, typified by their elongated, twisted nature when compared with larval galleries in uncolonized bark (see Fig. 2a). Galleries affected by Phomopsis were also characterized by their frequent association with modified zone lines. Rather than appearing as the irregular ovals shown in Fig. 1a, zone lines often enclosed an entire gallery system, outlining each larval mine separately (Fig. 1b).

Behaviour of beetles in choice breeding experiments also suggested that *Phomopsis*-colonized bark was usually avoided as breeding material when an alternative was offered. The mean number of maternal galleries per 100 cm² of bark was similar in both *Phomopsis* and control logs in forced conditions, whereas in choice breeding experiments the number of galleries cut in control logs was over twice that attempted in *Phomopsis* logs (Table 1).

Thus *Phomopsis* is clearly able to exert a twofold effect on scolytid beetles by decreasing numbers of viable offspring in brood trees, or by eliminating potential breeding material and therefore intensifying competition for any uncolonized bark. Both factors must invariably have some role in reducing vector populations but we can only speculate on how much this may affect the continuing spread of Dutch elm disease.

One of the major factors which must undoubtedly govern the effectiveness of *Phomopsis* as a control agent is its frequency of occurrence, possibly as a latent inoculum in the dead outer bark of healthy trees. High levels of such inoculum would favour

^{*} Some living larvae (<20) still remaining in log.



Fig. 2 a, Breeding galleries of S. multistriatus produced in the bark of wych elm (U. glabra) with no Phomopsis colonization. Typically each sallery consists of rows of larval mines (or galleries) radiating outwards from points of egg laying along the length of a central mother gallery. Many of the larval mines terminate in oval pupal chambers. $\times \sim 0.25$. b, Sparse and abnormal breeding galleries of S. multistriatus in the bark of wych elm colonized by *Phomopsis*. Such galleries commonly show abortive or elongated and contorted larval mines. $\times \sim 0.25$.

rapid colonization of trees if they succumbed to Dutch elm disease. Phomopsis has frequently been found in the outer bark of healthy elms, but survey data have shown that it is largely limited to the elms in western and northern parts of Britain (J. F. W. and J. N. Gibbs, in preparation). It is therefore more likely to contribute to natural disease control in these areas. Furthermore, the massive colonization of elm bark required to exert an appreciable effect on beetle breeding has only been observed in wych elm, and it seems that Phomopsis is more strongly assoclated with this species of elm than any other in Britain. The fact that wych elm comprises the main part of elm populations in northern England, Wales and Scotland, also suggests that Phomopsis may be effective only in the north and west.

It has long been known that the spread of Dutch elm disease has been slower in northern England and in Scotland⁵. Previously this has been ascribed to various factors, including a smaller and more scattered elm population, a climate less favourable to the vectors and the preponderance of wych elm rather than English elm (*Ulmus procera*), the latter predominating in the south^{3.6}. It now seems that *Phomopsis* in an additional, and in some areas possibly the major factor in slowing the spread of the disease in the north.

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- Gibbs, J. N. & Brauer, C. M. Nature 241, 38-383 (1973)
- er, C M. Nature 281, 78-80 (1979)
- Gribbs, J. N. Arisere J. 3, 110–114 (1978)

 Detch Elm Disease Control (East Sussex County Council Publ. No. P/151, 1977).
- Peaca, T. R. Bull. Fer. Convent. Level 33 (1960) Radfarn, D. B. Scatt For 31, 105-109 (1977) Webber, J. F. thess, Univ. Wales (1960)

Regenerative and passive membrane properties of isolated horizontal cells from a teleost retina

Daniel Johnston* & Dominic Man-Kit Lam†

Program in Neuroscience, "Department of Neurology and †Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030, USA

Neurones in the intact retina are interconnected by a complex network of chemical and electrical synapses. The electrical interactions between many types of retinal neurones make it difficult to determine the active and passive membrane properties of the individual cells. A direct way to examine such properties is to dissociate the retina into single cells1-3 and to record intracellularly from identified, isolated neurones in the absence of neural connections. Such an approach has been used to study the electrophysiological properties of several classes of neurones in the vertebrate retins³⁻³. We have combined the method developed previously for examining the neurochemistry of individual retinal cells^{1,2} with a single-electrode clamp technique^{6,7} to investigate the biophysical properties of isolated horizontal cells from the teleost retina. Our results suggest that the specific membrane capacity of these cells is $\sim 1 \mu F \text{ cm}^{-2}$, and that the specific membrane resistivity ranges from ~5,000 to 20,000 Ω cm². In isolation, these cells can show regenerative voltage responses which are most probably calcium-dependent.

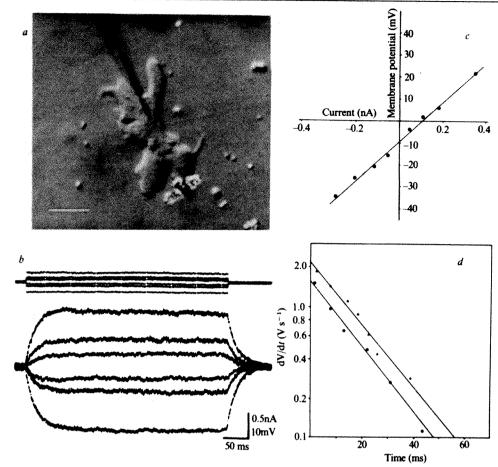


Fig. 1 Morphology and passive membrane properties of an isolated cone horizontal cell from the catfish retina. These cells were prepared by incubating isolated retinae with free Ringer's solution supplemented with 5 mM EGTA and 1 mg ml-1 (Worthington) at room temperature for 60-90 min. The retinae were then washed with Ca²⁺-free Ringer's solution containing 1 mg ml⁻¹ bovine serum albumin and trans-Ringer's solution containing ferred to a 15-ml conical tube. Isolated cells were obtained by gently pipetting the retinae through a wide-bore Pasteur pipette in 2 ml of a medium containing 90% 0.3 M sucrose and 10% Ringer's solution. The cell suspension was kept on ice for 2 min before the top 1 ml was transferred to 9 ml oxygenated Ringer's solution (120 mM NaCl, 4 mM KCl, 2 mM NaHCO₃, 5 mM CaCl₂, 5 mM glucose, 15 mM HEPES buffer, pH 7.7) supplemented with 10 µg ml⁻¹ deoxyribonuclease². a, Photomicrograph of a cone horizontal cell before impalement, with a microelectrode above. The surface area of this cell was calculated to be 16,000 µm². b, Voltage transients (bottom traces) recorded intracellularly from the cell shown in a in response to 400-ms constant current pulses (top traces). The cell was at its resting potential of -10 mV. c, Steady-state V-I plot from data shown in b. The slope indicates an input resistance of 89.6 MO. d. The first derivative of two voltage transients was determined graphically as described elsewhere^{7,13} and its log plotted against time. •, Data points from a 10-mV depolarizing voltage transient; A, data points from a 15mV hyperpolarizing transient. The data points can be fitted by single exponentials, suggesting isopotentiality for this cell. The average time constant was 17.7 ms.

In our electrophysiological studies, we started by isolating cone horizontal cells from retinae of the channel catfish for three reasons. First, the catfish retina contains only one type of cone (maximum absorbance 620 nm) and therefore only one type of cone horizontal cell that can be easily distinguished, even in isolation, from the more stellate rod horizontal cells. This ensures that our recordings are from a homogeneous population of horizontal cells. Second, the somata of these horizontal cells, which are probably GABA ergic⁹, receive input from dopaminergic terminals (D.M.K.L., in preparation). Therefore this preparation has a potential use in analyses of dopaminergic responses in a neurone from the vertebrate central nervous system. Third, these cells are relatively large in size (50–100 µm in diameter), making it possible to use low-impedance microelectrodes for voltage-clamp studies.

Catfish retinae were dissociated into single cells using a procedure described elsewhere². For electrophysiological studies, 0.2 ml of the isolated cell suspension was placed on a depression slide and the cells were allowed to sediment for 5 min. Under Nomarski interference optics, cone horizontal cells were impaled with 30-50 MΩ KCl-filled micropipettes. The single-electrode clamp system (SEC) used for currentclamp of these cells is described in detail elsewhere^{6,7}. Briefly, the SEC switches a single microelectrode rapidly (3 kHz) between current-passing and voltage-recording modes. The technique allows for the passage of larger transmembrane currents, and for a more accurate measurement of the membrane potential during current passage, than is possible using standard Wheatstone bridge arrangements. Each cell was photographed before intracellular recording. Surface area measurements were determined by projecting an enlarged image of the cell onto graph paper. The cells were assumed to be flat disks with a thickness of 10-15 µm, which was estimated from the depth of focus.

The results shown here are based on intracellular recordings of >92 cone horizontal cells. Figure 1a is a photomicrograph of a cone horizontal cell from which an intracellular recording was

made. Cone horizontal cells are easily identified by two criteria: they are the largest cells in the retina^{2.8} and even in isolation, they still have the amoeboid shapes characteristic of horizontal cells in the intact retina and can be easily distinguished from other isolated cells^{2,8}. Figure 1b-d gives data on the passive membrane properties of the cell shown in Fig. 1a. The isolated cone horizontal cells used here usually had an amoeboid shape with numerous fine processes. We generally chose to impale the larger cells with the simplest morphology that could be clearly and unequivocally identified, but that were free of long processes. For the cell in Fig. 1a, 400-ms constant current pulses were applied and the resulting voltage transients recorded (see Fig. 1b). Figure 1c shows a plot of the steady-state voltage-current (V-I) relationship, obtained from transients such as those shown in Fig. 1b. In this particular cell, the V-I plot is linear over the voltage range -40 to +20 mV. However, few cells showed completely linear V-I plots over a wide voltage range. We typically measured a decrease in input resistance with large depolarizations or hyperpolarizations.

To calculate the specific membrane capacity of these cells, it was necessary to determine accurately the membrane time constant. Figure 1d shows the logarithm of the first derivative of two low-amplitude voltage transients (from Fig. 1b), one depolarizing and the other hyperpolarizing, plotted against time. The data points fit well to single exponentials with time constants of 17.3 and 18.2 ms, respectively. As we could detect only a single exponential in the low-amplitude voltage transients, the cell behaved as a single isopotential compartment, composed of a parallel resistor and capacitor. Moreover, the time constants calculated from the small depolarizing (10 mV) and hyperpolarizing (15 mV) transients are similar, which supports our assumption that the membrane resistivity is effectively ohmic in this narrow voltage range.

With the information from Fig. 1, the specific membrane properties of this cell can be calculated. The surface area (A) was estimated from Fig. 1a as $16,000 \mu m^2$. The input resistance (R_N) was $89.6 M\Omega$, and the average membrane time constant (τ_m) was

17.7 ms. These values yield a specific membrane resistivity (R_m) of 14,330 Ω cm² and a specific membrane capacity (C_m) of 1.2 μ F cm⁻².

In most cells, depolarizing current pulses revealed a regenerative voltage response as defined by an increasing positive slope in the membrane potential transient. An example of such a regenerative response is shown in Fig. 2. The resting membrane potentials of impaled cells from different preparations were quite variable, ranging from -10 to -100 mV. However, as most isolated cells in a given preparation had similar resting potentials, all with large input resistances, we believe that these variations in resting potentials are not the result of damage due to the microelectrode, but are most likely due to variations in the retinae used or in the isolation procedures. Cells with low resting potentials generally showed regenerative voltage responses if a steady hyperpolarizing current was applied before the depolarizing pulses. The hyperpolarization probably reduced delayed rectification and closed the voltage-dependent channels that are responsible for the regenerative response. The cell shown in Fig. 2 had a resting membrane potential of -25 mV, but the voltage transients were obtained when the cell was hyperpolarized to -65 mV. The passive properties of this cell were $R_N = 250 \,\mathrm{M}\Omega$ at $-25 \,\mathrm{mV}$, $A = 3.740 \,\mu\text{m}^2$, $R_m = 9.350 \,\Omega \,\text{cm}^2$, $r_m = 9.6 \,\text{ms}$ and $C_m = 9.6 \,\text{ms}$ $1.0 \, \mu \text{F cm}^{-2}$

The addition of tetrodotoxin (TTX, 10 µg ml⁻¹) to the bathing solution of these cells had no apparent effect on the regenerative responses. To test whether Ca2+ might be involved in the regenerative responses, we applied CoCl2, MnCl2 or NlCl2 by pressure injection to the bathing solution; all three of these divalent cations have been reported to block inward Ca2+ currents in various systems 10,11. We found a rapid and complete blockade of all regenerative voltage responses with the application of any one of these divalent cations at a final concentration of ~10 mM, although Ni²⁺ seemed to be the most effective. Figure 3a shows the regenerative voltage response from a cell bathed in $10 \mu g \text{ ml}^{-1}$ TTX. The transients in Fig. 3bwere taken a few minutes after applying 10 mM NI2+; the regenerative response could no longer be elicited. Similar results were obtained in six other cells. These experiments demonstrate that the regenerative voltage responses recorded in isolated cone horizontal cells are TTX-insensitive and probably Ca2+dependent.

The results from a number of isolated cells show that they have an average specific membrane capacity of $1.3\pm0.3~\mu F\ cm^{-2}$ (mean \pm s.d., n=7). A C_m of $1~\mu F\ cm^{-2}$ has been suggested to be a biological constant, as this value has been found for all carefully studied cells in both vertebrate and invertebrate nervous systems $^{12.13}$. Our results agree with this hypothesis. However, it should be emphasized that our data may have several sources of error. First, the sample of cells is limited and biased towards larger cells with the simplest morphologies. Second, the assumption of uniform thickness for each cell and the inaccuracy associated with measuring depth could increase our surface area estimate by 10-20%. Third, the measurement

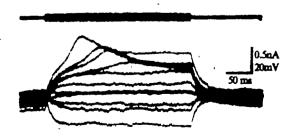


Fig. 2 Regenerative voltage responses from a cone horizontal cell. The resting potential of this cell was $-25\,\mathrm{mV}$. With the application of steady hyporpolarizing current, which brought the membrane potential to $-65\,\mathrm{mV}$, regenerative responses could be obtained in response to $300\mathrm{-ms}$ constant current pulses (top traces). The passive membrane properties of this cell, determined as described in Fig. 1 legend, were: $R_{\mathrm{H}} = 250\,\mathrm{M}\Omega$, $A = 3,740\,\mathrm{\mu m^2}$, $R_{\mathrm{H}} = 9,350\,\Omega\,\mathrm{cm^2}$, $r_{\mathrm{H}} = 9.6\,\mathrm{ms}$ and $C_{\mathrm{m}} = 1.0\,\mathrm{\mu F}\,\mathrm{cm}^{-8}$.

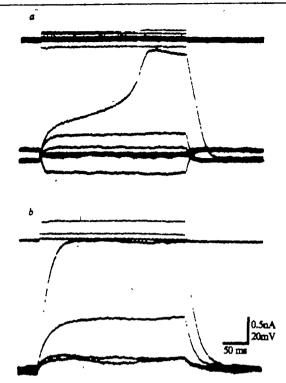


Fig. 3 Regenerative voltage responses blocked by Nl^{3+} . a, A regenerative voltage transient in response to a 300-ms constant current pulse. The resting potential of this cell was \sim -50 mV. Steady hyperpolarizing current brought the cell to \sim 90 mV, from which the pulses shown were given. The cell was bathed in 10 μ g mi⁻¹ TTX. b, The same cell several minutes after the application of 10 mM Nl^{3+} . No regenerative responses could be recorded with the cell at \sim 90 mV. Steady depolarizing and hyperpolarizing currents were passed to bring the cell to several different membrane potentials. The application of depolarizing current pulses from any of these different membrane potentials could not elicit a regenerative voltage response.

of the membrane time constant could be influenced by voltagedependent currents. This last source of error would be most critical in those cells with the largest specific membrane resistivities.

With the exception of an apparent lack of TTX-sensitive sodium channels, the active and passive membrane properties of cone horizontal cells seem to be similar to those described in various neurones from the central and peripheral nervous systems of both vertebrate and invertebrate species. Our results suggest that catfish cone horizontal cells, in isolation, can show regenerative behaviour that is probably Ca2+-dependent. The leak current in vivo, caused by the extensive electrotonic couplings among horizontal cells, might be expected to subtract from a voltage-dependent inward current and prevent the appearance of regenerative voltage responses. Accurate estimates of membrane resistivity are also difficult to make in the intact retina because of these electrical couplings between horizontal cells 14. For example, the input resistances of horizontal cells in intact retinae were reported to be of the order of 500 k Ω (ref. 14), a value about 200 times lower than that found in isolated horizontal cells. For these reasons, the ability to record from isolated cells is crucial for the study of the biophysical properties of these neurones.

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Lum, D. M.K. Prec. nam. Acad. Sci. U.S.A. 69, 1987 (1972).
 Lum, D. M.K. Matter 264, 345-347 (1975); Cold Spring Hark. Symp. quant. Biol. 40,

571-579 (1976).

- Drujan, B. D. & Svaetichin, G. Vision Res. 12, 1777-1784 (1972). Kaneko, A., Lam, D. M.K. & Wiesel, T. N. Brain Res. 105, 567-572 (1976). Bader, C. R., Macleish, P. R. & Schwartz, E. A. Proc. natn. Acad. Sci. U.S.A. 75,
- Johnston, D., Hablitz, J. J. & Wilson, W. A. Nature 286, 391–393 (1980).
 Johnston, D. Cell. molec. Neurobiol. 1, 41–55 (1981).

- Naka, K.-I. & Carraway, N. R. G. J. Neurophysiol. 38, 53-71 (1975). Lam, D. M.K., Lasater, E. M. & Naka, K.-I. Proc. nam. Acad. Sci. U.S.A. 75, 6310-6313
- 10. Hagiwara, S. in Membrane-A Series of Advances Vol. 3 (ed. Eisenman, G.) 359-381 (Dekker, New York, 1975).
- Akaike, N., Lee, K. S. & Brown, A. M. J. gen. Physiol. 71, 509-531 (1978)
- Cole, K. S. Membranes, Ions and Impulses (University of California Press, Berkeley, 1968).
 Brown, T. H., Perkel, D. H., Norris, J. C. & Peacock, J. H. J. Neurophysiol. 45, 1-15 (1981).
- 14. Kaneko, A. J. Physiol., Lond. 213, 95-105 (1971).

Inhibition of basophil histamine release by anti-inflammatory steroids

Robert P. Schleimer, Lawrence M. Lichtenstein & Elizabeth Gillespie

Department of Medicine, Division of Clinical Immunology. Johns Hopkins University School of Medicine at The Good Samaritan Hospital, Baltimore, Maryland 21239, USA

The release of histamine from human leukocytes is a useful in vitro model for studying allergic disease1. Many of the drugs used in the treatment of allergy and asthma (for example, isoprenaline and theophylline) are effective inhibitors of in vitro histamine release². However, the anti-inflammatory steroids have not been found to inhibit the in vitro release of histamine from mast cells or basophils activated by immunological stimuli³⁻⁶. In view of the fact that the in vivo anti-allergic effects of steroids occur only 12-24 h after administration, we have re-examined the effects of these drugs on IgE-mediated histamine release from human basophils after prolonged incubations. We report here a time-dependent inhibition of histamine release from human basophils by glucocorticosteroids. The relative activity of a series of steroids as inhibitors of histamine release correlates well with the relative in vivo anti-inflammatory activity of the same compounds. These results suggest that the basophil may be an in vivo target of the anti-inflammatory steroids and that inhibition of this cell type may account for some of the activity of steroids in preventing inflammation.

Human leukocytes were prepared from the blood of normal volunteers by dextran sedimentation as described elsewhere⁸ except that sterile conditions were used. Cells were cultured in the presence or absence of steroids for up to 24 h, as described in Fig. 1 legend, after which they were washed and challenged with either anti-IgE or buffer control. Dexamethasone acetate, at concentrations up to 10⁻⁶ M, did not reduce the total cellular histamine content in incubations of 24 h. Total leukocyte viability, as assessed by Trypan blue exclusion, was always greater than 70% and usually greater than 80% after 24-h culture with or without up to 10^{-6} M dexamethasone acetate. In initial experiments, the drug in use was present during the wash and challenge procedures. However, it was determined that its omission during wash and challenge steps did not affect the extent of inhibition of histamine release, and it was therefore not included in most of the experiments described here.

The time course of onset of dexamethasone-induced inhibition of histamine release is shown in Fig. 1. Control release in the absence of steroid was 28%. Preincubation of cells with dexamethasone acetate for up to 2 h produced no inhibition of histamine release during the subsequent 45-min challenge. However, inhibition was noted after 4 h of incubation, and increased linearly for 24 h. Similar results were obtained with anti-IgE in three experiments. In 21 experiments using a 24-h incubation, histamine release in the control groups, stimulated with $0.1 \,\mu g \, ml^{-1}$ anti-IgE, was $34 \pm 3.3 \,\%$ (mean \pm s.e.m.) whereas that from cells exposed to $10^{-7} \, M$ dexamethasone acetate was $12.9 \pm 1.4\%$. In two experiments, using the cells of

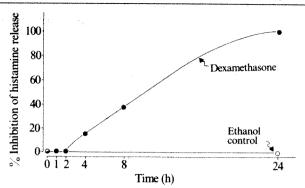


Fig. 1 Human leukocytes were collected from adult volunteers, sedimented in sterile dextran/EDTA, washed twice in sterile saline and cultured in 2-ml aliquots in multi-well tissue culture plates for 24 h in RPMI 1640 medium supplemented with penicillin/streptomycin, glutamine, 25 mM HEPES and 7% autologous serum. Dexamethasone acetate in ethanol at 10^{-2} M, or ethanol alone, was diluted in medium to a final concentration of 10added at various times so that total duration of exposure to the drug was as indicated. At the end of the culture period, cells were washed in PIPES-buffered saline, pH 7.3, containing 110 mM NaCl, 5 mM KCl and 25 mM PIPES, and resuspended in PIPESbuffered saline containing 1 mM CaCl₂ and 1 mM MgCl₂. Cells were then challenged with either 0.1 µg ml⁻¹ anti-IgE (a gift of Dr K. Ishizaka) or buffer control (spontaneous release) at 37 °C for 45 min. Histamine released into the supernatant was measured using an automated fluorometric technique²¹. Per cent histamine release was calculated as the fraction (×100) of total cellular histamine released into the challenge medium after subtraction of the spontaneous release (<5%). Per cent inhibition of histamine release was calculated as the reduction (expressed on a percentage basis) compared with control release induced by drug treatment.

ragweed-sensitive donors, histamine release stimulated by anti-IgE was inhibited by over 80% after a 24-h incubation with 10⁻⁷ M dexamethasone acetate.

To ascertain whether the in vitro activity of steroids parallels their in vivo effects, several steroids were tested for activity as inhibitors of basophil histamine release using 24-h incubation periods. The inhibition of histamine release by four antiinflammatory steroids showed a rank order of triamcinolone acetonide > dexamethasone acetate > 9α -fluorocortisone > hydrocortisone (Fig. 2). This order of potency is consistent with the anti-inflammatory potency of these compounds in man⁹, and the IC₅₀ of these and other steroids shown in Table 1 are similar to their binding constants for the steroid receptor 10,11. The sex steroids, β -oestradiol, testosterone and progesterone, as well as the cortisone metabolite, tetrahydrocortisone, were essentially inactive as inhibitors of histamine release. Prednisone, a cortisone analogue that is active in vivo, was inactive as an inhibitor of histamine release, whereas prednisolone, in two experiments, was an effective inhibitor with an approximate IC_{50} of 2.2×10^{-8} M. This observation is consistent with the hypothesis that prednisolone, the major metabolite of pred-

Table 1 Potency of various steroids as inhibitors of histamine release

Compound	IC ₅₀ (M)	n	Relative potency
Triamcinolone acetonide	3.1×10^{-9}	6	48
Dexamethasone acetate	7.8×10^{-9}	6	19
Prednisolone	2.2×10^{-8}	2	6.8
9α-Fluorocortisone	4.5×10^{-8}	4	3.3
Hydrocortisone	1.5×10^{-7}	8	1
Progesterone	$>10^{-5}$	1	< 0.01
β-Oestradiol	$>10^{-5}$	2	< 0.01
Testosterone	$>10^{-5}$	2	< 0.01
Prednisone	$>10^{-5}$	2	< 0.01
Tetrahydrocortisone	$> 10^{-5}$	3	< 0.01

IC₅₀, molar concentration of drug that inhibits histamine release by 50%, n, Number of experiments from which data are derived. Potency is relative to hydrocortisone.

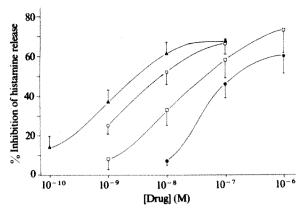


Fig. 2 Inhibition of basophil histamine release by triamcinolone acetonide (\triangle), dexamethasone acetate (\bigcirc), 9α -fluorocortisone (\square) and hydrocortisone (.). All steroids were dissolved in ethanol at 10-2 M and subsequently diluted in medium. Assay and calculations were performed as described in Fig. 1 legend and in the text. Data shown represent the mean ± s.e.m. inhibition of at least four experiments for each drug tested (see Table 1).

nisone, is responsible for the in vivo activity of prednisone 12,13. The relative and absolute potencies of the steroids tested are similar to their potencies as inhibitors of arachidonic acid release from guinea pig lung¹⁴ and transformed mouse fibroblasts¹⁵, as well as their potencies as inhibitors of lymphocyte mitogenosis 16 and macrophage plasminogen activator release¹⁷. Note that results similar to ours have recently been obtained using mouse peritoneal mast cells (M. Daëron, A. Sterk and T. Ishizaka, personal communication).

The mechanism of the steroid effect is unclear, but the observation that basophil activity is inhibited by glucocorticosteroids raises several important questions. First, it becomes necessary to determine to what extent a steroid-mediated inhibition of basophil activity plays a part in the abrogation of those delayedtype hypersensitivities which involve basophils 18,19. Second, as hydrocortisone inhibits histamine release at concentrations which are found naturally in the circulation, it becomes necessary to determine what role circulating glucocorticoids have in the regulation of basophil activity. Third, questions concerning the biochemical mechanism by which the steroids inhibit histamine release can be approached using a recent technique for obtaining human basophils at high purity20. Finally, it becomes necessary to ask what role the inhibition of basophil activity has in the glucocorticosteroid alleviation of allergic disease and asthma.

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- 1. Lichtenstein, L. M., Marone, G., Thomas, L. L. & Malveaux, F. J. invest. Derm. 71, 65-69
- Bourne, H. R. et al. Science 184, 19-28 (1974)
- Bourne, H. K. et al. Science 184, 19-28 (1974).

 Norn, S. Acta pharmac. tox. 22, 369-378 (1965).

 Schmutzler, W. & Freundt, G. P. Int. Archs Allergy appl. Immun. 49, 209-212 (1975).

 Thomas, R. O. & Whittle, B. J. R. Br. J. Pharmac. 57, 474P-475P (1976).

 Lewis, G. P. & Whittle, B. J. R. Br. J. Pharmac. 61, 229-235 (1977).

 Liddle, G. W. Clin. Pharmac. Ther. 2, 615-635 (1961).

 Lichtenstein J. M. & Color A. G. L. van Med. 329, 527, 530 (1964).

- Lichtenstein, L. M. & Osler, A. G. J. exp. Med. 120, 507-530 (1964).

 Gilman, A. G., Goodman, L. S. & Gilman, A. The Pharmacological Basis of Therapeutics 6th edn (Macmillan, New York, 1980).
- Ballard, P. L., Carter, J. P., Graham, B. S. & Baxter, J. D. J. clin. Endocr. Metab. 41, 290-304 (1975).

- Dausse, J. P. et al. Molec. Pharmac. 13, 948-955 (1977).
 Meikle, A. W., Weed, J. A. & Tyler, F. H. J. clin. Endocr. Metab. 41, 717-721 (1975).
 Peterson, R. E., Pierce, C. E., Wyngaarden, J. B., Bunim, J. J. & Brodie, B. B. J. clin. Invest. 37, 1301-1312 (1957).
- 14. Blackwell, G. J., Flower, R. J., Nijkamp, F. P. & Vane, J. R. Br. J. Pharmac. 62, 79
- Tam, S., Hong, S. L. & Levine, L. J. Pharmac. exp. Ther. 203, 162-168 (1977).
 Cantrill, H. L., Waltman, S. R., Palmberg, P. F., Zink, H. A. & Becker, B. J. clin. Endocr. Metab. 40, 1073-1077 (1975).
 Vassalli, J. D., Hamilton, J. & Reich, E. Cell 8, 271-281 (1976).
- Dvorak, A. M., Mihm, M. C. & Dvorak, H. F. J. Immun. 116, 687-695 (1976).
 Askenase, P. W., Haynes, D. & Hayden, B. J. J. Immun. 117, 216-224 (1976).
 MacGlashan, D. W. & Lichtenstein, L. M. J. Immun. 124, 2519-2521 (1980).
- Siraganian, R. P. Analyt. Biochem. 57, 383-388 (1974).

Lipoxygenase products modulate histamine release in human basophils

Stephen P. Peters, Marvin I. Siegel, Anne Kagey-Sobotka & Lawrence M. Lichtenstein

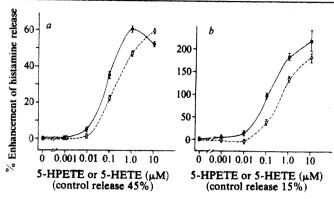
The Johns Hopkins University School of Medicine, Department of Medicine, Divisions of Clinical Immunology and Pulmonary Medicine, Baltimore, Maryland 21239 and Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709, USA

Arachidonic acid released from cellular phospholipids is metabolized by two major enzyme systems1. The cyclooxygenase pathways are responsible for the production of prostaglandins, thromboxanes and prostacyclin while the lipoxygenase pathways form a series of monoperoxy- and monohydroxyeicosatetraenoic acids. The 5-lipoxygenase pathway eventually leads to the production of both leukotriene B, one of the most potent chemotactic molecules known, and SRS-A (slow-reacting substance of anaphylaxis-leukotrienes C and D). Our earlier studies of the lipoxygenase enzyme(s) suggested that a product(s) of lipoxygenase(s) is both necessary for histamine release from human basephils and blunts endogenous control mechanisms²⁻⁴. To test this hypothesis, we have studied the effect of exogenously added products of lipoxygenase pathways on antigen-induced histamine release from human basophilic leukocytes in vitro. The present report demonstrates that 5-hydroperoxyeicosatetraenoic acid (5-HPETE), at sub-micromolar concentrations, causes both a dose-dependent enhancement of histamine release and a reversal of the inhibition of histamine release caused by hormones and other agonists which act via adenylate cyclase. The reduced product of 5-HPETE, 5-hydroxyeicosatetraenoic acid (5-HETE) has both activities, but is approximately 3 to 10 times less potent.

As studies of the oxidation of arachidonic acid by cyclooxygenase and lipoxygenase pathways have been pursued, evidence has accumulated that products of the lipoxygenase pathways and other fatty acid hydroperoxides: (1) enhance anaphylactic mediator release from guinea pig lung^{5,6}, (2) are chemotactic for polymorphonuclear leukocytes^{7-1,4}, (3) can inhibit both leukotriene biosynthesis15 and platelet cyclooxygenase activity¹⁶, (4) enhance IgE-mediated release of histamine in rat mast cells¹⁷, (5) are incorporated into neutrophil phospholipids and triglycerides¹⁸ and induce degranulation of these cells¹⁹, and (6) may be involved in inflammatory and pyretic reactions in vivo²⁰

The nonspecific lipoxygenase inhibitor, 5,8,11,14-eicosatetraynoic acid, and 5,8,11-eicosatriynoic acid, a relatively specific lipoxygenase inhibitor, inhibit basophil histamine release caused by antigen-IgE interactions, C5a peptide, fMet peptides and the ionophore, A23187, in a complete and dose-dependent fashion^{2,3}. In addition, arachidonic acid and pharmacologic concentrations of indomethacin and other nonsteroidal anti-inflammatory drugs both potentiate histamine release and reverse the inhibition of histamine release caused by drugs and hormones that act via adenylate cyclase, while failing to affect the action of drugs which increase cyclic AMP levels by other mechanisms²⁻⁴. These data, together with the finding that the phospholipase A₂ inhibitor, p-bromophenacyl bromide, also causes a complete and dose-dependent inhibition of histamine release⁴, led to the hypothesis that a lipoxygenase product(s) of arachidonic acid, generated by phospholipase A2, was both obligatory for histamine release and negatively modulated the signal generated by receptors linked to adenylate cyclase.

Figure 1 shows the dose-response relationships for the enhancement of histamine release by 5-HPETE and 5-HETE. 5-HPETE had half-maximal effect at 0.10 µM, and a peak effect



Enhancement of basophil histamine release by 5-HPETE and 5-HETE. Peripheral blood leukocytes from atopic adult volunteers who had given informed consent were isolated by dextran sedimentation of venous blood anticoagulated with EDTA as previously described². The histamine release assay was performed in a total volume of 0.5 ml in buffer containing 25 mM PIPES, 110 mM NaCl, 5 mM KCl, 0.003% human serum albumin, 1.0 mM MgCl₂ and 1.0 mM CaCl₂. Incubations were carried out for 45 min at 37 °C after a 5-min preincubation of cells with drug or lipoxygenase product before addition of antigen, either ragweed antigen E or rye grass, group I. The lipoxygenase product, 5-HPETE, was prepared as described by Porter et al.²²; 5-HETE was prepared from 5-HPETE by reduction with NaBH4. These lipoxygenase products were added to 12×75-mm polypropylene reaction tubes as an ether solution which was evaporated under a stream of nitrogen immediately before use. Leukotrienes C and D were a gift from Dr Joseph Rokach of Merck, Sharp and Dohme and stored frozen as an aqueous solution at -70 °C. Dimaprit was a gift of Dr M. Parsons of Smith Kline and French. Histamine released was quantified by fluorometric assay as previously described^{23,24} and corrected for spontaneous release, typically <5% of the total histamine content. Experiments were carried out in duplicate or triplicate with cells from 3-10 atopic donors. None of the lipoxygenase products caused spontaneous histamine release. In the experiment above, assays were performed in triplicate with either 5-HPETE (1) or 5-HETE (1), Control histamine release was 45% (a) and 15% (b). Error bars indicate s.e.m.

at 1.0 µM. 5-HETE had a similar effect but was 60-80% as active as 5-HPETE in enhancing histamine release at a concentration of 1 µM. Examination of the linear portion of the enhancement dose-response curve shows that it requires 3 to 10 times more 5-HETE than 5-HPETE to cause an equivalent enhancement of histamine release. The enhancement observed is, of course, a function of the magnitude of the control histamine release. In a series of 20 experiments, the enhancement ranged from 15 to 60% when histamine release was 40-70% of total cellular histamine (Fig. 1a), and from 190 to 340% when histamine release was 10-15% of total cellular histamine (Fig. 1b). In a series of six experiments, 11-HPETE and 15-HPETE were 100 ± 40 times less active than 5-HPETE in enhancing release. The stable hydroperoxide, 13-hydroperoxylinoleic acid, showed no enhancement at this concentration in four of six experiments. In six experiments, other products of the 5-lipoxygenase pathway, leukotrienes C and D, at concentrations of 0.5-3 µM, caused no consistent enhancement (or inhibition) of antigen-induced histamine release. These results are in contrast to the observations of Schellenberg and co-workers, who showed differential effects for leukotriene C and D on 48/80-induced histamine release in rat mast cells²¹ Other lipoxygenase products, including the potent chemotactic molecule, leukotriene B¹²⁻¹⁴, were not available for evaluation.

Figure 2 demonstrates the previously reported specific reversal by indomethacin of the inhibition caused by the histamine type 2 agonist, dimaprit². The activity of 5-HPETE is essentially the same as that of indomethacin. 5-HETE also reversed the inhibition caused by dimaprit but to a lesser extent (data not shown). Similar reversal was seen in five of five experiments with 5-HPETE and four of five experiments with 5-HETE. In simul-

taneous experiments, neither indomethacin (as previously reported) nor 5-HPETE reversed the inhibition caused by dibutyryl cyclic AMP or isobutyl methylxanthine, agonists which increase cyclic AMP levels by mechanisms other than activation of adenylate cyclase (that is, directly or by phosphodiesterase inhibition). Thus, the effect of 5-HPETE in specifically reversing the inhibition of histamine release caused by agonists which act via adenylate cyclase is the same as that previously reported for indomethacin and arachidonic acid.

The mechanism by which antigen-IgE interactions cause mediator release from basophils and mast cells has been extensively studied^{2-4,17-19}. The bulk of the evidence suggests that stimulation of the basophil occurs by cross-linking cell-surface IgE molecules followed by phospholipase A2 activation leading to the production of arachidonic acid which is then metabolized by the lipoxygenase pathway(s) leading to histamine release. The present study provides direct evidence that specific 5lipoxygenase products (5-HPETE and 5-HETE) are able to modulate histamine release in human basophils; they can both enhance antigen-induced histamine release and specifically reverse the inhibition caused by agents that increase cyclic AMP levels via an effect on adenylate cyclase. Products of the 11- and 15-lipoxygenase pathways are much less active in this regard and leukotrienes C and D are inactive. The naturally occurring hydroperoxy derivatives of arachidonic acid are relatively unstable in aqueous solutions, being converted, usually within minutes, to the corresponding hydroxy compound. The present report demonstrates that both the relatively unstable 5-hydroperoxy derivative of arachidonic acid and the stable 5-hydroxyeicosatetraenoic acid have the ability to modulate histamine release in human basophils. Whether further metabolism of these compounds occurs during the in vitro incubation is unknown. Our interpretation assumes that exogenously added material will have a similar effect to endogenously generated products.

The mechanism by which lipoxygenase products enhance histamine release is unknown, although it is tempting to speculate that these products may be incorporated into membrane phospholipids and then induce degranulation, analogous to the mechanism proposed for human neutrophils^{18,19}. The ability of other lipoxygenase products to modulate enzymes involved in inflammatory reactions, including the inhibition of platelet cyclooxygenase activity¹⁶ and the inhibition of the biosynthesis of leukotrienes¹⁵, argues strongly for the importance of these compounds in inflammatory processes. Whether other lipoxygenase products, particularly leukotriene B, are capable of modulating histamine release in human basophils remains to be tested.

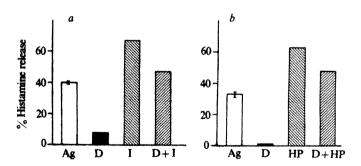


Fig. 2 Reversal of dimaprit inhibition of histamine release by indomethacin (a) and 5-HPETE (b). Cells were preincubated for 5 min with dimaprit (2 μM), indomethacin (2 μM), 5-HPETE (1 μM) or combinations of the above for 5 min before addition of antigen. Assays containing drug or lipoxygenase products were performed in duplicate; in general, replicate assays varied by <10%. Ag, antigen controls; D, dimaprit; I, indomethacin; HP, 5-HPETE. Error bars indicate range of histamine release for antigen controls.

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- Samuelsson, B., Granström, E., Green, K., Hamberg, M. & Hammarström, S. A. Rev. Biochem. 44, 669-695 (1975).
- Marone, G., Kagey-Sobotka A. & Lichtenstein L. M. J. Immun. 123, 1664-1667 (1979).
 Marone, G., Hammarström S. & Lichtenstein, L. M. Clin. Immun. Immunopath. 17, 117-122 (1980)
- 4. Marone, G., MacGlashan, D. W. Jr, Kagey-Sobotka, A. & Lichtenstein, L. M. in Advances in Allergology and Immunology (eds Oehling, A. et al.) 147-154 (Pergamon, New York,
- 5. Adcock, J. J., Garland, L. G., Moncada, S. & Salmon, J. A. Prostaglandins 16, 163-177
- 6. Adcock, J. J., Garland, L. G., Moncada, S. & Salmon, J. A. Prostaglandins 16, 179-187
- Turner, S. R., Tainer, J. A. & Lynn, W. S. Nature 257, 680-681 (1975).
 Goetzl, E. J., Woods, J. M. & Gorman, R. R. J. clin. Invest. 59, 179-183 (1977).
 Goetzl, E. J., Weller, P. F. & Sun, F. F. J. Immun. 124, 926-933 (1980).
 Goetzl, E. J. & Sun, F. F. J. exp. Med. 150, 406-411 (1979).

- Gotzl, E. J., Hill, H. R. & Gorman, R. R. Prostaglandins 19, 71-85 (1980).
 Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. & Smith, M. J. H. Nature 286, 264-265 (1980).
- 13. Palmer, R. M. J., Stepney, R. J., Higgs, G. A. & Eakins, K. E. Prostaglandins 20, 411-418 (1980)
- 14. Goetzl, E. J. & Pickett, W. C. J. Immun. 125, 1789-1791 (1980).
- GOEZI, E. J. & PICKEII, W. C. J. Immun. 125, 1789-1791 (1980).
 Vanderhoek, J. Y., Bryant, R. W. & Bailey, J. M. J. biol. Chem. 255, 10064-10065 (1980).
 Siegel, M. I., McConnell, R. J., Abrahams, S. L., Porter, N. A. & Cuatrecasas, P. Biochem. biophys. Res. Commun. 89, 1273-1280 (1979).
 Stenson, W. F., Parker, C. W. & Sullivan, T. J. Biochem. biophys. Res. Commun. 96, 1045-1052 (1980).
- 18. Stenson, W. F. & Parker, C. W. Prostaglandins 18, 285-292 (1979)
- 19. Stenson, W. F. & Parker, C. W. J. Immun, 124, 2100-2104 (1980)
- Siegel, M. I., McConnell, R. T. & Cuatrecasas, P. Proc. natn. Acad. Sci. U.S.A. 76, 3774-3778 (1979).
- 21. Schellenberg, R. R., Johnston, M. E., Bach, M. K. & Hanna, C. J. Fedn Proc. 40, 1014
- Porter, N. A., Legan, J. & Kontoyiannidou, V. J. org. Chem. 44, 3177-3181 (1979).
 Lichtenstein, L. M. & Osler, A. G. J. exp. Med. 120, 507-530 (1964).
- 24. Siraganian, R. P. Analyt. Biochem. 57, 383-394 (1974).

Type-specific protective immunity evoked by synthetic peptide of Streptococcus pyogenes M protein

Edwin H. Beachey*†, Jerome M. Seyer‡, James B. Dale*, W. Andrew Simpson* & Andrew H. Kang*‡

The Veterans Administration Medical Center and the Departments of *Medicine, †Microbiology and ‡Biochemistry, University of Tennessee School of Medicine, Memphis, Tennessee 38104, USA

The M protein on the surface of Streptococcus pyogenes cells enables the organisms to resist ingestion and killing by phagocytic cells in the blood of the non-immune host. In the immune host, type-specific antibodies against the M protein neutralize the antiphagocytic effect and allow the rapid elimination of any invading streptococcci having the same serotype of M protein. Recent studies in our laboratories of the primary structure of one of these M proteins (type 24) have indicated that the molecule is composed of repeating covalent structures, each of which contains protective antigenic determinants. These results suggest that only a small portion of the M protein molecule may be required to produce primary protective immunity against \hat{S} . pyogenes. We now report that this notion is well founded. Our findings may have a bearing on the development of M-protein vaccines to protect against streptococcal infections causing acute rheumatic fever and rheumatic heart disease2.

The mechanism whereby streptococcal infections give rise to complications such as rheumatic fever remains a mystery³. Because the sera of some patients with rheumatic fever show serological cross-reactivity between heart tissue antigens and certain streptococcal antigens3, it has been feared that immunization with intact M-protein vaccines may lead to

rheumatic heart disease. However, we have recently found that rabbits and mice immunized with cyanogen bromide fragments (CB6 or CB7) of type 24 M protein containing only 35 amino acid residues each developed opsonic and protective antibodies against type 24 streptococci4. One of these peptides (CB7) has now been chemically synthesized (S-CB7) and we report here protective immunity in laboratory animals induced by S-CB7 covalently linked to polylysine. In addition, we present evidence that one of the protective determinants resides in a peptide fragment of CB7 containing only 12 amino acid residues.

The CB7 polypeptide fragment of type 24 M protein and a dodecapeptide starting at residue 18 and ending at residue 29 (S18-29CB7) were synthesized⁵ and purified by reverse-phase HPLC on Ultrasphere ODS2 (Whatman) columns (Penninsula laboratories). The S18-29CB7 peptide overlaps two subpeptides derived from trypsin digestion of lysyl-blocked CB7 (see below). Amino acid analyses confirmed the identity of the synthetic peptides. Automated Edman degradation 1,4 to the penultimate residue gave the amino acid sequence of S-CB7 as

> Asn-Phe-Ser-Thr-Ala-Asp-Ser-Ala-Lys-Ile-Lys-Thr-Leu-Glu-Ala-Glu-Lys-Ala-Ala-Leu-Ala-Ala-Arg-Lys-Ala-Asp-Leu-Glu-Lys-Ala-Leu-Glu-Gly-Ala-Met

This sequence is identical to that of the native CB7 fragment except that the COOH-terminal residue of S-CB7 is methionine, not homoserine4. The amino acid sequence of the S18-29CB7 peptide was found to be identical to the corresponding segment of CB7 except for the addition of a glycine tripeptide at the COOH terminal, which is used as a leash in the synthesis of the peptide (Penninsula Laboratories).

Three rabbits immunized with 25 nmol of S-CB7 emulsified in complete Freund's adjuvant (CFA)4 developed antibody titres at 6 weeks of 1:400, 1:1,280 and 1:25,600, respectively, as determined by enzyme-linked immunosorbent (ELISAs)2.6. However, only the serum showing the highest ELISA titre was capable of opsonizing type 24 streptococci (data not shown). Therefore, three additional rabbits were immunized with 25 nmol of CB7 covalently conjugated to polylysine (molecular weight (MW) =35,000) and emulsified in CFA. The sera of all three rabbits showed good antibody responses as measured by ELISAs or opsonic antibody assays (Fig. 1). In bactericidal assays (see Fig. 1 legend) using types 5, 6 and 24 streptococci, the immune sera were able to promote phagocytosis and killing of only the homologous type 24 streptococci, indicating that the humoral responses to the synthetic peptide fragment are type-specific.

Agar gel diffusion tests using the immune rabbit sera gave precipitin arcs between the polylysine conjugates of the synthetic and the native CB7, as well as with the intact pepsinextracted type 24 M protein (pep M24) molecule, which further confirms that the synthetic and native CB7 peptide fragments are immunochemically identical. Neither type 5 nor type 6 M protein was immunoreactive with anti-CB7 antiserum. None of the S-CB7 immune sera tested were reactive with frozen sections of human heart tissue assayed by immunofluorescence as previously described2.

To demonstrate the protective capacity of the antisera against S-CB7, mice were passively immunized with a pool of the immune rabbit sera and challenged after 24 h with live type 24 or type 6 streptococci. The results (Table 1) clearly show the type-specific protective capacity of the immune sera and indicate that the S-CB7 peptide contains at least one protective antigenic determinant of type 24 streptococci.

To determine whether protective antigenic determinants resided in yet smaller peptide fragments, the CB7 peptide was cleaved at its arginine residue by trypsin, after blocking lysyl residues with recrystallized maleic anhydride (to a molar excess

of 20 over the total number of lysyl residues)4,7 After digestion with trypsin (TPCK (tosyl-phenylethyl-chloromethyl-ketone) -treated, Worthington) at an enzyme/substrate ratio of 1:50 (w/w) in 0.05 M NH₄HCO₃, pH 8.3, the lysyl residues were demaleylated using pyridine/acetate (1:10), pH 3.0 at 60 °C for 6 h. The demaleyated peptides were then HPLC-separated on a column of Ultrasphere ODS2 equilibrated with 0.01 M phosphate buffer, pH 7.2, and eluted on a gradient of 0-40% acetonitrile8. In this way, a 12-residue COOH-terminal and a 23-residue NH₂-terminal peptide were purified and then tested for their ability to inhibit opsonic antibodies4 (see Fig. 1 legend). For 50% inhibition of opsonization of type 24 streptococci. 9 nmol of the 23-residue, NH₂-terminal peptide and 20 nmol of the 12-residue, COOH-terminal peptide of CB7 were needed. compared with only 1.6 nmol of a mixture of the two peptides and 0.8 nmol of the uncleaved CB7 peptide. The greater activity

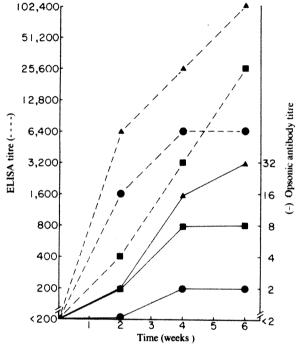


Fig. 1 Immune responses in three rabbits (△, ■, ●) as measured by ELISAs (---) or opsonic antibody (- assavs after immunization with 24 nmol of S-CB7 conjugated to polylysine and emulsified in complete Freund's adjuvant (CFA) followed a week later by the same dose emulsified in CFA. Each symbol represents a different rabbit (see also Table 1 legend). Sera collected at 2-week intervals were assayed for antibodies to type 24 M protein adsorbed to the walls of plastic cuvettes by the ELISA method as previously described^{2,6}, except that semi-automatic methods were used (EIA PR50 automatic analyser and injector, Gilford). ELISA titres are expressed as the reciprocal of the highest dilution of serum giving an absorption >0.1 at 405 nm. Opsonic antibodies were assayed as described elsewhere¹. Briefly, the test mixture consisted of 0.4 ml of fresh heparinized (10 U ml⁻¹) human blood, 0.05 ml of a standard suspension of streptococci and 0.05 ml of various dilutions of test serum. The number of streptococcal units per leukocyte was ~10. The percentage of neutrophilic leukocytes counted that ingested one or more bacteria was estimated by microscopic examination of stained smears prepared from a drop of test mixture after incubation for 30 min. The opsonic antibody titres are expressed as the reciprocal of the highest twofold dilution of test serum in three separate tests that promoted phagocytosis of streptococci in ≥10% of the neutrophils counted after incubation at 37 °C for 30 min; the same organisms in the presence of preimmune rabbit serum were phagocytosed by ≤2% of neutrophils in each test. Antisera giving titres >1:4 all produced phagocytosis in the range 40-70% when undiluted. The results of these phagocytosis tests were confirmed by indirect bactericidal tests performed as previously described¹⁸. Type specificity of the sera was confirmed by the failure of the S-CB7 immune sera to promote phagocytosis and killing of heterologous type 5 and type 6 streptococci.

Table 1 Protection of mice against challenge infections with type 24 streptococci by sera of rabbits immunized with S-CB7

	LD ₅₀ in mice challenged with:			
Serum used to immunize mice passively	Type 6 streptococci	Type 24 streptococci		
Preimmune serum Pooled (three rabbits) immune anti-S-CB7 serum	<500 (2/15) <500 (2/15)	<500 (3/15) 3,500,000 (14/15)		

Three rabbits were immunized intracutaneously with 25 nmol of S-CB7 conjugated to polylysine (MW \approx 35,00) and emulsified in CFA as previously described⁴. The initial immunizing dose was followed 1 week later by the same dose emulsified in incomplete Freund's adjuvant and injected subcutaneously. Preimmune and immune sera, obtained before immunization and 6 weeks after the initial immunizing dose, respectively, were pooled and white Swiss mice injected intraperitoneally with 0.2 ml of either serum. The mice were challenged 24 h later by the same route with various doses $500-4 \times 10^6$ colony-forming units of type 6 or type 24 streptococci. The survivals were recorded over a 7-day period and are shown in parentheses as the number of survivors per number of challenged mice.

of the mixture of the two peptide fragments of CB7 indicates that CB7 contains at least two distinct type-specific protective determinants, and that one of these resides in a peptide containing only 12 amino acid residues. The synthetic dodecapeptide overlapping these two peptides (residues 18–29) had no opsonic inhibitory effect in doses as high as 100 nmol, suggesting that neither immunodeterminant is included in this dodecapeptide. However, the possibility that the COOH-terminal triglycine residues may interfere with antibody binding at that end of the dodecapeptide has not been excluded.

In contrast to the type specificity of the humoral immune responses to S-CB7, the cellular immune responses were highly cross-reactive. The lymphocytes of S-CB7-immunized rabbits were equally responsive to a heterologous type 5 M protein and an homologous type 24 M protein (Table 2). Moreover, immunization of rabbits with the synthetic dodecapeptide (S18-29CB7), although not providing effective humoral immunity, induced cellular immunity to both serotypes of M protein similar to that seen after immunization with S-CB7 (Table 2). The lymphocytes from none of the animals responded to S-CB7 or S18-29CB7, indicating that these peptides were of insufficient molecular size to elicit the *in vitro* blastogenic response of sensitized lymphocytes.

Recently Audibert et al. 10 actively immunized laboratory animals against diptheria toxin using a chemically synthesized oligopeptide. However, we believe that our results are the first to show that a synthetic polypeptide antigen can raise antibodies which promote phagocytosis and killing of a bacterial pathogen. The synthetic CB7 of type 24 streptococcal M protein represents a 35-amino acid fragment of the parent molecule, which consists of 376 amino acid residues¹. The molecule has previously been shown to consist of repeating covalent structures, the first 20 residues of CB7 being indentical to the corresponding regions of four other peptide fragments (CB3, CB4, CB5 and CB6)¹. Two additional fragments (CB1 and CB2), each with a MW of ~10,000, have NH₂-terminal amino acid sequences which are different from the smaller peptide fragments but are identical to each other and to the intact pepsin-extracted M protein (pep M24) molecule for at least the first 27 residues^{1,1}

In addition, the 12 COOH-terminal residues of CB1 and CB2 were found to be identical to those of each of the smaller peptides (unpublished data). It was particularly intriguing that one of the protective determinants was shown to reside in this 12-residue peptide, which is repeated seven times in the pep M24 molecule. Because of the repetitive nature of the primary structure of the M protein molecule it is not surprising that antibody directed against such a small segment of the molecule proved to be opsonic and presumably protective. The repetition of the determinant would provide multiple sites of interaction

Table 2 Blastogenic responses of lymphocytes from rabbits immunized with S-CB7 or S18-29CB7 fragments of type 24 M protein

		Mean c.p.m. (±s.e.m.) of lymphocytes cultured with:				
Rabbit	Immunizing antigen	Control	M5	M24		
8026 8027 8028	S-CB7 S-CB7 S-CB7	98 ± 23 181 ± 28 156 ± 31	$3,743 \pm 411$ $12,684 \pm 1,454$ $43,391 \pm 3,094$	4,721±479 829±124 36,449±5,416		
7919 7921 8020	S18-29CB7 S18-29CB7 S18-29CB7	415 ± 27 135 ± 31 705 ± 59	$11,521 \pm 3,081$ $17,056 \pm 3,081$ $12,487 \pm 918$	$12,038 \pm 243$ $3,575 \pm 1,265$ $4,916 \pm 362$		
8007 8008	CFA (control) CFA (control)	76 ± 102 31 ± 11	64 ± 12 153 ± 25	40 ± 2 83 ± 25		

Heparinized (100 U ml⁻¹) peripheral blood was obtained from rabbits 2-6 weeks after immunization (see Table 1 legend) by cardiac puncture. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation as described elsewhere ^{17,19}. Lymphocytes were washed three times and resuspended in RPMI 1640 (Gibco) supplemented with penicillin (100 U ml⁻¹), streptomycin (100 µg ml⁻¹), L-glutamine (2 mM) and HEPES buffer (25 mM). Lymphocytes (2 × 10⁵) were incubated at 37 °C with 50 µg per culture of each antigen tested in 96-well microculture plates (Falcon Plastics) in a total volume of 200 µl supplemented with 5% heat-inactivated fetal calf serum. Control cultures were incubated in the same volume of medium without antigen. Eighteen hours before collecting, 1 μCi of ³H-thymidine (specific activity 2 Ci mmol⁻¹, Research Products International) in 25 μl culture medium was added to each well. All cultures were collected after 5 days using multiple automated sample harvester and the cells assayed for radioactivity in a liquid scintillation counter as described elsewhere 17.20. CFA, complete Freund's adjuvant. Control animals were injected with the same volume of CFA emulsified in 0.15 M NaCl without antigen.

with the immunoglobulin molecules. Such interaction at multiple sites seems to be necessary for optimal opsonization of M protein-containing streptococcal cells^{†2}.

The significance of the cell-mediated cross-reactions between synthetic peptide fragments and heterologous M proteins is unclear; structural similarities between the M proteins 11,13-16 may account for these cross-reactions as well as for the high degree of cellular immunity to various M proteins recently observed in lymphocytes from human adults as well as cord blood of newborn infants¹

The immunogenicity of small peptide fragments is encouraging for the development of safe and effective vaccines against those streptococcal infections that initiate rheumatic fever and rheumatic heart disease2. The efficacy of very small peptides would permit disposal of a large portion of the M protein molecule and, therefore, should reduce the chances of eliciting immunological cross-reactions against host tissues. Thus, the continued identification of peptide structures responsible for protective immunity should yield a pool of small peptides that may eventually be synthesized and administered safely to humans as vaccine broadly protective against many serotypes of streptococci, particularly against those strains that trigger post-streptococcal sequelae.

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- 1. Beachey, E. H., Seyer, J. M. & Kang, A. H. Proc. natn. Acad. Sci. U.S.A. 75, 3163-3167
- 2. Beachey, E. H., Stollerman, G. H., Johnson, R. H., Ofek, I. & Bisno, A. L. J. exp. Med. 150,
- Stollerman, G. H. Rheumatic Fever and Streptococcal Infection (Grune & Stratton, New
- Beachey, E. H., Seyer, J. M. & Kang, A. H. J. biol. Chem 255, 6284-6289 (1980).
- Merrifield, R. B. J. Am. chem. Soc. 85, 2149-2155 (1963).
 Dale, J. B., Ofek, I. & Beachey, E. H. J. exp. Med. 151, 1026-1038 (1980)
- Butler, P. J. G., Harris, J. I., Hartley, B. S. & Leberman, R. Biochem. J. 112, 679-689

- (1908).

 8. River, J. E. J. Liq. Chromatogr. 1, 343-353 (1978).

 9. Singh, B. et al. J. Immun. 124, 1336-1342 (1980).

 10. Audibert, F. et al. Nature 289, 593-594 (1981).

 11. Beachey, E. H. & Seyer, J. M. Bacterial Vaccines (eds Robbins, J. B. et al.) (Decker, New York, in the press).
- 12. Fischetti, V. A. J. exp. Med. 146, 1108-1123 (1977).
- Beachet, E. H., Seyer, J. M. & Kang, A. H. in Streptococcal Diseases and the Immune Response (eds Zabriskie, J. B. & Read, S. E.) 149-160 (Academic, New York, 1980).
- 14. Seyer, J. M., Kang, A. H. & Beachey, E. H. Biochem biophys. Res. Commun. 92, 546-553
- Manjula, B. N. & Fischetti, V. A. J. Immun. 124, 261–267 (1980).
 Manjula, B. N. & Fischetti, V. A. J. exp. Med. 151, 695–707 (1980).

- Dale, J. B., Simpson, W. A., Ofek, I. & Beachey, E. H. J. Immun. 126, 1499-1505 (1980).
 Beachey, E. H. & Stollerman, G. H. J. exp. Med. 134, 351-365 (1971).
 Boyum, A. Scand. J. clin. Lab. Inness. 21, 77-89 (1968).
- 20. Beachey, E. H. et al. J. Immun. 122, 189-195 (1979).

Binding of MLR precursors requires immunosorbents which present both self- and alloantigen

Heather Y. Schnagl & William Boyle

Department of Microbiology, University of Melbourne, Parkville, Victoria 3052, Australia

The response of T lymphocytes to antigens of the major histocompatibility complex (MHC) in a species is commonly measured by two assays. The mixed lymphocyte reaction (MLR) measures proliferating lymphocytes (Tp), and cell-mediated cytolysis (CMC) measures the cytotoxic T cells (T_c) generated, which can destroy tissue cells bearing the alloantigens against which the response is directed1. Tp and Tc are thought to be derived from different precursors, the Tp-P and Te-P respectively, and each seem to have different requirements for activation. Initially it was claimed that Tp-P and Tc-P were coded to respond to antigens specified by different genes in the MHC2 but this distinction has since been shown not to be absolute³⁻⁶. We previously demonstrated that Tp-P and Tc-P had different antigen-binding characteristics as T.-P could be bound in a haplotype-specific manner to allogeneis monolayers but $T_{\mathfrak{p}}\text{-}P$ were not bound in these conditions. We report here further experiments which show that these two populations have qualitatively distinct antigen recognition properties, by demonstrating that Tp-P will bind only when self- plus alloantigen are presented by a monolayer, whereas Tc-P will bind when alloantigen alone is present.

Our original observation that monolayers of preincubated spleen cells specifically absorbed cytotoxic precursors, Tc-P, but failed to bind precursors of proliferative cells, T_p-P, has been confirmed in many experiments. Because the proliferative response seen in MLR reactions had been shown to be strongest when stimulated by H-2I region antigens, it seemed possible that T_p-P did not bind because monolayers of spleen cells were used and not all these cells expressed Ia determinants. Poly-Llysine monolayers of B-lymphocyte blasts were therefore prepared from B10 mice, then spleen cells from B10.BR mice incubated on these for 1 h. The nonadherent cells were recovered and cultured for MLR and CMC production against X-irradiated B10 and B10.D2 cells. Table 1 shows that the results were the same as those obtained with spleen cell monolayers; T_c-P were bound but not T_p-P. Similar experiments

Table 1 Absorption on B-cell blast monolayers and its effect on the proliferative and cytotoxic response of the nonadherent B10.BR responder population to stimulation with X-irradiated B10 spleen cells

Responder	Absorbing LPS blast monolayer	Stimulator	Proliferative Response (c.p.m. ³ H-T)	Cytoto H-2 ^b target	xic titre H-2 ^d target
B10.BR		X-B10	10.500 ± 950	16.7	5.7
B10.BR	****	X-B10.D2	19.800 ± 1.500	<1	23.8
B10.BR		X-B10.BR	480 ± 60	-	
B10.BR	B 10	X-B10	10.800 ± 680	<1	1.7
B10.BR	B 10	X-B10.D2	16.900 ± 660	<1	23.3
B10.BR	B 10	X-B10.BR	500 ± 50		
X-B10.BR	B10	X-B10	230 ± 20		
X-B10.BR	B 10	X-B10.D2	130 ± 10		

B10 spleen cells (3×10⁸) were cultured in 75 cm² flasks (Falcon no. 3024) in a final volume of 75 ml of Eagle's minimum essential medium plus non-essential amino acids, supplemented with 10% v/v fetal calf serum (FCS) and 7×10^{-5} M 2-mercaptoethanol (MEM/FCS) containing $10\,\mu\mathrm{g}\,\mathrm{ml}^{-1}$ of lipopolysaccharide (LPS, Difco no. 3122-25) at 37 °C with 10% CO₂ in air for 3 days to generate B-cell blasts. After washing, $1-2\times10^7$ blast cells were attached to poly-L-lysine-coated dishes as described previously. B10.BR responder spleen cells, or in one case X-irradiated B10.BR (X-B10.BR) spleen cells as controls, were absorbed on the resultant monolayers as previously described. Briefly, B10.BR responder spleen cells (2×10^7) in 2 ml MEM/FCS were added to each monolayer. The dishes were centrifuged at 100g for 5 min then incubated at 37 °C with 10% CO2 in air for 1 h. The nonadherent B10.BR responder cells were recovered by brief agitation, washed once, and 2×10^7 were resuspended in 8 ml MEM/FCS and divided equally between two 25 cm² flasks (Falcon no. 3013). Control cultures containing 107 unfractionated B10.BR spleen cells in 4 ml MEM/FCS were similarly prepared. To each flask were added 107 X-irradiated (1,000 rad) stimulator spleen cells in 1 ml. Stimulator cells of the monolayer strain (X-B10, H-2b) were added to one of each pair of flasks representing the nonadherent population from a single dish, while stimulators of an unrelated strain (X-B10.D2, H-2d) or of the responder strain (X-B10.BR) were added to the remaining flask. All cultures were prepared in duplicate and incubated upright at 37 °C with 10% CO₂ in air for 5 days. To determine the proliferative response, duplicate 0.3-ml samples of each culture after 3 days incubation were transferred to a microtitre tray (Sterilin no. M29 ARTL). After centrifugation at 100g for 5 min, half the supernatant was discarded and 25 µl of medium containing 0.5 µCi of ³H-thymidine (³H-T) were added to each well. After incubation in a humidified box with 10% CO2 in air for 18 h, cultures were transferred to microtubes and treated according to the method of Smart et al. 16. Results obtained were expressed as c.p.m. 3H-T and represent the mean ±s.d. of quadruplicate determinations. Cytotoxicity was determined after 5 days incubation using a microassay described elsewhere¹⁷. Duplicate cultures were pooled, washed and resuspended in 0.3 ml RPMI 1640 supplemented with 10% v/v FCS (RPMI/FCS). All T_c populations were titrated against ⁵¹Cr-labelled tumour target cells of both stimulator haplotypes. The results obtained were expressed as % lysis ±s.d. of triplicate determinations. Per cent specific lysis represents the % lysis minus the spontaneous release of label by target cells incubated with medium alone. The cytotoxic titre for a given Te population was alculated as the reciprocal of the highest dilution of the Te population which gave 15% specific lysis of the target concerned.

using mouse embryonic fibroblasts which have also been shown to express I-region antigens^{8,9} also failed to show depletion of T_p-P .

Although T-cell responses to MHC alloantigens have always been considered not to require self-antigen presentation, in the absence of any other effective system for binding T_p-P, we examined the effect of using absorbent monolayers which copresented both allo- and self-antigen. Table 2 shows the results of absorbing B10.BR spleen cells separately on to monolayers composed of preincubated splenic lymphocytes of B10.BR, BALB/c or (B10.BR×BALB/c)F₁ hybrid origin. Neither the B10.BR nor BALB/c monolayers bound T_p-P, as shown by the unimpaired MLR response of the nonadherent B10.BR cells when stimulated with X-irradiated BALB/c or B10 cells. The BALB/c monolayers were adequate as shown by the fact that, although T_p-P were not bound, T_c-P directed against BALB/c were bound (data not shown). However, B10.BR cells which had been exposed to the (B10.BR×BALB/c)F₁ monolayer were found to be depleted of both T_c-P and T_p-P when stimulated by BALB/c alloantigens. The T_p-P depletion was apparently specific as the T_p response to B10 alloantigens was unimpaired.

To ensure that the reduction in ability to respond to BALB/c antigens by the B10.BR cells, which were nonadherent to the $(B10.BR \times BALB/c)F_1$ monolayer, was due to binding of T_p -P and not to induction of a suppressor effect, non-F1 monolayer-

adherent B10.BR cells were added to normal B10.BR spleen cells and stimulated with X-irradiated BALB/c cells. As can be seen in Table 3, no evidence was found for suppression by the B10.BR cells that were nonadherent to the F_1 monolayer. An alternative explanation of the results is that T_p-P were either bound or inactivated nonspecifically by exposure to the F monolayer and that the apparent specificity of the undiminished anti-B10 response was attributable to F, monolayer cells which were mounting an anti-B10 response. However, this was not the case as the cells which incorporated ³H-T on stimulation by B10 alloantigens were susceptible to complement-mediated lysis with anti-B10.BR serum but not with anti-BALB/c serum (Table 4). It was therefore concluded that the reduction in proliferation was due to binding of the T_p-P to the F₁ monolayer.

The specificity of T_p-P depletion was confirmed and investigated further by exposing B10.BR spleen cells to monolayers of B10.BR, BALB/c, B10, (B10.BR×BALB/c)F₁, $(B10.BR \times B10)F_1$ or $(B10 \times BALB/c)F_1$ spleen cells. The results are summarized in Table 5. Absorption of anti-BALB/c T_p -P occurred only on (B10.BR × BALB/c) F_1 monolayers. The depletion is specific for the anti-BALB/c response as the nonadherent B10.BR cells gave an undiminished MLR response to B10 alloantigen stimulation. The specificity of the depletion technique is further illustrated by the demonstration that absorption on a (B10.BR × B10)F₁ monolayer depleted the anti-B10 proliferative response but not the anti-BALB/c response. This experiment also defines further the self requirement, because B10.BR were not depleted of anti-BALB/c T_p-P when exposed to a monolayer of (B10× BALB/c)F₁, indicating that the self-antigen recognized must be coded in the H-2 region. This has been confirmed by further experiments (data not shown) in which B10 responder cells

Table 2 Absorption of B10.BR responders on preincubated (B10.BR× BALB/c)F₁ monolayers specifically depletes the proliferative response of the nonadherent responder population to stimulation with X-irradiated BALB/c spleen cells

Responder	Absorbing monolayer	Stimulator	Proliferative response (c.p.m. ³ H-T)
B10.BR		X-BALB/c	$6,560 \pm 480$
B10.BR		X-B10	$5,310 \pm 510$
B10.BR	****	X-B10.BR	500 ± 80
B10.BR	B10.BR	X-BALB/c	$6,710 \pm 310$
B10.BR	B10.BR	X-B10	$5,450 \pm 470$
B10.BR	B10.BR	X-B10.BR	510 ± 50
B10.BR	BALB/c	X-BALB/c	7.890 ± 960
B10.BR	BALB/c	X-B10	$6,420 \pm 400$
B10.BR	BALB/c	X-B10.BR	510 ± 60
B10.BR	$(B10.BR \times BALB/c)F_1$	X-BALB/c	310 ± 120
B10.BR	$(B10.BR \times BALB/c)F_1$	X-B10	$5,850 \pm 610$
B10.BR	$(B10.BR \times BALB/c)F_1$	X-B10.BR	390 ± 40

B10.BR responder spleen cells were absorbed onto B10.BR, BALB/c (H-2d) or (B10.BR × BALB/c)F₁ spleen cell monolayers (preincubated for 4 h) as described in Table 1. The resulting nonadherent responder cells were stimulated with X-irradiated BALB/c (X-BALB/c), B10 (X-B10) or B10.BR (X-B10.BR) spleen cells. After 3 days incubation the proliferative response was determined as described in Table 1 legend.

Table 3 Inability of B10.BR responders nonadherent to (B10.BR×BALB/c)F₁ preincubated monolayers to suppress the proliferative response of normal, unfractionated B10.BR responders to stimulation with X-irradiated BALB/c

	Responder popul	ation	
B10.BR	X-B10.BR	B10.BR nonadherent to F ₁ * monolayer	Proliferative response (c.p.m. ³ H-T)
$\begin{array}{c} 5 \times 10^6 \\ 5 \times 10^6 \end{array}$	5×10 ⁶	5×10 ⁶	$2,930 \pm 250$ $2,780 \pm 110$
	5×10^{6}	5×10^{6}	230 ± 70

B10.BR responder cells nonadherent to a (B10.BR × BALB/c)F; monolayer (5×10^6) were mixed with an equal number of unfractionated B10.BR spleen cells. These were stimulated with 10^7 X-irradiated BALB/c spleen cells as were control cultures containing 5×106 unfractionated B10.BR responder spleen cells and 5×10^6 X-irradiated B10.BR spleen cells. * $F_1 = (B10.BR \times BALB/c)F_1$.

could be depleted of anti-BALB/c Tp-P by exposure to a (B10×BALB/c)F₁ spleen monolayer but not a (B10.BR× BALB/c)F₁ monolayer.

The specificity of binding of T_n-P by monolayers presenting self-plus alloantigen has been tested using a number of parental and F₁ strains. These have confirmed the general observation that T_p-P were bound only when the responder and absorbing F₁ monolayer shared a common haplotype, and the depletion was specific for the haplotype of the other parent. In addition, we have found that monolayers composed of mixtures of responder and stimulator spleen cells would not absorb anti-stimulator T_p-P. The level of depletion achieved by absorption on semiallogeneic F₁ monolayers has varied for different combinations of responder-monolayer mouse strains but has been consistent for any single combination. This is not surprising as we found that even for T_c-P binding, the level of depletion depends on the time of preincubation before the monolayer is constructed and this differs from strain to strain. All the experiments reported here using F₁ cells have involved only a 4-h preincubation which may not be optimal for all combinations.

Table 4 Proliferative response of the B10.BR nonadherent population to stimulation by X-irradiated B10 is not due to detached (B10.BR×BALB/c)F1 monolayer cells

	Preincubated	Co.	Total	Proliferative response (c.p.m. ³ H-T)
Responder	monolayer	Stimulator	Treatment	(c.p.m. ri-1)
B10.BR		X-B10	C' only	$4,980 \pm 220$
B10.BR		X-B10	Anti-BALB/c+C'	$4,560 \pm 190$
B10.BR		X-B10	Anti-B10.BR+C'	$2,280 \pm 90$
F,*		X-B10	C' only	$6,680 \pm 290$
F,		X-B10	Anti-BALB/c+C'	$3,050 \pm 120$
F,		X-B10	Anti-B10.BR+C'	$3,100 \pm 110$
B10.BR	F ₁	X-B10	C' only	$4,380 \pm 210$
B10.BR	\mathbf{F}_{1}	X-B10	Anti-BALB/c+C'	$4,550 \pm 180$
B10.BR	$\mathbf{F_i}$	X-B10	Anti-B10.BR+C'	$1,740 \pm 80$

B10.BR, (B10.BR \times BALB/c)F $_1$ or B10.BR responders that were nonadherent to preincubated (B10.BR \times BALB/c)F $_1$ monolayers were stimulated with Xirradiated B10 (X-B10) spleen cells. Samples of 3-day cultures were incubated with 3H-T as described in Table 1 legend. After labelling, replicate samples were pooled, washed and resuspended in 0.2 ml RPMI/FCS. To three 50-µl aliquots the following were added: (1) 50 µl of RPMI/FCS; (2) 50 µl of a 1:4 dilution of B10.BR anti-BALB/c serum; (3) 50 µl of a 1:4 dilution of a BALB/c anti-B10.BR serum. After incubation for 30 min the tubes were centrifuged and supernatants discarded; 0.2 ml of a 1:8 dilution of rabbit complement (C') was added to each tube and incubated for a further 30 min. After washing twice in RPMI/FCS the samples were collected and processed as described in Table 1

legend. * $F_1 = (B10.BR \times BALB/c)F_1$.

Table 5 Specific depletion of Tp-P requires the responder and monolayer strains to share an H-2 haplotype

Responder	Absorbing monolayer	Stimulator	Proliferative response (c.p.m. minus control values)
B10.BR		X-BALB/c	$11,020 \pm 710$
B10.BR		X-B10	$20,660 \pm 860$
B10.BR	B10.BR	X-BALB/c	$10,770 \pm 810$
B10.BR	B10.BR	X-B10	$19,770 \pm 1,020$
B10.BR	BALB/c	X-BALB/c	$10,950 \pm 720$
B10.BR	BALB/c	X-B10	$19,750 \pm 690$
B10.BR	B 10	X-BALB/c	$11,080 \pm 570$
B10.BR	B 10	X-B10	$18,020 \pm 790$
B10.BR	$(B10.BR \times BALB/c)F_1$	X-BALB/c	80 ± 70
B10.BR	$(B10.BR \times BALB/c)F_1$	X-B10	$19,340 \pm 900$
B10.BR	$(B10.BR \times B10)F_1$	X-BALB/c	$10,480 \pm 330$
B10.BR	$(B10.BR \times B10)F_1$	X-B10	900 ± 110
B10.BR	$(B10 \times BALB/c)F_1$	X-BALB/c	$10,370 \pm 520$
B10.BR	$(B10 \times BALB/c)F_1$	X-B10	$20,640 \pm 1,020$

B10.BR responder spleen cells were absorbed, as described in Table 1 legend, on monolayers of B10.BR, BALB/c, B10, (B10.BR \times BALB/c) F_1 , (B10.BR \times B10)F₁, or (B10 × BALB/c)F₁ spleen cell monolayers that had been preincubated for 4 h. The resultant nonadherent responder cells were stimulated with Xirradiated BALB/c, B10 or B10.BR spleen cells. After 3 days incubation the proliferative response was determined as described in Table 1 legend. The results are expressed as c.p.m. minus control values (stimulated with X-B10.BR).

The importance of the experiments reported here is that, in addition to clarifying the distinction between antigen recognition by T_p-P and T_c-P, they present the first evidence of the need for self-antigen recognition by T cells coded to respond to alloantigens of the MHC. It is already well established that T cells responsive to non-MHC antigens require the extraneous antigen to be presented together with self-antigens 10-15. This distinction has been the basis of suggestions that alloreactive cells might, in fact, include multiple clones coded to self plus various extraneous antigens. This view will require some revision if the need for self-plus alloantigen demonstrated here is found to be generally applicable to the activation of cells which proliferate in response to MHC alloantigens. It should be stressed that in our experiments we have measured binding of precursors, not activation, but preliminary tests suggest that the same features apply to activation.

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- Wagner, H., Röllinghoff, M. & Nossal, G. J. V. Transplantn Rev. 17, 3-36 (1973).
- Bach, F. H. et al. Science 180, 403-406 (1973).
 Widmer, M. B., Alter, B. J., Bach, F. H. & Bach, M. L. Nature new Biol. 242, 239-241
- 4. Klein, J., Hauptfeld, M. & Hauptfeld, V. Immunogenetics 1, 45-56 (1974)
- Nabholz, M. et al. Eur. J. Immun. 5, 594-599 (1975). Wagner, H., Götze, D., Ptschelinzew, L. & Röllinghoff, M. J. exp. Med. 142, 1477-1487
- Schnagl, H. Y. & Boyle, W. Nature 279, 331-333 (1979)
- Hämmerling, G. J., Mauve, G., Goldberg, H. & McDevitt, H. O. Immunogenetics 1, 428-437 (1975).
- 9. McDevitt, H. O., Delovitch, T. L., Press, J. L. & Murphy, D. B. Transplantn Rev. 30, 197-235 (1976).
- Katz, D. H., Hamaoka, T. & Benacerraf, B. J. exp. Med. 137, 1405-1418 (1973).
 Zinkernagel, R. M. & Doherty, P. C. Nature 248, 701-702 (1974).
- Shearer, G. M., Rhen, T. C. & Garbarino, C. A. J. exp. Med. 141, 1348–1364 (1975).
 Gordon, R. D., Simpson, E. & Samelson, L. E. J. exp. Med. 142, 1108–1120 (1975).
- Bevan, M. J. exp. Med. 142, 1349-1364 (1975).
 Miller, J. F. A. P., Vadas, M. A., Whitelaw, A. & Gamble, J. Proc. natn. Acad. Sci. U.S.A. 73, 2486-2490 (1976).
- 16. Smart, I. J., Boyle, W. & Morris, P. J. Tissue Antigens 11, 20-29 (1978).
- 17. Schnagl, H. Y. & Boyle, W. J. immun. Meth. 24, 79-88 (1978)

Adrenaline activation of phosphofructokinase in rat heart mediated by α -receptor mechanism independent of cyclic AMP

M. G. Clark & G. S. Patten

Commonwealth Scientific and Industrial Research Organization, Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000, Australia

We recently reported that adrenaline treatment of the perfused rat heart produced a marked activation of phosphofructokinase when assayed at low substrate concentration using a new isotopic assay2. As muscle phosphofructokinase has been reported to be phosphorylated by the cyclic AMP-dependent protein kinase3, activation of phosphofructokinase might be expected to result from phosphorylation mediated by the β -receptor/adenylate cyclase/cyclic AMP-dependent protein kinase sequence. We now report that this is not so, but that activation of phosphofructokinase in the perfused rat heart occurs independently of phosphorylase activation, by a cyclic AMP-independent mechanism, and that this process seems to be mediated predominantly through the α -adrenergic receptors. We believe this represents the first example showing that not only in liver 4.5 but also in a muscular tissue, occupancy of an α -adrenergic receptor changes the activity of a glycolytic enzyme. In addition, the present findings imply a coordination of muscle glycolysis with hepatic glucose output.

Figure 1 shows the effect of varying ATP concentration on extract phosphofructokinase activity from control and adrenaline-treated hearts. The adrenaline-activated form of the enzyme as expressed in crude heart extracts displays higher activity than the control form at all ATP concentrations. This is most pronounced at ATP concentrations > 0.5 mM, where the control form is almost totally inhibited. We have subsequently used this difference in sensitivity to inhibition by ATP to define an 'activity ratio' for phosphofructokinase where the control (unactivated) form of the enzyme is 0.2 ± 0.1 and the activated form of the enzyme is 0.6 ± 0.1 , representing the activity of phosphofructokinase determined at 1 mM ATP divided by that at 0.1 mM ATP (both at 0.01 mM hexose 6-phosphate).

Table 1 shows the effect of 5-min perfusions with adrenergic agonists or antagonists on the activity ratios of phosphofructokinase and glycogen phosphorylase in heart. For phosphofructokinase the basal activity ratio of 0.21 was increased about threefold by both adrenaline $(10^{-5} \, \mathrm{M})$ and $10^{-5} \, \mathrm{M}$ phenylephrine and remained elevated following gel filtration of the heart extracts (results not shown). Neither $10^{-5} \, \mathrm{M}$ isoprenaline nor $10^{-5} \, \mathrm{M}$ phenoxybenzamine had any effect, but $10^{-5} \, \mathrm{M}$ propranolol increased the ratio to 0.35. As the increase produced by adrenaline was blocked by phenoxybenzamine but not by propranolol, it was concluded that activation of phosphofructokinase was mediated predominantly through the α -receptor. This view was further supported by the observation that the marked increase in phosphofructokinase activity ratio produced by $10^{-5} \, \mathrm{M}$ phenylephrine was also blocked by $10^{-5} \, \mathrm{M}$ phenoxybenzamine.

In contrast to a predominantly α -receptor-mediated activation of phosphofructokinase, the activation of phosphorylase was mediated by a β -receptor mechanism. Adrenaline $(10^{-5} \, \mathrm{M})$ and $5 \times 10^{-7} \, \mathrm{M}$) and $10^{-5} \, \mathrm{M}$ isoprenaline each increased the phosphorylase activity ratio (a/a+b) approximately fourfold. $10^{-5} \, \mathrm{M}$ Phenylephrine had a marginal effect. Neither $10^{-5} \, \mathrm{M}$ propranolol nor $10^{-5} \, \mathrm{M}$ phenoxybenzamine had any stimulatory effect. The increases produced by adrenaline and isoprenaline were totally blocked by the β -antagonist, propanolol $(10^{-5} \, \mathrm{M})$, but the adrenaline-mediated increase in phosphorylase activity ratio remained unaffected by the α -antagonist, phenoxybenzamine $(10^{-5} \, \mathrm{M})$.

The marginal increase in phosphorylase activity ratio produced by the highest concentration of phenylephrine (10^{-5} M) was blocked by 10^{-5} M phenoxybenzamine. However, lower concentrations of phenylephrine ($\leq 10^{-6} \text{ M}$) significantly increased the activity ratio of phosphofructokinase but did not increase that of phosphorylase (Table 1).

Figure 2 shows the time course effects of 10^{-5} M isoprenaline, 10^{-5} M phenylephrine and 10^{-5} M naphazoline on the activity ratios of phosphofructokinase and phosphorylase as well as the

Table 1 The effect of adrenergic agonists and antagonists on the activity ratios of phosphofructokinase and glycogen phosphorylase

* *		
Additions	Phosphofructokinase	Phosphorylase
None	0.21 ± 0.01 (4)	0.16 ± 0.004 (4)
10 ⁻⁵ M adrenaline	$0.60 \pm 0.10 (14)$	0.68 ± 0.027 (10)
5×10 ⁻⁷ M adrenaline	0.42 ± 0.02 (3)	0.60
10 ⁻⁵ M adrenaline	0.57 ± 0.05 (3)	0.14 ± 0.031 (3)
+10 ⁻⁵ M propanolol		
10 ⁻⁵ M adrenaline	0.43	0.66
+10 ⁻⁵ M phenoxybenzamine		
5×10 ⁻⁷ M adrenaline	0.23 ± 0.02 (3)	0.58
+ 10 ⁻⁵ M phenoxybenzamine		
10 ⁻⁵ M propranolol	$0.35 \pm 0.10(3)$	0.16 ± 0.004 (3)
10 ⁻⁵ M phenoxybenzamine	0.18 ± 0.06 (3)	0.15 ± 0.015 (3)
10 ⁻⁵ M isoprenaline	0.24 ± 0.04 (3)	0.72 ± 0.036 (3)
105- M isoprenaline	0.34 ± 0.02 (3)	0.15 ± 0.007 (3)
+ 10 ⁻⁵ M propranolol		
10 ⁻⁵ M phenylephrine	0.62 ± 0.01 (3)	0.31 ± 0.018 (3)
10 ⁻⁶ M phenylephrine	0.40 ± 0.02 (3)	0.17 ± 0.005 (3)
10 ⁻⁷ M phenylephrine	$0.37 \pm 0.09(3)$	0.16 ± 0.017 (3)
10 ⁻⁵ M phenylephrine	0.35 ± 0.03 (3)	0.16 ± 0.016 (3)
+ 10 ⁻⁵ M phenoxybenzamine		

The values are means ± s.e.m. with the number of hearts in parentheses

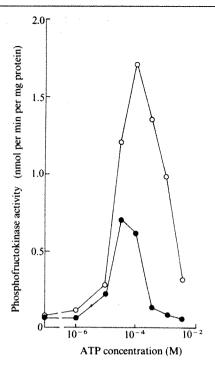


Fig. 1 Effect of ATP concentration on phosphofructokinase activity in extracts from control (and adrenaline-treated () hearts. Hearts of adult male hooded Wistar rats (200-250 g) that had been fed ad libitum on a standard diet were perfused in the Langendorff manner11. The perfusion medium contained 118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄, 1.2 mM phosphate, 25 mM NaHCO₃, 3.0 mM CaCl₂ and 0.5 mM Na₂EDTA and was equilibrated with $O_2 + CO_2$ (95 + 5) to give a pH and pO₂ of 7.4 and 450 mm Hg, respectively. After a 10-min non-recirculating perfusion (pressure 60 mm Hg, temperature 37 °C) the hearts were perfused in a recirculating manner with identical medium containing hormone or saline. After the appropriate time of perfusion (in this case 5 min) the hearts were freezeclamped using tongs that had been precooled in liquid N2. The powdered hearts were kept at -70°C. Cell-free extracts of frozen hearts were prepared by homogenizing 1 part by weight of powdered heart with 10 vol of homogenizing buffer (100 mM Tris-HCl, pH 7.4, which contained 2 mM dithiothreitol) using an Ultra-Turrax homogenizer (speed 20,000 r.p.m. for 30 s). Consistent results for phosphofructokinase activity were obtained when frozen heart powder was added to preweighed tubes containing homogenizing buffer that had been frozen in liquid N2 and the combined mixture homogenized during thawing at 5 °C. Thawing of the powdered heart before homogenization resulted in abnormally high phosphofructikinase activities, presumably due to the action of endogenous activators. The homogenate was centrifuged for 30 s at 8,000g and kept on ice. In these conditions the activity increased by ~10% per hour. 'Control' heart before homogenization resulted in abnormally high phosphofructokinase activities, presumably due to the action of endogenous siological fructose 6-phosphate concentration using a newly developed radioisotopic procedure². The assay has been previously shown to be specific for phosphofructokinase and to give values for maximal catalytic activity identical to those obtained using the conventional spectrophotometric assay of 16 nmol per min per mg protein (ref. 2). The assays were conducted in disposable plastic tubes (70×10 mm) at 30 °C and contained 10 µM glucose 6-phosphate, 3.3×10^4 d.p.m. [5-3H]glucose 6-phosphate (prepared from [5-3H]glucose2), ATP as indicated, 10 mM MgCl2 and 20 µ1 of cell-free supernatant (0.075-0.1 mg of protein) in a total volume of 0.2 ml of homogenizing buffer. Reactions were started by the addition of the extract and terminated by the addition of 0.5 ml of Dowex 1×8 (200-400 mesh) borate resin (1:1 in water), vortexed, then centrifuged for 3 min at 1,300g. A 0.25-ml portion of the supernatant (containing only ³H₂O) was mixed with 2 ml of a Triton X-based water-accepting scintillant and counted for radioactivity. The activity of phosphofructokinase is expressed in nmol of [5-³H]hexose 6-phosphate converted to ³H₂O per min per mg of protein, and was calculated from the d.p.m. in 3H2O and the specific radioactivity of hexose 6-phosphate. The specific radioactivity of hexose 6-phosphate was calculated from the d.p.m. added as [5-3H]glucose 6-phosphate and the sum of unlabelled glucose 6-phosphate and fructose 6-phosphate (added plus endogenous). Glucose 6-phosphate and fructose 6-phosphate were determined on neutralized perchlorate extracts of heart 12. The total concentration of hexose 6-phosphate in the assays was 10.7×10^{-6} and 14×10^{-6} M for control and adrenaline-treated extracts, respectively. Glycogen phosphorylase was measured using the filter disk assay of Gilboe et al. 13 as modified by Birnbaum and Fain 14 and was determined on extracts that had been passed through columns $(0.7 \times 2.5 \text{ cm})$ of Sephadex G-25. Protein was determined using the procedure of Lowry et al. 15 with bovine serum albumin as the standard. A total of four hearts (two control and two adrenalinetreated) were used. Mean values are shown.

tissue content of cyclic AMP. 10⁻⁵ M isoprenaline had no significant effect on the activity ratio of phosphofructokinase over the 15-min perfusion period. However, within 30 s there were marked increases in both cyclic AMP concentration and the activity ratio of phosphorylase. Both remained close to the maximal value throughout the 15-min non-recirculating perfusion. Phenylephrine (10⁻⁵ M), generally considered as predominantly an α -agonist but with some β activity in the rat⁵ increased the activity ratio of phosphofructokinase from 0.2 at 0 min to a maximal value of 0.65 at 1.5 min. The activation was similar to the time course previously reported for adrenaline1. Phenylephrine also produced an increase in the activity ratio of phosphorylase (100%). Naphazoline (10⁻⁵ M), an α -agonist⁷, increased the activity ratio of phosphofructokinase in a similar time-dependent manner to phenylephrine, reaching a maximum value of 0.55 at 1.5 min. Naphazoline had no effect on phosphorylase or cyclic AMP concentration.

The present findings suggest that short-term regulation of phosphofructokinase in rat heart by catecholamines is mediated by α -adrenergic receptors. This process may operate in anticipation of increased glucose released from the liver8 when catecholamines are released into the blood in emergency situations. The β -receptor component in the heart would lead to increased glycogenolysis, contractile rate and force. The α receptor component would separately permit increased flux through glycolysis from either myocardial glycogen stores or extra-myocardial glucose. Such a mechanism for α -receptormediated, cyclic AMP-independent control of phosphofructokinase would complement the observation by Keely et al.⁶ of an α -mediated glucose transport system in rat heart.

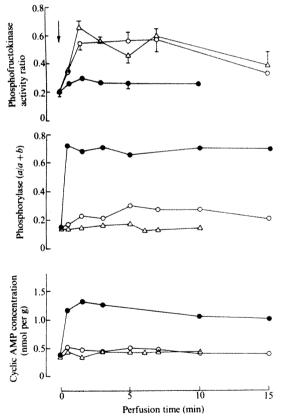


Fig. 2 Time course for the effects of α - and β -agonists on perfused rat heart. Hearts were perfused as described in Fig. 1. At the time indicated (arrow), medium containing 10^{-5} M isoprenaline (\blacksquare), 10^{-5} M naphazoline (△) or 10⁻⁵ M phenylephrine (○) was introduced. Non recirculating perfusion was continued until the hearts were freeze-clamped. The activity ratios of phosphofructokinase and glycogen phosphorylase were determined as described in Fig. 1 and the text. Solutions of agonists were freshly prepared in isotonic saline and mixed with warm (37 °C) oxygenated buffer immediately before introduction into the perfusion apparatus. Cyclic AMP was determined16 on neutralized perchlorate extracts of the frozen heart powders. Where indicated mean values ± s.e.m. have been calculated (bars) from at least three hearts. Other values are the mean of duplicate determinations from single hearts.

Activation of phosphofructokinase in rat heart by α -agonists seems to involve mechanisms quite different from those controlling glucose output in rat liver. Exton and co-workers8 have provided strong evidence that α -receptor stimulation in liver leads to Ca2+ release from intracellular stores. This in turn is believed to activate the Ca2+-dependent phosphorylase kinase that phosphorylates and activates phosphorylase. The present study indicates that activation of phosphorylase and phosphofructokinase are separate, suggesting a unique mechanism for phosphofructokinase activation. Furthermore, any increase in intracellular Ca2+ concentration should enhance contractility. In the present study, the α -agonist naphazoline, which activated phosphofructokinase, had no visable effect on either heart rate or force.

The mechanism by which α -agonsits activate heart muscle phosphofructokinase is unknown. It seems unlikely that changes in activity can be related to changes in the concentration of intracellular effectors1 even though a new activator of the liver enzyme has recently been described^{9,10}. Covalent modification including phosphorylation3 requires serious consideration as the change in activity is stable to gel filtration or dialysis (unpublished observations), providing buffers contain EDTA and sulphydryl reagents.

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- Clark, M. G. & Patten, G. S. J. biol. Chem. 256, 27-30 (1981).
- Clark, M. G. & Patten, G. S. J. molec. cell. Cardiol. 12, 1053-1064 (1980). Riquelme, P. T., Hosey, M. M., Marcus, F. & Kemp, R. G. Biochem. biophys. Res. Commun. 85, 1480-1487 (1978).
- S., 1400-140 (1976). Feliú, J. E., Hue, L. & Hers, H. G. Proc. natn. Acad. Sci. U.S.A. 73, 2762-2766 (1976). Kemp, B. E. & Clark, M. G. J. biol. Chem. 253, 5147-5154 (1978). Keely, S. L., Corbin, J. D. & Lincoln, T. Molec. Pharmac. 13, 965-975 (1977).
- Keely, S. L. Coffell, J. & Ellindon, J. Nobleck, Pharmacological Basis of Therapeutics 4th edn (eds Goodman, L. S. & Gilman, A.) 478-523 (Macmillan, New York, 1970).
 Exton, J. H. Biochem. Pharmac. 28, 2237-2240 (1979).
 Van Shaftingen, E., Hue, L. & Hers, H. G. Biochem. J. 192, 897-901 (1980).
 Claus, T. H., Schlumf, J., Pilkis, J., Johnson, R. A. & Pilkis, S. J. Biochem. biophys. Res.

- Commun.98, 359-366 (1981)
- Neely, J. R., Liebermeister, H., Battersby, E. J. & Morgan, H. E. Am. J. Physiol. 36, 413-459 (1974).
- 12. Hohorst, H. J. in Methods of Enzymatic Analysis (ed. Bergermeyer, H. U.) 134-138 (Academic, New York, 1965).
- (Gilboe, D. P., Larson, K. L. & Nuttall, F. Q. Analyt. Biochem. 47, 20–27 (1972). Birnbaum, M. J. & Fain, J. N. J. biol. Chem. 252, 528–535 (1977).
- 15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265-275
- 16. Gilman, A. G. Proc. natn. Acad. Sci. U.S.A. 67, 305-312 (1970).

Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor

K. Tsuruta, E. A. Frey, C. W. Grewe, T. E. Cote, R. L. Eskay* & J. W. Kebabian†

Experimental Therapeutics Branch, NINCDS, and *Laboratory of Clinical Science, NIMH, National Institutes of Health, Bethesda, Maryland 20205, USA

Available evidence suggests the existence of multiple categories of dopamine receptor¹⁻⁴. According to the classification scheme used here⁵, dopamine receptors can be divided into two general categories, D-1 and D-2. The dopamine receptors in the bovine parathyroid gland⁶⁻⁸ and carp retina^{9,10} exemplify the former category, and the dopamine receptors on the mammotrophs^{11,12} and melanotrophs^{13,14} of the pituitary gland exemplify the latter. Stimulation of a D-1 receptor enhances the formation of cyclic AMP, whereas stimulation of a D-2 receptor does not 4.5. The availability of dopaminergic agonists which specifically activate either a D-1 or a D-2 receptor would facilitate pharmacological investigation of the physiological responses to dopamine; however, to date, an agonist specific for the D-2 receptor has not

[†] To whom correspondence should be addressed.

been available. Dopamine and other catecholamines stimulate both types of receptor⁶⁻¹⁴; ergolines (such as lergotrile and lisuride) stimulate the D-2 receptor¹³⁻¹⁵ but antagonize the D-1 receptor^{8,16-18}; some agonists acting on the D-2 receptor (such as apomorphine) both stimulate and block the D-1 receptor^{6,8}. Although one report claims that S-584 may selectively stimulate the dopamine receptor on mammotrophs¹⁹, according to another, S-584 stimulates the striatal dopamine-sensitive adenylate cyclase²⁰. Several recently synthesized compounds containing various portions of the ergoline ring system stimulate the D-2 receptor on mammotrophs²¹. We report here that one of these ergoline analogues, LY-141865 or '38, R = pro'²¹, stimulates the D-2 receptor in the intermediate lobe (IL) of the rat pituitary gland but does not interact with the D-1 receptor in carp retina.

The melanotrophs of the IL synthesize and secrete α -melanocyte-stimulating hormone (α -MSH). Stimulation of the dopamine receptor in the rat IL inhibits basal release of α -MSH^{13,14}. LY-141865 mimics the ability of dopamine and dopaminergic agonists to diminish the basal release of α -MSH from the IL. Thus, during a 3-h incubation of dispersed cells derived from the neurointermediate lobe, LY-141865 decreased, in a dose-dependent manner, the basal release of α -MSH (Fig. 1 α). Half-maximal inhibition was achieved with \sim 30 nM LY-141865, maximal inhibition with a concentration \geqslant 1 μ M.

The melanotrophs of the rat IL also possess a β -adrenoreceptor, stimulation of which enhances adenylate cyclase activity¹⁵. Stimulation of the dopamine receptor diminishes basal adenylate cyclase activity and inhibits in a non-competitive manner the (-)isoprenaline-stimulated adenylate cyclase activity^{13-15,22}. Like other dopaminergic agonists, LY-141865 diminished the basal adenylate cyclase activity (Fig. 2, inset A) and the (-) isoprenaline-stimulated enzyme activity (Fig. 2 left, and inset B). Half-maximal inhibition of either basal or (-)isoprenaline-stimulated adenylate cyclase activity was achieved with ~30 μM LY-141865; maximal inhibition was achieved at concentrations $\geq 100 \,\mu\text{M}$ (Fig. 2 left, and inset B). Like other dopaminergic agonists, LY-141865 decreased the maximal response to (-)isoprenaline but did not affect the molar potency of (-)isoprenaline. Fluphenazine or (-)sulpiride (dopaminergic antagonists) prevented the inhibitory effects of LY-141865 (Fig. 2 left and insets A and B). Stimulation of the β -adrenoreceptor enhances the release of α -MSH; like other dopaminergic agonists, LY-141865 diminished the ability of B-adrenergic agonists to enhance α -MSH release (Fig. 1b).

To examine the actions of LY-141865 on rat IL adenylate cyclase in the absence of β -adrenergic agonists, the drug was tested on rat IL tissue previously treated with cholera toxin. Adenylate cyclase activity was substantially greater in homogenates of cholera toxin-treated tissue than in homogenates of either fresh or control tissue (Table 1). The dopamine receptor remained functional after treatment of IL tissue with cholera toxin. Both dopamine and apomorphine (a dopaminergic agonist) markedly diminished enzyme activity in homogenates of cholera toxin-treated tissue (Table 2); (-)sulpiride or fluphenazine (dopaminergic antagonists) prevented the inhibitory effect of both dopaminergic agonists. Like dopamine and apomorphine, LY-141865 decreased adenylate cyclase activity in homogenates of cholera toxin-treated tissue (Fig. 2, right); half-maximal inhibition was achieved with 30 µM LY-141865 (Fig. 2, insert C). The inhibitory effect of LY-141865 was attenuated in a dose-dependent manner by spiroperidol or (-)sulpiride; (-)sulpiride was 100-fold more potent than the (+)sulpiride (Fig. 2, right).

To determine whether LY-141865 is a specific D-2 agonist, the compound was tested on the dopamine-sensitive adenylate cyclase of carp retina, a tissue displaying an unusually large (four- to fivefold) dopamine-induced stimulation of adenylate cyclase activity^{9,10}. In this test system, LY-141865 displayed no activity, either as a dopaminergic agonist or as a dopaminergic antagonist (Table 3); lergotrile failed to stimulate adenylate

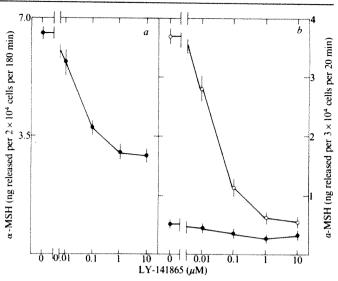


Fig. 1 LY-141865 inhibits release of α -MSH from dispersed neurointermediate lobe cells. a, Incubations were performed for 180 min in the presence of the indicated concentrations of LY-141865 (trans-(\pm)-4,4a,5,67,8,8a,9-octahydro-5-propyl-2H-pyrazolo-(3,4-g)quinoline). b, Incubations were performed for 20 min in the absence (\bigoplus) and the presence (\bigcirc) of $0.1~\mu$ M(-)isoprenaline and the indicated concentrations of LY-141865. Previously described procedures 13 were used for the preparation of dispersed neurointermediate lobe cells, the experimental incubations and the assay of α -MSH.

cyclase activity but diminished the stimulatory effect of dopamine.

These results indicate that LY-141865 discriminates between the D-1 and D-2 receptors. Clearly LY-141865 does not interact with the D-1 receptor: it did not stimulate the D-1 receptor in the carp retina, and unlike lergotrile, LY-141865 did not block this receptor²³. It is also clear that LY-141865 stimulates

Table 1 Cholera toxin increases adenylate cyclase activity in rat IL

Tissue	Adenylate cyclase activity (pmol cyclic AMP per mg protein per min)			
Fresh	43 ± 2			
2 h control	47 ± 21			
2 h + 30 nM cholera toxin	361 ± 29			

IL tissue was dissected and adenylate cyclase activity assayed as described in Fig. 2 legend. Tissue was homogenized and adenylate cyclase activity assayed either immediately after dissection (fresh) or after incubation for 2 h at 37 °C in 5 ml of RPMI 1640 medium (equilibrated with a mixture of 95% O_2 and 5% CO_2) containing 25 mM HEPES, $100~U~ml^{-1}$ penicillin, $100~\mu g~ml^{-1}$ streptomycin, and cholera toxin as indicated. Data represent mean $\pm s.e.m.$ for five replicate homogenates.

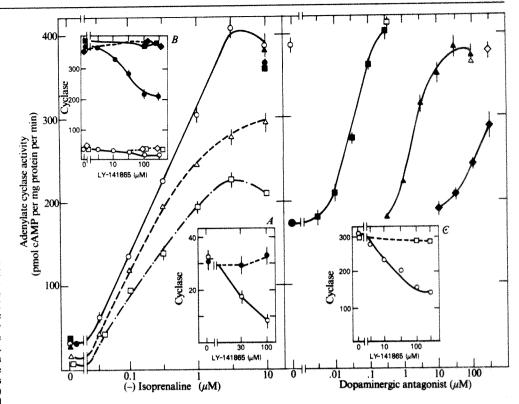
Table 2 Dopaminergic inhibition of adenylate cyclase activity in cholera toxin-treated rat IL

Adenylate cyclase activity

	(pmol cyclic AMP per mg protein					
	Antagonist					
Agonist	None	Fluphenazine (10 µM)	(-)Sulpiride (100 μM)			
None Dopamine (100 μM) Apomorphine (10 μM)	433±14 106±3 150±3	465±10 453±7 490±7	420 ± 4 475 ± 9 500 ± 11			

IL tissue was dissected and incubated for 2 h in the presence of 30 nM cholera toxin as described in Table 1 legend. At the end of the incubation, the tissue was homogenized and adenylate cyclase activity assayed in the presence of the indicated concentrations of test substances, as described in Fig. 2 legend. Data represent mean ±s.e.m. for four replicate samples in a single experiment.

Fig. 2 Left: LY-141865 decreases basal and (-)isoprenaline-stimulated adenylate cyclase activity in rat IL. Adenylate cyclase activity was assayed in the presence of the indicated concentrations of (-)isoprenaline alone (O) or in combination with 30 µM LY-141865 (Δ), 100 μM LY-141865 (□), 10 μM fluphenazine (●), 10 µM fluphenazine and 30 µM LY-141865 (▲) or 10 µM 100 µM fluphenazine and 141865 (). Inset A: Data for basal adenylate cyclase activity are replotted with a larger scale for the ordinate. Adenylate cyclase activity was assayed in the presence of the indicated concentrations of LY-141865 alone (O) or in combination with 10 uM fluphenazine (). Inset B: In a separate experiment, adenylate cyclase activity was assayed in the presence of the indicated concentrations of LY-141865 alone (O) or in combination with 10 µM fluphenazine (\Box), 100 μ M (-)sulpiride (\diamondsuit), 3 μ M (-)isoprenaline (\spadesuit), 3 μ M (-)isoprenaline and 10 μ M fluphenazine (\blacksquare) or 3 µM (-)isoprenaline and 100 µM (-)sulpiride (◆). Right: Dopaminergic antagonists reverse the inhibition by LY-141865 of adenylate cyclase activity in cholera toxin-treated IL tissue. IL tissue was treated with cholera toxin (30 nM, 2 h) as described in Table 1 and adenylate cyclase activity was determined in the absence of drugs (O) or in the presence of the indicated



concentrations of spiroperidol (□), (-)sulpiride (△), (+)sulpiride (⋄), 100 µM LY-141865 (♠) or 100 µM LY-141865 in combination with the indicated concentrations of spiroperidol (□), (-)sulpiride (♠) and (+)sulpiride (♠). Inset C: In a separate experiment, IL tissue was treated with cholera toxin (30 nM, 2 h) and adenylate cyclase activity determined in the presence of the indicated concentrations of LY-141865 alone (O) or in combination with 100 μ M (-)sulpiride (\square). Adenylate cyclase activity was determined using a previously described assay system¹⁵ containing: 80 mM, Tris-HCl (pH 7.4), 1 mM MgSO₄; 0.8 mM EGTA; 10 mM theophylline; 0.25 mM ATP; 0.01 mM GTP; intermediate lobe tissue, 2.5-3.5 μ g of protein; and drugs as indicated in Tables 1 and 2 and Figs 1 and 2. The assay system was incubated for 10 min at 30 °C, and the reaction stopped by boiling for 2 min. Cyclic AMP concentration was determined by the method of Brown et al.27, protein content by the method of Lowry et al.28.

the D-2 dopamine receptor in the rat IL because (1) LY-141865 mimics the ability of dopamine and dopaminergic agonists to decrease basal or (-)isoprenaline-stimulated release of α -MSH; (2) LY-141865 mimics the inhibitory actions of dopamine and dopaminergic agonists on adenylate cyclase activity in homogenates of fresh or cholera toxin-treated tissue; and (3) the inhibitory effects of LY-141865 on adenylate cyclase activity are reversed by dopamine antagonists, including the selective D-2 antagonist, (-)sulpiride²⁴. LY-141865 is substantially more potent on intact cells of the rat IL than in the cell-free adenylate cyclase assay system (compare potency in Figs 1 and 2); the reasons for this discrepancy are unknown. The previous

demonstration²¹ that LY-141865 lowers the level of serum prolactin in reserpine-treated male rats is in accord with the conclusion that LY-141865 is an agonist on the D-2 receptor. The availability of drugs selectively stimulating either the D-1

receptor (such as SKF 38393A)25 or the D-2 receptor (such as

LY-141865) may facilitate pharmacological analysis of the

physiological responses to dopamine. Furthermore, because

Table 3 LY-141865 fails to stimulate adenylate cyclase of fish retina

Adenylate cyclase activity (pmol cyclic AMP per mg protein per min)

m .		rest compound	
Dopamine concentration (µM)	None	LY-141865 (100 μM)	Lergotrile (100 μM)
0 10	$11.1 \pm 0.7 \\ 35.5 \pm 1.7$	9.4 ± 0.8 36.3 ± 2.4	6.2 ± 1.1 12.5 ± 0.7

Retinal tissue was obtained from goldfish purchased at a local pet store. Adenylate cyclase activity was assayed as described in Fig. 2 legend. Data represent mean ±s.e.m. for four replicate samples obtained in a single experiment.

LY-141865 penetrates the blood-brain barrier after its systemic administration²¹, it may be of use in the treatment of parkinsonism (as are the dopaminergic ergots²⁶).

Note added in proof: Euvard et al. (Neuropharmacology 19, 376-386; 1980) describe two other compounds which seem to act as selective agonists on the D-2 receptor.

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- Cools, A. R. & Van Rossum, J. M. Psychopharmcologia 45, 243-254 (1976).
 Titeler, M., Weinreich, P., Sinclair, D. & Seeman, P. Proc. natn. Acad. Sci. U.S.A. 75, 1153-1156 (1978). Costal, B. & Naylor, R. J. Life Sci. 28, 215-229 (1981).
- Costat, B. & Nayot, K. J. Cipe Sci. 22, 127–129 (1975).

 Spano, P. F. et al. in Dopaminergic Ergot Derivatives and Motor Function (eds Fuxe, K. & Calne, D. B.) 159–171 (Pergamon, Oxford, 1979).

 Kebabian, J. W. & Calne, D. B. Nature 277, 93–96 (1979).

 Brown, E. M., Carroll, R. J. & Aurbach, G. D. Proc. natn. Acad. Sci. U.S.A. 71, 1113–1117
- Attie, M. et al. Endocrinology 107, 1776-1781 (1980).
- 8. Brown, E. M. et al. Molec. Pharmac. 18, 335-340 (1980).
 9. Redburn, D., Clement-Cormier, Y. C. & Lam, D. M. K. Life Sci. 27, 23-31 (1980).
- Watling, K. J., Dowling, J. E. & Iversen, L. L. Nature 281, 578-580 (1979).
 MacLeod, R. M. in Frontiers in Neuroendocrinology Vol. 4 (eds Martini, L. & Ganong, W. F.)
- 169-194 (Raven, New York, 1976). Caron, M. G. et al. J. biol. Chem. 253, 2244-2253 (1978).
- Caron, M. G. et al. J. biol. Chem. 253, 2224-223 (1976).
 Munemura, M., Eskay, R. L. & Kebabian, J. W. Endocrinology 106, 1795-1803 (1980).
 Munemura, M. et al. Endocrinology 107, 1676-1683 (1980).
 Cote, T. E. et al. Endocrinology 107, 108-116 (1980).
 Kebabian, J. W., Calne, D. B. & Kebabian, P. R. Commun. Psychopharmac. 1, 311-318

- Schmidt, M. J. & Hill, L. E. Life Sci. 20, 789-798 (1977).
- Pieri, L., Keller, H. H., Burkard, W. & DaPrada, M. Nature 272, 278-280 (1978). Mowles, T. F., Burghardt, B., Burghardt, C., Charnecki, A. & Sheppard, H. Life Sci. 22, 2103-2112 (1978).
- 20. Miller, R. J. & Iversen, L. L. Naunyn-Schmiedebergs Archs Pharmak. 282, 213-216 (1974).
- Bach, N. J. et al. J. med. Chem. 23, 481-491 (1980). Cote, T. E., Grewe, C. W. & Kebabian, J. W. Endocrinology 108, 420-426 (1981)
- Vatling, K. J. & Dowling, J. E. J. Neurochem. 36, 559-568 (1981).
 Spano, P. F., Stefanini, E., Trabucchi, M. & Fresia, P. in Sulpiride and Other Benzamides (eds Spano, P. F. et al.) 11-31 (Raven, New York, 1979).
 Setler, P. E., Sarau, H. M., Zerkle, C. L. & Saunders, H. L. Eur. J. Pharmac. 50, 419-430
- 26. Thorner, M. O., Fluckiger, E. W. & Calne, C. B. Bromocriptine: A Clinical and Pharmacological Review, 1-189 (Raven, New York, 1980). 27. Brown, B. L. et al. Biochem. J. 121, 561-562 (1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265-275 (1951).

Trypanocidal activity of daunorubicin and related compounds

J. Williamson & T. J. Scott-Finnigan

Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA. UK

M. A. Hardman & J. R. Brown*

Department of Pharmaceutical Chemistry, School of Pharmacy, Leicester Polytechnic, PO Box 143, Leicester LE1 9BH, UK

The anthracycline antibiotic daunorubicin is, in vitro, one of the most potent trypanocidal agents reported1; it permanently abolishes the infectivity of African trypanosomes at less than nanomolar concentrations. In contrast, other anthracyclines such as doxorubicin and nogalamycin must be present in millimolar concentrations to achieve the same effect (Table 1). Despite its uniquely high activity in vitro, daunorubicin is totally inactive against trypanosomes in infected rodents1. An investigation of this lack of in vivo activity suggested that uptake into trypanosomes is only transient². We therefore tried to prolong exposure of trypanosomes to the drug by coupling it to a carrier macromolecule. The use of such a carrier should delay removal of the drug from the blood and simultaneously exploit the endocytotic properties of trypanosomes. Ferritin and albumin were chosen as potential carriers as these proteins are endocytosed by trypanosomes3,4. We report here that when the drug was attached by a stable linkage, activity was not maintained, but when attached by a labile covalent linkage the preparation was active both in vitro and in vivo, indicating the desirability of testing other macromolecules, such as dextran⁵, as carriers for trypanocidal drugs.

As none of the daunorubicin analogues tested (see Table 1. and Fig. 1) reproduce the high in vitro trypanocidal activity of daunorubicin, the loss of activity of this drug in vivo must be overcome. We first tested a DNA-daunorubicin complex⁶ but it was inactive. As this product acts as a slow-release form of drug? rather than a true carrier, covalent attachment of drug to possible carriers was then investigated. Bovine serum albumin (BSA) and ferritin were chosen as carriers because of the likelihood of their endocytosis by trypanosomes^{3,4}. The first albumin conjugate was prepared by oxidative cleavage of the amino sugar of the drug to yield an aldehyde which was reacted with the protein to give a Schiff's base—this was then reduced with borohydride⁸. The product was almost inactive (Table 1), possibly due to destruction of the amino sugar unit or to the stability of the linkage. Therefore a second conjugate⁸ was prepared using glutaraldehyde to form Schiff's bases simultaneously with the amino group of daunorubicin and a free amino group of the protein. This product retained activity in vitro (Table 1) and was significantly active in vivo (Table 2). As Schiff's bases are susceptible to hydrolysis, the rate of drug release from the conjugate in aqueous solution (pH 7.4) at 37 °C was monitored (by size-exclusion HPLC); release seemed to be a first-order process with a half life of ~100 h.

The retention of activity only when the carrier-binding linkage is labile suggests that the drug must be released before it can exert its effects. Because of the success of albumin as a carrier, we also prepared ferritin conjugates in which daunorubicin was linked by the glutaraldehyde method⁸. These were also found to have high activity in vitro and in vivo (Tables 1 and 2). These glutaraldehyde-coupled daunorubicin-albumin and daunorubicin-ferritin complexes were the first daunorubicin preparations to clear trypanosomes from the blood of infected mice for an extended period. For 20-30 h after treatment, the proportion of abnormal trypanosomes rose to almost 100%, the highest proportions coinciding with a sharp fall in parasitaemia.

Table 1 In vitro activity of daunorubicin and related compounds against Trypanosoma rhodesiense

	Loss of motility	Loss of in Temporary	nfectivity Permanent
a Daunorubicin and its			
analogues			
(1) Daunorubicin	4	11	10
hydrochloride			
(2) Doxorubicin hydro-	8	>10	4
chloride			
(3) 4-Demethoxydaunorubicin	5	5	<3
hydrochloride			
(4) 4'-Deoxydaunorubicin	5	6	3
hydrochloride			
(5) Marcellomycin	10	10	4
(6) N-Trimethyldaunorubicin	<3	3	<3
chloride			
(7) Doxorubicin-14-stearate	3	<3	3
hydrochloride			
(8) AD32	3	5	<3
(9) Dibenzyldaunorubicin	<3	<3	<3 . 3 . 3 . 4
(10) Dihydrochloride	4	7	. 3
(11) Hydrochloride	8	>9	3
(12) Dihydrochloride	7	7	3
(13) Dihydrochloride	7	.10	4
b Daunorubicin-protein			
complexes			
Daunorubicin-BSA (P)			
(22 μg drug per mg)	4	4	<4
Daunorubicin-BSA (G)			
(18 µg drug per mg)	<4	>10	>10
Daunorubicin-ferritin (G)			
(55 μg drug per mg)	4	10	6
Daunorubicin-ferritin (C)			
(38 µg drug per mg)	<3	>9	4

In these experiments we used the monomorphic Liverpool strain of *T. rhodesiense* ¹¹. For loss of motility and infectivity, the values given are maximum titres (log₁₀ M⁻¹) producing the effect; drugs with titres <3 are considered inactive. The assay method is given elsewhere¹. P denotes that the periodate oxidation coupling method⁸ was used, G that the glutaraldehyde coupling method⁸ was used, and C that the daunorubicin is sequestered in the iron core. Compound sources: 1-4, Dr F. Arcamone; 5, Dr W. T. Bradner; 6, Dr E. M. Acton; 7, was prepared by an adaptation of the method described for doxorubicin-14-octanoate ¹²; 8, Dr M. Israel; 9, The Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI; 10, Dr K. C. Murdock.

Table 2 Activity of daunorubicin-carrier complexes in mice infected with T. rhodesiense

Compound	Dose of drug or drug component (mg per kg)	Proportion of mice cleared of infection
Daunorubicin	30	0/4*
Daunorubicin-DNA	30	0/5*
	(30	7/11*
	15	11/13*
Daunorubicin-BSA (G)	7.5	10/11
	3.8	0/11
	(1.9	0/11
Daunorubicin-ferritin (G)	30	8/10
Daunorubicin-ferritin (G)	15	11/13
Daunorubicin-ferritin (C)	15	0/13

Mice with a patent parasitaemia were given a single intraperitoneal dose of the compound being tested 24-48 h after infection. Control mice were uniformly dead 4-5 days after infection. Generally, the amounts of the conjugates available were small and consequently the numbers of mice used in the tests were limited. G denotes conjugation by the glutaraldehyde method⁸; C denotes non-covalent binding of drug in the ferritin iron core.

^{*} Present address: Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD, UK.

^{*} Some deaths due to drug toxicity at this dose.

	R1	R ²	Rª	R*
(1) Daumorubicin	OCH ₃	н	OH	NH2
(2) Doxorubicin	OCH ₃	ОН	он	NH.
(3) 4-Demethoxydaunorubicin	н	H	ОН	NH ₂
(4)4'-Deoxydaunorubicin	OCII3	Н	H	NH3
(6) N-Trimethyldaunorubicin chloride	OCH ₃	Н	OH	N(CH _b) ₃ C1
(7) Doxorubicin-14-stearate	OCH ₃	OCO(CH ₂) _{1 s} CH ₃	ОН	NH ₃
(8) AD 32	OCH ₃	OCO(CH ₂) ₃ €H ₃	OH	HNCOCF 9
(9) Dibenzyldaunorubicin	осна	H	ОН	N(CH2C6H5) 2

$(10) R^1 = R^2 = NH(CH_2)_2NH$	H ₂ CH ₂ OH	R*	Spe	R*	æ	он		
(11) $R^4 = NHCH(CH_3)(CH_2)_3$	N(CH ₂ CH ₃) ₂	R a	gst	R3	æ	R"	sate:	17
(12) $R^1 = R^3 = NHCH(CH_3)$	CH ₂) ₃ N(CH ₂ CH ₃) ₃	R2	362	R*	520	H		
(13) R1 = R4 = NHCH(CH ₄)(CHa)aN(CHaCHa)a	R a	28	R3	ştir.	Н		

Fig. 1 Structures of the compounds tested for trypanocidal activity.

Fluorescence microscopy showed that the parasites took up the complexed drug progressively for up to 12 h after treatment and retained it thereafter, unlike the transient uptake peaking at 1.5-2 h of the free drug. This altered pattern of uptake suggests that drug is taken up as the drug-protein complex; the pre-

paration thus seems to act as a true endocytotic carrier.

Ferritin contains an iron (Fe³⁺) core surrounded by 24 subunits of apoferritin and can be reconstituted from apoferritin in the presence of Fe3+ or Fe2+ and oxidizing agents9. As daunorubicin forms a chelate with iron, preparation of a daunorubicin-ferritin complex by sequestration of the drug in the iron core of ferritin was attempted by reconstitution of ferritin from apoferritin and Fe²⁺ with a weak oxidizing agent, in the presence of daunorubicin. A high uptake of drug was achieved and despite the non-covalent nature of the complex, the drug was not released on gel chromatography of the complex in 1M NaCl or in 2% SDS in 8M urea; the product was however less active than the covalently bound preparation and showed no in vivo activity (Tables 1 and 2).

The need for new drugs active against African trypanosomiases, both human and animal, is widely recognized 10. Our results clearly demonstrate that daunorubicin has considerable potential as a trypanocide as: (1) it shows unparalleled trypanocidal activity in vitro; (2) it can be conjugated to macromolecular carriers to give soluble complexes which retain activity in vivo; (3) it is structurally distinct from existing trypanocides and so should lack cross-resistance with these drugs; and (4) it has considerable capacity for structural variation.

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- Williamson, J. & Scott-Finnigan, T. J. Antimicrob. Agents Chemother. 13, 735 (1978).
- Brown, J. E., Brown, J. R., & Williamson, J. Pharm. Pharmac. (submitted).
 Brown, K. N., Armstrong, J. A. & Valentine, R. C. Expl Cell Res. 39, 129 (1965).

- Fairlamb, A. H. & Bowman, I. B. R. Expl Parasit. 43, 353 (1977).

 Bernstein, A. et al. J. natn. Cancer Inst. 60, 379 (1978).

 Trouet, A., Deprez-de Campaneere, D., de Smedt-Malengreaux, M. & Atassi, G. Eur. J. Cancer 10, 405 (1974).

- 7. Hulhoven, R. & Harvengt, C. Biomedicine 33, 44 (1980)
- Hurwitz, E., Levy, R., Wilchets, M., Arnon, R. & Sela, M. Cancer Res. 35, 1175 (1975). Crichton, R. Angew. Chem. 12, 57 (1973).
- The African Trypanosomiases Tech. Rep. Ser. 635 @WHO/FAO, Geneva, 1979).
 Yorke, W., Adams, A. R. D. & Murgatroyd, F. Ann. trop. Med. Parasit. 23, 501 (1929).
- 12. Arcamone, F. et al. J. med. Chem. 17, 335 (1974).

Interactions of daunomycin and melanotropin-daunomycin with DNA

Gary Wiesehahn*, Janos M. Varga† & John E. Hearst*

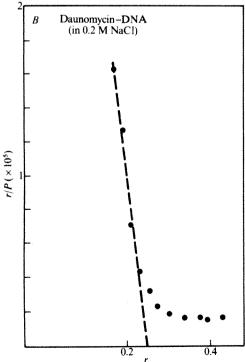
- * Department of Chemistry and Laboratory of Chemical Biodynamics, University of California, Berkeley, California 94720, USA
- † Departments of Biochemistry and Dermatology, Yale Medical School, Yale University, New Haven, Connecticut 06520, USA

The specific recognition of fluorescein-melanotropin and melanotropin-ferritin-fluorescein conjugates by melanotropin (MSH) receptors on the surface of murine melanoma cells suggested the possibility of specific site-directed chemotherapy of melanomas¹⁻³. An MSH-daunomycin conjugate has been shown to be more toxic to mouse melanoma cells than free daunomycin, and, at the same concentration, it was not toxic to mouse 3T3 fibroblast cells. Unconjugated daunomycin was equally toxic to both cell lines4. We report here investigation of the interactions of purified DNA with free daunomycin and with MSH-daunomycin conjugate using spectrophotometry and also an agarose gel assay that detects the unwinding of DNA due to ligand binding. We found an association constant of $4.8\times$ 106 M⁻¹ and an unwinding angle of 10±1.5° for daunomycin, but no detectable binding and an association constant of $<4.8 \times$ 103 M-1 for MSH-daunomycin conjugate. We conclude that the cytotoxicity of MSH-daunomycin is not due to its binding to DNA.

The cytotoxicity of daunomycin is thought to be due to its strong interaction with cellular DNA^{5.6}, thereby interfering with both RNA and DNA synthesis^{7.8}. The experiments described here were designed to determine whether the interaction of daunomycin with DNA is affected by the covalent coupling of daunomycin to MSH. The change in the absorption spectrum of daunomycin as increasing amounts of supercoiled Col El DNA are added is shown in Fig. 1A. With increasing concentration of DNA, the absorption maximum shifts gradually from 480 nm to 505 nm and the absorption of daunomycin decreases towards a limit that represents the spectrum of daunomycin in the fully complexed form. There appears to be an isosbestic point at ~545 nm. Assuming independent binding sites, Scatchard analysis⁹ will result in the following expression:

$$\frac{r}{P} = K_{APP} \times (B_{APP} - r)$$

Following the nomenclature of Bresloff and Crothèrs¹⁰, r is the ratio of the concentration of bound ligand to the total concentration of DNA base pairs, K_{APP} is the apparent association constant for the interaction of DNA and ligand, B_{APP} is the



number of apparent binding sites for ligand per base pair and P the concentration of free ligand.

A Scatchard plot calculated from the spectrophotometric results on daunomycin binding is shown in Fig. 1B. The plot reveals a $K_{\rm APP}$ of $4.8\times10^6\,{\rm M}^{-1}$ and a $B_{\rm APP}$ of 0.24 for the binding of daunomycin to DNA. These values are in reasonable agreement with published values of $6.6\times10^6\,{\rm M}^{-1}$ and 0.36 for daunomycin binding to calf thymus DNA in 0.1 M Tris-HCl (pH 7.0)⁶.

In contrast, Fig. 1C shows that minimal spectral change (only a very slight initial depression in the absorption profile) occurred when Col El DNA was added to the MSH-daunomycin conjugate. Thus no binding was detected by this method.

An additional test for DNA binding is the unwinding assay in which supercoiled DNA is relaxed with nicking-closing enzyme in the presence of compounds that bind DNA. The result is a complex between drug and covalently closed DNA with the DNA in a relaxed conformation. On removal of the drug the DNA becomes superhelical, and the number of superhelical turns can be determined by agarose gel electrophoresis¹¹.

Figure 2A illustrates a comparison of DNA-unwinding assays with daunomycin (lanes a-f) and MSH-daunomycin conjugate (lanes g-l) at comparable drug/DNA ratios. Daunomycin clearly has a large effect on the superhelical density of DNA,

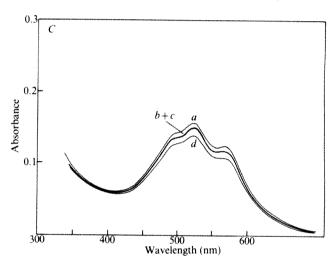
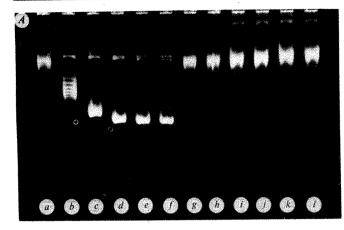


Fig. 1 A, effect of supercoiled colicin El (Col El) plasmid DNA. isolated from E. coli JC 411 thy (Col El), on the absorption spectrum of daunomycin. The incubation mixture contained 2.89 × 10⁻⁵ M daunomycin in 0.04 M Tris-HCl, pH 8.0, 5 mM NaOAc, 0.5 mM EDTA, 0.2 M NaCl. Absorbance was measured using a 1-cm path length quartz cuvette in a Cary 118 spectrophotometer. at the following DNA concentrations (M): curve a, zero; b, 2.57×10^{-5} ; c, 1.03×10^{-4} ; d, 2.14×10^{-4} . These concentrations are in moles of DNA base pairs per litre. B, Scatchard plot of the data in A, plotted according to the method of Muller and Crothers¹ determined the ratio of free to bound daunomycin at intermediate points by assuming the absorption spectrum is a linear combination of the free and fully complexed daunomycin spectra. The absorbance at 470 nm was used for this calculation as at this wavelength the difference between free and bound daunomycin is greatest. For example, we assumed that the ratio of free to bound daunomycin is 2:1 when the absorption at 470 nm is one-third of the way from the absorption of free daunomycin to the absorption of fully complexed daunomycin. Knowing the total concentration of daunomycin, it is then simple to calculate the concentrations of free and bound daunomycin. The absorption data were corrected for the dilution due to addition of DNA solution. C, effect of supercoiled Col El DNA on the absorption spectrum of MSHdaunomycin conjugate. The incubation mixture contained 2.11 × M conjugate in the buffer described in A. The absorbance was measured at the following DNA concentrations (M): curve a, 0; b, 1.03×10^{-5} ; c, 2.57×10^{-5} ; d, 3.48×10^{-4} . The slight depression in the absorbance spectrum is due to dilution of the conjugate on addition of the DNA. The conjugate was prepared as described by Varga et al.4.



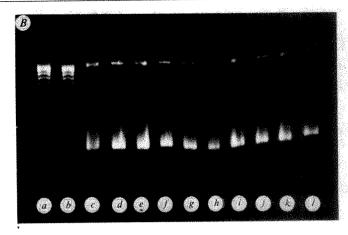


Fig. 2 A, agarose slab gel of Col El DNA $(4.3 \times 10^{-5} \text{ M})$ relaxed with nicking-closing enzyme in the presence of increasing amounts of daunomycin (lanes a-f) or MSH-daunomycin conjugate (lanes g-l). Lanes: a, no daunomycin present; b, 2.05×10^{-6} M; c, 4.10×10^{-6} M; d, 6.14×10^{-6} M; e, 8.19×10^{-6} M; f, 1.02×10^{-5} M; g, no conjugate present; h, 1.49×10^{-6} M; f, 1.02×10^{-6} M; f, 1.02reacted with nicking-closing enzyme in the presence of increasing amounts of daunomycin-MSH conjugate. Lanes, a, no conjugate present; b, 7.50×10^{-6} M; c, 2.25×10^{-5} M; d, 3.75×10^{-5} M; e, 5.25×10^{-5} M; f, 6.75×10^{-5} M; g, 8.25×10^{-5} M; h, 9.75×10^{-6} M; i, 1.12×10^{-4} M; j, 1.28×10^{-4} M; k, 1.42×10^{-4} M; l, 1.58×10^{-4} M. If we assume that the binding of one molecule of conjugate would unwind DNA by 10° as does the binding of unconjugated daunomycin, then the binding of 1% of the conjugate molecules (at a base pair/conjugate ratio of 1:1) would cause about a two-band shift in the DNA distribution on the agarose gel $(64 \times 10^{\circ} = 640^{\circ})$ or 1.8 superhelical turns). This two-band shift should be easily detected on the gel. As no shift was observed, we conclude that the ratio of bound to unbound conjugate is <0.01. This ratio can be compared with the ratio of 12 bound daunomycin molecules per unbound daunomycin molecule calculated from the Scatchard equation at low values of r. Thus, the bound/unbound ratio for the conjugate is at least three orders of magnitude less than for daunomycin, and $K_{APP} < 4.8 \times 10^3 \,\mathrm{M}^{-1}$ for the conjugate.

whereas the conjugate has no detectable effect. Using the binding constant $4.8 \times 10^6 \,\mathrm{M}^{-1}$ and assays such as are shown in Fig. 2A, we have determined an unwinding angle of $10 \pm 1.5^{\circ}$ for each bound daunomycin molecule following the method previously described¹². This again agrees with the value of $12 \pm 3.3^{\circ}$ (corrected to an ethidium unwinding angle of 28°) published previously by Waring¹²

As shown in Fig. 2B, increasing the concentration of MSHdaunomycin conjugate in an attempt to detect low-level binding to DNA does not result in the production of a population of DNA molecules of intermediate superhelical density as in lanes b and c of Fig. 2A. It results instead in inhibition of nickingclosing activity (lanes c-l, Fig. 2B). The highest concentration of MSH-daunomycin conjugate that produced fully relaxed Col El DNA corresponded to one conjugate molecule per base pair (~6.400 conjugate molecules per Col El DNA molecule). The inhibition of nicking-closing enzyme by MSH-daunomycin conjugate seems to be due to an interaction between the nicking-closing enzyme and the conjugate because increased concentrations of enzyme require increased concentrations of conjugate for inhibition. This inhibition seems to be mainly due to MSH and occurs at rather high concentrations of conjugate (50 μM) (data to be published elsewhere).

We could not detect any binding of MSH-daunomycin conjugate to DNA which results in a shift of the absorption spectrum of daunomycin nor any which distorts the DNA helix enough to be detected in a DNA-unwinding assay. We conclude from the DNA-unwinding assay that the association constant for binding of the conjugate to DNA is $<4.8\times10^3$ M⁻¹. This lack of binding is consistent with the observation of Zunino et al.6, who showed that the N-acetylation of the sugar ring of daunomycin reduces the affinity for DNA by more than two orders of magnitude. The addition of the large MSH molecule to the split sugar ring (which also results in the removal of the primary amino group) might be expected to decrease the affinity of daunomycin to DNA even more.

The killing of mouse melanoma cells by melanotropindaunomycin conjugate must therefore be explained by a mechanism other than direct binding of the conjugate to DNA. It has recently been shown that adriamycin, a structural analogue of daunomycin, perturbs the cell membrane by lowering the temperature of gel-to-liquid transition¹³. Thus the site of

action of melanotropin-daunomycin might be the cell membrane. However, the effects of transglutaminase inhibitors on the toxicity of the conjugate strongly suggest an effect on internalization. It has been demonstrated that inhibitors of transglutaminase inhibit receptor-mediated endocytosis of polypeptide hormones¹⁴. If endocytosis of melanotropindaunomycin is essential for toxicity, we could expect that inhibitors of transglutaminase would prevent the toxic effects of the conjugate. We have, indeed, found that ammonium acetate (1 mM), methylamine (0.1 mM) and chloroquine (10⁻⁷ M) all reduce the toxic effects of MSH-daunomycin by about 50 % 15 As direct binding of the conjugate to DNA does not explain its toxic effect, we must therefore consider two alternative explanations—that the free and conjugated toxins kill the cells by different mechanisms, or that the internalized MSHdaunomycin is metabolized to a form that can bind to DNA and thereby interfere with transcription and replication. We think the second mechanism more likely.

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- 1. Varga, J. M., DiPasquale, A., Pawelek, J., McGuire, I. S. & Lerner, A. B. Proc. natn. Acad. Sci. U.S.A. 71, 1590-1593 (1974). Varga, J. M., Moeilmann, G., Fritsch, P., Godawska, E. & Lerner, A. B. Proc. natn. Acad.
- Sci. U.S.A. 73, 559-562 (1976).
- Lerner, A. B., Moellmann, G., Varga, J. M., Halaban, R. & Pawelck, J. Cold Spring Harb Conf. on Cell Proliferation 6, 187-197 (1979). Varga, J. M., Asato, N., Lande, S. & Lerner, A. B. Nature 267, 56-58 (1977):
- Penco, S. Chimica Ind., S. Paulo 50, 908 (1968)
- Zunino, F., Gambetta, R., DiMarco, A. & Zaccara, A. Biochim. biophys. Acta 277, 489-498 (1972)
- Ward, D. C., Reich, E. & Goldberg, I. H. Science 149, 1259-1263 (1965)
- Hartmann, G., Goller, H., Koschel, K., Kersten, W. & Kersten, H. Biochem. Z. 34%,
- Scatchard, G. Ann. N. Y. Acad. Sci. 51, 660-672 (1949).
- Bresloff, J. L. & Crothers, D. M. J. molec. Biol. 95, 103-123 (1975)
- Wiesehahn, G. & Hearst, J. E. Proc. natn. Acad. Sci. U.S.A. 75, 2703-2707 (1978). Waring, M. J. molec. Biol. 54, 247-279 (1970).
- Tritton, T. R., Murphree, S. A. & Sartorelli, A. C. Biochem. biophys. Res. Commun. 84,
- Davies, P. J. A. et al. Nature 283, 162-167 (1980). Varga, J. M., Asato, N., Wiesehahn, G. & Hearst, J. E. Pigment Cell 6 (in the press).
- 16. Muller, W. & Crothers, D. M. J. molec. Biol. 35, 251-290 (1969)

Enteropancreatic circulation of digestive enzymes does not exist in the rat

Gerhard Rohr*, Horst Kern† & George Scheele‡

* Medizinische Universitätsklinik and † Institut für Anatomie und Zellbiologie, Universität, D-3550 Marburg/Lahn, FRG ‡ Department of Cell Biology, Rockefeller University, New York, New York 10021, USA

The hypothesis of an enteropancreatic circulation 1-3, that digestive enzymes are absorbed intact across the intestinal epithelium, are circulated through the bloodstream and resecreted by the exocrine pancreas, has generated considerable discussion4-6 and has been accepted by a number of medical scientists⁷⁻⁹. However, the data presented in support of this hypothesis do not demonstrate with certainty that labelled proteins introduced into the bathing medium^{1,3}, intestinal tract^{1,2} or bloodstream² appear unmodified in pancreatic juice. We have now critically examined that part of the hypothesis which proposes that pancreatic secretory proteins are taken up from the bloodstream and secreted intact into the pancreatic juice. Injection of ³⁵S-methionine-labelled rat pancreatic amylase into the blood circulation of unanaesthetized rats resulted in the appearance of radioactivity in all exocrine pancreatic proteins and showed a distribution which was indistinguishable from that following the intravenous administration S-methionine. These findings are not consistent with the direct transport of amylase from the blood circulation to the pancreatic ductal lumen, but are consistent with metabolic degradation of the polypeptide probe and reutilization of the free 35S-methionine for the synthesis and secretion of the entire complement of pancreatic exocrine proteins. In contrast, analysis of bile by similar methods indicated the presence of a haematobiliary pathway, which accounted for the excretion of 0.8% of exocrine pancreatic proteins injected into the blood circulation.

35S-methionine-labelled rat pancreatic amylase was injected into the blood circulation of nonanaesthetized rats and the appearance of radioactivity in pure pancreatic juice was followed with time and further analysed by two-dimensional isoelectric focusing/SDS-gel electrophoresis followed by fluorography. Rats (250-300 g) were prepared for study as follows. The pancreatic duct was cannulated with a 0.6-mm PVC catheter, fixed with a surgical ligature and routed subcutaneously to the nape of the neck. Bile flow into the intestinal tract was re-established during the surgical procedure using a short cannula of the same size. After the operation rats were maintained in restraining cages, which allowed exercise and free access to food and water. Rats were studied 6-18 h after the operation when pancreatic secretion rates were stable, usually between 450 and 500 µl h⁻¹. As the control, ³⁵S-methionine was injected into the tail vein of nonanaesthetized rats.

Trichloroacetic acid (TCA)-insoluble radioactivity first appeared in pancreatic juice 1.5 h after the injection of ³⁵S-labelled amylase (Fig. 1). Radioactivity increased slowly thereafter and peaked at 6 h. In contrast, after the injection of ³⁵S-methionine, TCA-insoluble radioactivity appeared in pancreatic juice within 30 min and peaked at 2 h. In both conditions, we observed no difference between TCA-insoluble and total radioactivity, indicating that free methionine was not released into the ductal fluid. These findings clearly differ from those of previous investigators who claimed that pancreatic proteins were transported from the interstitial space of the exocrine pancreas to the duct lumen within 15 min.

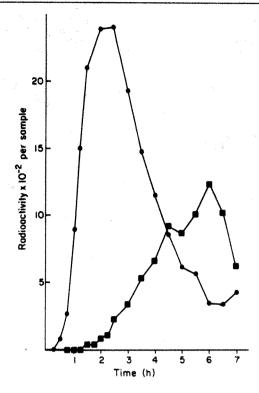
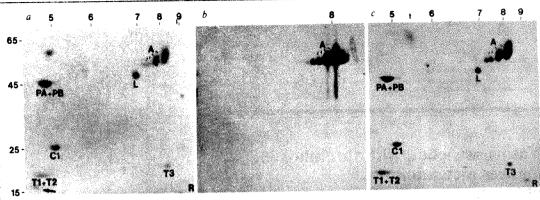


Fig. 1 Appearance of radioactivity in pancreatic juice as a function of time after intravenous administration of ³⁵S-methionine-labelled rat pancreatic amylase (2×10⁶ c.p.m.) compared with ³⁵S-methionine (1.5×10⁷ c.p.m.). TCA-insoluble radioactivity is shown on the ordinate for 100-μl samples collected after the injection of amylase (■) or for 10-μl samples after the injection of ³⁵S-methionine (●). Radiolabelled amylase was prepared as follows. Exocrine pancreatic proteins contained in rat pancreatic lobules were labelled with ³⁵S-methionine for 3 h as previously described for the dog pancreas ¹². A crude zymogen granule fraction was isolated by differential sedimentation and proteins contained within it were liberated by treatment with 0.2 M NaHCO₃, pH 8.5. ³⁵S-methionine-labelled amylase was precipitated with shellfish glycogen by the procedure of Schramm and Loyter ¹³. The glycogen amylase precipitate was washed twice as described previously ¹¹ and solubilized in normal saline.

Radioactive protein(s) injected into the blood circulation and those appearing in pure pancreatic juice were separated by two-dimensional isoelectric focusing/SDS-gel electrophoresis using the procedure of Bieger and Scheele 10 and analysed by Coomassie blue staining and fluorography. Figure 2a shows the Coomassie blue-staining pattern of proteins found in rat pancreatic juice. Most of these proteins have been identified according to actual or potential enzyme activity (J. Schick, H. Kern and G. Scheele, unpublished observations) by methods previously described for the guinea pig pancreas¹¹. Figure 2b shows the distribution of radioactive proteins injected into the blood circulation and confirms that the injected material contained exclusively ³⁵S-methionine-labelled rat pancreatic amylase. Figure 2c shows that radioactivity is distributed among all exocrine proteins in pancreatic juice at the 6-h time point (maximal radioactivity). After injection of 35S-methionine, pancreatic juice showed a similar distribution of radioactive proteins as judged by both fluorography and liquid scintillation spectroscopy following excision of Coomassie blue-stained spots and solubilization of the acrylamide pieces (data not shown). Samples of pancreatic juice collected earlier, 3-6 h after the injection of radiolabelled amylase, also showed a similar distribution of radioactivity among all secreted proteins.

The per cent radioactivity precipitated with shellfish glycogen allowed us to quantify the distribution of amylase among

Fig. 2 Analysis of proteins injected into the blood circulation or collected in 65 pancreatic juice by twodimensional isoelectric focusing/SDS-gel electrophoresis. a Shows the blue-staining Coomassie of proteins pattern contained in rat pancreatic juice; b shows a fluorograph of 35S-methionine-labelled 25 rat pancreatic proteins precipitated with shellfish glycogen and subsequently injected into the blood



circulation; and c shows a fluorograph of proteins contained in pancreatic juice 6 h after injection of the radiolabelled material presented in b. After intravenous administration of 35 S-methionine, the distribution of radioactive proteins secreted into the pancreatic juice showed a fluorographic pattern similar to that observed in c. Each of the gels in panels a-c were impregnated with diphenylexazole as required for fluorography. Proteins are labelled according to their actual or potential enzyme activities: A, amylase; L, lipase; PA, procarboxypeptidase A; PB, procarboxypeptidase B; C, chymotrypsinogen; T, trypsinogen; and R, ribonuclease. The arrow in a identifies soybean trypsin inhibitor added to the samples of pancreatic juice to prevent autoactivation of pancreatic proteins during their analysis by the two-dimensional gel procedure. The upper abscissae give the isoelectric points of proteins; the left ordinate gives the apparent molecular weight ×10⁻²

radioactive proteins injected into the blood circulation and those secreted into pancreatic juice collected between 2 and 7 h during the experiment. Although the radioactivity injected was exclusively represented by amylase (99.0%), only 30.7% of the radioactivity which subsequently appeared in pancreatic juice was represented by this enzyme. This contribution by amylase was identical to that observed in a crude zymogen granule fraction after labelling of rat pancreatic lobules with 3 methionine (29.9%). As the distribution of radioactivity after injection of 35S-labelled amylase was indistinguishable from that resulting from injection of 35S-methionine, we conclude that the amylase injected into the blood circulation was taken up by tissues in the rat and degraded to individual amino acids and that the resulting free ³⁵S-methionine, circulated through the

7 8 65 45 25 15

Fig. 3 Analysis of proteins contained in bile by two-dimensional isoelectric focusing/SDS-gel electrophoresis. Rat pancreatic proteins were labelled with ³⁵S-methionine as described in Fig. 1 legend. After injection of the entire complement of radioactive proteins into the blood circulation, bile samples were collected at 5-min intervals. a Shows the Coomassie blue-staining pattern and b the fluorographic pattern of proteins contained in the bile sample collected between 20 and 30 min. Radioactive pancreatic proteins observed in bile are labelled by abbreviations described in Fig. 2 legend. The radioactive protein with an isoelectric point of 4.8 and M_r (molecular weight) ~72,000 in b represents an acidic glycoprotein normally found in low levels in rat pancreatic secretion. The upper abscissae give the isoelectric points of proteins and the left ordinate gives the apparent molecular weight ×10-

bloodstream, was used for synthesis of the entire complement of secretory proteins by the exocrine pancreas. No degradation was observed when 35S-labelled amylase was incubated with rat serum at 37 °C for 7 h. Presumably the bulk of amylase was taken up by the reticuloendothelial system because rat pancreatic amylase contains no attached carbohydrate. These findings indicate that rat pancreatic amylase is not directly transported from the blood circulation to the pancreatic juice. We therefore conclude that this component of the proposed enteropancreatic circulation for digestive enzymes does not exist.

Other studies strongly support our conclusions. Injection of 35S-methionine-labelled guinea pig pancreatic proteins into the blood circulation of the rat resulted in the appearance of TCAinsoluble radioactivity in pancreatic juice after I h. Separation of proteins contained in pancreatic juice by the two-dimensional gel procedure, which discriminates guinea pig from rat exocrine pancreatic proteins, followed by fluorography, indicated that radioactivity was exclusively associated with rat proteins. The sensitivity of our analysis using the methods described would have allowed the detection of 0.002% of radioactive guinea pig pancreatic proteins.

In contrast with our studies analysing pancreatic juice, the analysis of bile after injection of pancreatic proteins into the blood circulation indicated that 35S-labelled rat pancreatic proteins appeared rapidly, within 5 min, in biliary fluid. Figure 3 compares the Coomassie blue and fluorographic pattern of proteins contained in a sample of bile collected 20-30 min after injection of radioactive proteins into the blood circulation. Pancreatic proteins, which appeared in bile, represented 0.8% of the injected radioactivity and the appearance of negatively charged proteins was favoured over positively charged proteins (compare Figs 3b and 2c). These observations indicate not only the presence of a haematobiliary pathway for exocrine pancreatic proteins, but provide a powerful positive control for our studies into the possible existence of a haematopancreatic pathway. Taken together, our data convincingly demonstrate, to the level of 0.002%, that this latter putative pathway, at least in the rat, does not exist.

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- Liebow, C. & Rothman, S. S. Science 189, 472-474 (1975).
- Götze, H. & Rothman, S. S. Nature 257, 607-609 (1975). Isenman, L. D. & Rothman, S. S. Biochim. biophys. Acta 585, 321-332 (1979)
- Beynon, R. J. & Kay, J. Nature 260, 78-79 (1976). Rothman, S. S., Götze, H. & Liebow, C. Nature 260, 79 (1976).
- Diamond, J. M. Nature 271, 111-112 (1978).

- Heinrich, H. C., Gabbe, E. E., Brüggmann, J. & Icagic, F. Klin. Wschr. 57, 1295-97 (1979). Lake-Bakaar, G., Rubio, C. E., McKavaragh, S., Potter, B. J. & Summerfield, J. A. Clin. Sci. molec. Med. 56, 18 (1979); Gut 21, 580-586 (1980).
- 9. Harper, A. & Scratcherd, T. in The Exocrine Pancreas (eds Howat, H. T. & Sarles, H.) 63 (Saunders, London, 1979).
- Bieger, W. & Scheele, G. Analyt. Biochem. 109, 222-230 (1980).
 Scheele, G. J. biol. Chem. 250, 5375-5385 (1975).
- Scheele, G. A., Jacoby, R. & Carne, T. J. Cell Biol. 87, 611-628 (1980).
- 13. Schramm, M. & Loyter, D. Meth. Enzym. 8, 533-537 (1966)

Vasoconstriction with thromboxane A. induces ulceration of the gastric mucosa

B. J. R. Whittle, G. L. Kauffman & S. Moncada

Department of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK

Thromboxane A₂ (TXA₂) is formed from the fatty acid precursor, arachidonic acid (AA), by the sequential actions of the enzymes cyclooxygenase and thromboxane synthetase1. It is readily generated by platelets and is a potent vasoconstrictor and inducer of platelet aggregation 1,2. Studies in dogs show that the addition of AA to blood flowing through an incubation coil leads to production of TXA2, characterized by its instability and its ability to contract isolated strips of vascular tissue3. We now report that the formation of TXA2 from AA in blood can be inhibited by 1-benzylimidazole, a thromboxane synthetase inhibitor, and selectively by very low doses of indomethacin. In conditions of TXA2 inhibition, a vasodilator AA metabolite, presumably prostacyclin, is formed in the stomach. We show that TXA2 is a powerful gastric vasoconstrictor and can extensively damage the gastric mucosa, and suggest that it may be involved in the pathogenesis of certain ulcerative disorders of

Segments of acid-secreting fundic mucosa from the stomachs of pentobarbitone-anaesthetized dogs were encased in a lucite chamber as described by others⁴. The branches of the splenic artery and vein, which supplied the mucosal flap, were isolated, and all other vascular connections supplying the greater curvature of the stomach and the spleen were ligated. Each dog was heparinized (500 IU per kg), and the splenic artery was cannulated close to the mucosal flap and perfused (10 ml min⁻¹) with blood from a cannulated femoral artery with a constant flow roller-pump (Watson Marlow). Changes in perfusion pressure, indicating vascular resistance, were recorded with a transducer connected to the arterial blood line close to the stomach. Resting perfusion pressure was 80-100 mm Hg. Injection ports were sited in the arterial blood line, either close to the stomach (with a 3-s delay before reaching the stomach) or distally into a delay coil (allowing 30-s incubation in blood before the injected substances reached the stomach). Mean arterial blood pressure was measured via a cannula in a femoral artery and drugs could be administered intravenously (i.v.) through a cannula in a

Bolus injection of AA (25-200 µg intra-arterially (i.a.)) into the 30-s incubation coil produced a dose-dependent rise in gastric perfusion pressure, with 100 µg AA producing a nearmaximal increase of 56±9 mm Hg (mean±s.e.m. mean; 13 animals). This vasoconstriction was accompanied by localized blanching in the superficial mucosa and increased motility of the tissue within the chamber.

We investigated whether the increase in perfusion pressure induced by AA could be due to platelet aggregates blocking the microcirculation. Although some workers have shown that dog platelets do not aggregate when they form TXA2 from AA5

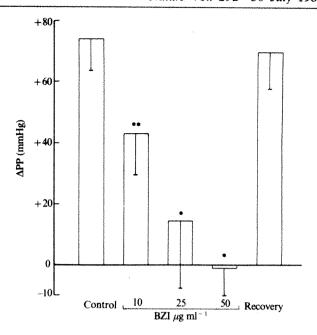


Fig. 1 Vasoconstriction in the canine gastric circulation by TXA₂ generated from arachidonic acid in blood, and its inhibition by 1-benzylimidazole in five dogs. AA (Grade I; Sigma) was stored in *n*-hexane (10 mg ml^{-1}) at $-20 \,^{\circ}$ C. After evaporation of the *n*hexane with N₂ from 1-ml aliquots, sodium hydroxide (0.5 M in methanol, 0.5 ml) was added and subsequently evaporated to dryness. The residue was dissolved (10 mg ml⁻¹) in 1 M Tris buffer (pH 10.5, 4°C), and stored in a light-proof container on ice. Control' represents response to AA (100 µg in 10-µl volume) injected i.a. into a delay coil so as to incubate with blood for 30 s before reaching the stomach. BZI (10-50 µg ml⁻¹ final concentration in blood) was infused i.a. (0.1 ml min) so as to incubate with blood 30 s before reaching injection site for AA. Results, shown as change in gastric perfusion pressure (ΔPP), are mean \pm s.e.m. of five experiments, where level of statistical difference from control (using paired data t-test) is shown as: *, P<0.05; **, P < 0.01.

infusions of very high doses of AA i.a. to the canine stomach, which elevate perfusion pressure⁷, may lead to thrombotic occlusion of the gastro-epiploic artery6. We have determined gastric arterio-venous (AV) differences in platelet counts in plasma after AA administration. Samples (2 ml) of blood collected in trisodium citrate (0.32%) were rapidly centrifuged in a modified Eppendorf bench centrifuge8 and the platelet count in the plasma determined in a Coulter counter. In four dogs, the AV platelet count after i.a. administration of 100 µg AA into the delay coil was not significantly (P > 0.05) different from that obtained without AA in control conditions, indicating that platelet aggregation with microemboli plugging of the gastric microcirculation is not a major factor in this vasoconstriction.

1-Benzylimidazole (BZI), like imidazole², is a selective inhibitor of TXA₂ formation in both human⁹ and dog¹⁰ platelets. To investigate its action, the hydrogen fumarate salt of BZI was dissolved in isotonic saline and infused into the delay coil so that it mixed with the blood for 30 s before reaching the AA injection site (thus incubating for 1 min in blood before reaching the stomach). In five dogs, BZI infusion (10-50 µg ml⁻¹, final concentration in the blood) alone had no effect on resting perfusion pressure in the gastric circulation. At 3 min after starting the infusion of BZI (10-50 µg ml⁻¹), the vasoconstrictor response to AA (100 µg into the delay coil) was dose-dependently reduced, presumably reflecting inhibition of TXA2 formation (Fig. 1). The BZI concentration reducing the vasoconstrictor response by 50% (13 µg ml⁻¹; 50 µM) is similar to that described for the inhibition of thromboxane formation from

Table 1 Effect of topical application for 1 h of acid (100 mM HCl) or acid (100 mM) plus sodium taurocholate (5 mM) either alone or during TXA₂-induced gastric vasoconstriction

	Anid	Acid+AA	Acid+Tauro	Acid+Tauro+AA
p.d. (mV) ΔH ⁺ (μmol) Lesion area (mm ²)	Acid -60 ± 3 -234 ± 202 0	-57±6 -8±239	$ \begin{array}{r} -44 \pm 3^{*} \\ -837 \pm 224^{*} \\ 25 \pm 11^{*} \end{array} $	$-43 \pm 2^{**}$ -403 ± 240 $278 \pm 55^{**}$ 4
n	- T	•		

AA was infused i.a. $(5 \,\mu\text{g ml}^{-1} \,\text{or}\, 50 \,\mu\text{g min}^{-1})$ into a 30-s incubation coil to generate TXA₂ for the latter 30 min of the study period. Results, which show the p.d., acid back-diffusion (ΔH^+) and lesion area of gastric mucosa over 1 h, are mean \pm s.e.m. of values from n degs. Significant differences from acid-alone group is shown by *, P < 0.05; **, P < 0.01.

 14 C-AA by canine platelets in vitro 10 . Infusion of BZI into the injection port close to the stomach failed to prevent the vaso-constrictor response to AA, indicating that the generation of TXA₂ was primarily in the blood and not in the stomach (Fig. 2). With 50 μg ml $^{-1}$ of BZI in the delay coil, a net vasodilator response to AA was observed in three of five experiments, suggesting that reduced TXA₂ formation in blood can unmask the biotransformation of AA to a metabolite, presumably prostacyclin 11 , which is a vasodilator in the stomach (Fig. 2). Indeed, even in the absence of BZI, close arterial administration of AA (100 μg), with only a 3-s delay before reaching the stomach, significantly (P < 0.01) reduced perfusion pressure (-23 ± 8 mm Hg, n = 8).

Within 3 min of terminating the BZI infusion to the incubation coil, the vasoconstrictor response to AA had returned to control values (Figs 1, 2). This system is thus useful for studying locally acting drugs without treatment of the whole animal.

The effect of a systemically administered cyclooxygenase inhibitor12 was also studied in six dogs. A low dose of indomethacin (25 µg per kg intravenously (i.v.)), given 5 min before injection of AA into the 30-s delay coil, significantly (P < 0.05) reduced the vasoconstrictor response (Fig. 3). With higher doses of indomethacin (50-500 µg per kg, i.v.) the vasoconstrictor response was converted into a vasodilator response, whereas with 1-5 mg per kg, i.v., indomethacin abolished all the vasoactive effects of AA. We conclude that low doses of indomethacin selectively inhibit the cyclooxygenase in platelets, thus reducing the formation of the vasoconstrictor, TXA2, while cyclooxygenase in the stomach retains its ability to transform AA into the vasodilator metabolite, prostacyclin. At higher doses, the cyclooxygenase in the stomach also becomes inhibited, with subsequent abolition of the vasodilator response. Such differential actions may explain the small vasodilation in

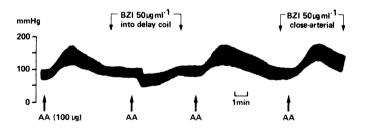


Fig. 2 Vasoconstriction in the canine gastric circulation induced by TXA₂, generated from AA (100 μ g in 10 μ l volume) injected into a 30-s delay coil. In this experiment, the vasoconstrictor response was converted to a vasodilator response when 1-benzylimidazole (BZI, 50 μ g ml⁻¹ final concentration in blood) was infused (0.1 ml min⁻¹) into the delay coil. BZI, infused locally to the stomach, failed to prevent the vasoconstrictor response to AA, indicating that TXA₂ was being generated in blood and not in the stomach.

the canine gastric circulation with infusion of high doses of AA after indomethacin pretreatment⁷.

As discussed previously for aspirin¹³, our present results in the dog suggest that indomethacin can selectively inhibit platelet cyclooxygenase *in vivo*. Whether such differential sensitivity would be of clinical value for the inhibition of platelet aggregation must await further studies as the more chronic administration of low doses of indomethacin may inhibit cyclooxygenase in most tissues. Previous studies with indomethacin at anti-inflammatory doses in rats did not reveal any selectivity between inhibitory effects on prostaglandin production in the inflammatory exudate and in the gastric mucosa (unlike sodium salicylate and the anti-inflammatory compound, BW755C) while the

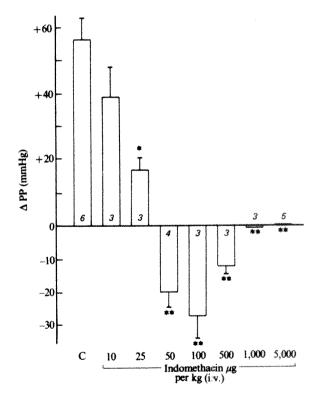


Fig. 3 Effect of indomethacin (10-5,000 μ g per kg) administered i.v. on the gastric vasoactive actions of AA (100 μ g; injected i.a. into a delay coil, so as to incubate with blood for 30 s before reaching the stomach). Indomethacin was dissolved in 5% w/v sodium bicarbonate solution, and the stock (20 mg ml⁻¹) diluted to desired concentration. Indomethacin in progressively increasing doses reduced the control (C) vasoconstriction with TXA2 generated from AA, and converted it into a vasodilator response, and subsequently abolished all vasoactive responses. Results, shown as increase or decrease in gastric perfusion pressure (Δ PP), are the mean \pm s.e.m. Number of animals used is shown in each bar.

* P < 0.02; **, P < 0.001.

inhibition of gastric cyclooxygenase was correlated with the production of gastric erosions¹

As mucosal ischaemia may be involved in the pathogenesis of gastric ulceration, we investigated whether the gastric vasoconstrictor actions of TXA2 could induce or enhance gastric damage. Although vasoconstriction induced in dogs by vasopressin alone does not induce marked erosion, it does potentiate the damage from topical application of a low concentration of bile salts4. We have therefore compared the gastric lesions after $1\ h$ topical application of acid (100 mM HCl) or acid plus the bile salt (100 mM HCl, 5 mM taurocholate) both alone, and during generation of the TXA₂ by infusion of AA (50 µg min⁻¹; 5 µg ml⁻¹ final concentration in blood) into the 30-s delay coil. This infusion produced a sustained increase in gastric perfusion pressure of 56 ± 7 mm Hg (n = 10), and is comparable with the AA concentration which generated TXA2 in dog blood, as detected by bioassay on vascular tissues3. In a randomized experiment in eight dogs, the acid back-diffusion (measured by titration) and electrical potential difference (p.d.) across the mucosa, indices of mucosal permeability15, were also determined in a two-compartment chamber.

Topical application of acid plus taurocholate for 1 h significantly increased acid loss and lowered p.d., but caused only slight gastric mucosal damage (measured in terms of lesion area with calipers) as shown in Table 1. Vasoconstriction induced by TXA2, generated from AA in blood, did not significantly alter acid back-diffusion or p.d. during application of topical acid, nor lead to observable damage (Table 1). However, in the presence of acid plus taurocholate, substantial damage of the mucosa was observed during vasoconstriction with TXA2. Necrosis and punctate bleeding became apparent within 10 min and large areas of the mucosal surface became damaged within 30 min (Table 1). After terminating the AA infusion, frank bleeding from the eroded surface was observed.

This work using TXA₂ confirms that vasoconstrictor agents can readily induce ulceration of the gastric mucosa in conditions where the mucosal surface had been in contact with low concentrations of bile salts^{4,16}. Although back-diffusion of acid alone into the mucosa seems to cause only slight gastric damage, concurrent vasoconstriction allows the accumulation of these back-diffusing hydrogen ions, leading to mucosal tissue acidification and thus necrosis. Our findings that the TXA2 generated from AA in blood is a powerful gastric vasoconstrictor and leads to extensive mucosal damage may implicate endogenous TXA₂ in the pathogenesis of gastric ulceration. Clinical conditions such as stress or shock might lead to local platelet activation with subsequent TXA2 generation in the stomach, or lead to an imbalance between prostacyclin-TXA2 levels, and hence induce gastric mucosal damage.

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- 1. Hamberg, M., Svensson, J. & Samuelsson, B. Proc. natn. Acad. Sci. U.S.A. 72, 2994-2998
- 2. Moncada, S. et al. Prostaglandins 13, 611-618 (1977)
- Mullane, K. M., Dusting, G. J., Salmon, J. A., Moncada, S. & Vane, J. R. Eur. J. Pharmac, 54, 217-218 (1979).
- Ritchie, W. P. Gastroenterology 68, 699-707 (1975)
- Chignard, M. & Vargaftig, B. B. Eur. J. Pharmac. 38, 7-18 (1976).
- Konturek, S. J., Mikos, E., Pawlik, W. & Walus, K. M. J. Physiol., Lond. 286, 15-28 (1979). Walus, K. M., Gustaw, P. & Konturek, S. J. Prostaglandins 20, 1089-1102 (1980).
- Whittle, B. J. R., Moncada, S., Whiting, F. & Vane, J. R. Prostaglandins 19, 605-627
- Tai, H. H. & Yuan, B. Biochem. biophys. Acta 539, 162-172 (1978). Harris, R. H. et al. Adv. Prostaglandin Thromboxane Res, 6, 457-461 (1980).
- Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. Nature 263, 663-665 (1976).
 Vane, J. R. Nature new Biol. 231, 232-235 (1971).

- Moncada, S. & Vane, J. R. New Engl. J. Med. 300, 1142-1147 (1979). Whittle, B. J. R., Higgs, G. A., Eakins, K. E., Moncada, S. & Vane, J. R. Nature 284, 271-273 (1980).
- Davenport, H. W. Gastroenterology 46, 245-253 (1964).
- Whittle, B. J. R. Br. J. Pharmac. 60, 455-460 (1977)

Mobility of polypeptide chain in the pyruvate dehydrogenase complex revealed by proton NMR

Richard N. Perham & Harry W. Duckworth*

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

Gordon C. K. Roberts

National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Recent studies of several small proteins by NMR spectroscopy and X-ray crystallography have clearly demonstrated significant internal mobility in their structures (see, for example, refs 1-9), which can involve not only amino acid side chains but also larger regions of polypeptide chain. Occasionally a plausible function for this mobility has been suggested 1,9, but there has been no conclusive evidence for a direct connection between intramolecular mobility and a defined step in an enzymatic mechanism. The pyruvate dehydrogenase (PDH) multienzyme complex of Escherichia coli (molecular weight (M_r) 4.5-6×10°) is one of the largest well defined assemblies of proteins known, comprising multiple copies of three different enzymes 10,11. The substrate is carried in thioester linkage by lipoyl-lysine residues of the lipoate acetyltransferase component, the structural core of the complex. The lipoyl-lysine residues act as swinging arms, carrying substrate between the catalytic centres of the three enzymes12-15 and between lipoic acid residues attached to different subunits in the lipoate acetyltransferase core 16-18. It has been conjectured that the lipoic acid-containing regions of polypeptide chain might be flexible 19,20 and therefore able to increase greatly the effective radius of a swinging arm¹⁹. We report here unexpectedly sharp lines in the 270-MHz proton NMR spectrum of the enzyme complex that are attributed to remarkable conformational mobility of large regions of polypeptide chain carrying the lipoic acid residues. This mobility would enhance the functional connection of active sites in a multisubunit structure.

A schematic mechanism of the PDH complex is shown in Fig. 1. A 270-MHz proton NMR spectrum of the intact complex is shown in Fig. 2a, the most striking feature of which is the appearance of several sharp resonance lines (between -0.6 and -3 p.p.m. from dioxan) attributable to the protein. The complex has an M_r of $\sim 6 \times 10^6$ (ref. 11), a diameter of at least 30 nm (refs. 10, 11) and a rotational correlation time of $\sim 10^{-5}$ s (ref. 14). A methylene proton moving with this correlation time would have a linewidth of \sim 8.5 kHz, and indeed there is an extremely broad envelope of resonance underlying the sharp lines in Fig. 2a. However, resonances with linewidths of only ~50 Hz must mean that some amino acid residues have substantial freedom to move rapidly with respect to the enzyme complex.

The most prominent sharp resonance in the spectrum is at -2.34 p.p.m., close to the positions of the methyl resonances of alanine (-2.32 p.p.m.) and threonine (-2.48 p.p.m.) in simple peptides. There is also a clear shoulder at -2.77 p.p.m., which is similar to the chemical shifts of the methyl protons of valine, leucine and isoleucine (-2.82 to -2.74 p.p.m.). Methyl groups in proteins are commonly found to rotate very rapidly about the carbon-carbon bond attaching them to the molecule^{1,2} However, this motion is not sufficient to explain the observed linewidths for these resonances. Rapid rotation of a methyl

^{*} Permanent address: Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Fig. 1 The reaction mechanism of the PDH multienzyme complex. E1 (pyruvate decarboxylase, EC 1.2.4.1), E2 (lipoate acetyltransferase, EC 2.3.1.12) and E3 (lipoamide dehydrogenase, EC 1.6.4.3) represent the three enzymatic activities. TPP, thiamine pyrophosphate; $R = CH_3 - ...$

group about this single axis can decrease the linewidth by no more than a factor of 4 (refs 21, 22) to give a predicted methyl linewidth of \sim 4 kHz in the present case—almost two orders of magnitude greater than that observed. It is clear that motion about several bonds must be involved, and that the sharp signals in Fig. 2a must arise from a region of the protein where the backbone, as well as the side chains, has substantial mobility.

It is impossible to integrate these spectra accurately owing to the obvious difficulties of drawing an appropriate baseline, but approximate integration of spectra obtained with a 5-s pulse interval indicates that several hundred protons per enzyme protomer contribute to the sharp lines in the spectrum. We define a protomer as 1/24th part of the enzyme complex, as the particle appears to be a 24-fold repeat of this structure, based on an E2 core of 24 polypeptide chains arranged with octahedral symmetry 10,11. Thus more than a few amino acids are involved and substantial regions of polypeptide chain must be mobile. The observed intensity ratio of the (Ala+Thr) to the (Val+ Leu + Ile) resonances is greater than would be expected from the amino acid composition of the complex (see refs listed in ref. 23), and in addition no sharp resonances from aromatic protons are observed. Clearly we are not observing a general mobility of all the polypeptide chains in the complex, but rather a high degree of mobility for specific regions of the primary structure

No change in the NMR spectrum was observed when the complex was converted to the acyl enzyme by treatment with pyruvate (0.11 mM), thiamine pyrophosphate (0.11 mM) and MgCl₂ (5.7 mM) in the absence of coenzyme A and NAD⁺ (see mechanism in Fig. 1). Likewise, no change was observed when the lipoic acid residues were reduced by treatment with NADH (0.15 mM) and NAD⁺ (0.05 mM) or when this reduced enzyme complex was acetylated by treatment with acetyl CoA (0.14 mM).

The PDH complex can be resolved into two subcomplexes: an E1-E2 subcomplex by selective removal of the E3 subunits in 4 M urea, and an E2-E3 subcomplex by selective removal of the E1 subunits at pH 10 (refs 24, 25). Both subcomplexes have a large M_r because they are based on the octahedral (24-chain) core of the E2 component. Their appearance on SDS-gel electrophoresis is shown in Fig. 3 and their proton NMR spectra in Fig. 2. The most prominent features of the spectrum of the intact complex are retained in the spectra of the subcomplexes. As the only type of polypeptide chain common to all three structures is that of lipoate acetyltransferase (E2), we infer that the most mobile regions of the intact PDH complex belong to this core enzyme.

The isolated E1 and E3 components are dimers, with M_r s of $\sim 200,000$ and 112,000 respectively¹⁹. Their proton NMR spectra (Fig. 4) differ from those of the intact complex and subcomplexes (Fig. 2), lacking the prominent (Ala+Thr) resonance at -2.34 p.p.m., but having clearly detectable resonances from aromatic protons. The lines in these spectra have apparent widths (~ 100 Hz) similar to those expected for molecules of this size^{1.26.27}, and there is no indication of substantial internal motion. These experiments, together with those on the subcomplexes, clearly implicate the E2 component as the source of the sharp resonances in the spectrum of the complex. Attempts to obtain a spectrum of the isolated E2 component were frustrated by its tendency to aggregate and precipitate at the high protein concentrations required.

Each E2 chain of the $E.\ coli$ complex contains two lipoic acid residues which become reductively acetylated by substrate (see Fig. 1) during the enzymatic reaction 16.17.28. Limited trypsin treatment of the complex releases fragments of the E2 polypeptide chain carrying these lipoyl groups, whereas E1 and E3 components remain bound to the residual part of the E2 core in an inactive complex which still has a very high $M_r^{19.20.29}$. This limited proteolysis suggested that the lipoic acid-containing

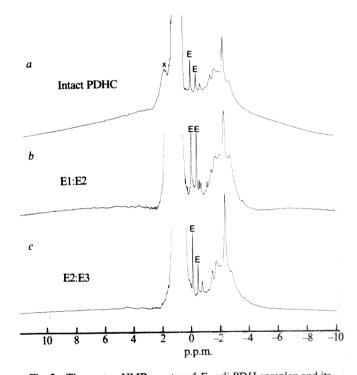


Fig. 2 The proton NMR spectra of E. coli PDH complex and its subcomplexes. Complexes were pelleted in the ultracentrifuge (3 h, 45,000g), dissolved in D₂O buffer, apparent pD 7.6, containing 20 mM potassium phosphate and 2 mM sodium EDTA, and dialysed exhaustively against the same buffer. Spectra were obtained at 270 MHz using a Bruker WH270 spectrometer. Quadrature phase detection was used, with a 6-kHz spectral width and 0.34-s pulse intervals. The free-induction decay was recorded in 4,096 data points and 2,000 decays were averaged. Before Fourier transformation, the free-induction decay was multiplied by an exponential corresponding to a line broadening of 5 Hz and the data table was extended to 8,192 points with zeros. Chemical shifts are expressed relative to internal dioxan. The peaks marked E arise from EDTA. a, Intact complex, 70 mg ml⁻¹; s_{20,w} (sedimentation coefficient corrected to water at 20 °C) of a portion of this sample, diluted to 3.0 mg ml⁻¹, was 58.7 S. The feature marked x is an instrumental artefact. b, E1-E2 subcomplex, prepared by resolution of intact complex on hydroxyapatite in 6 M urea, a method similar to that in ref. 24; 29 mg ml⁻¹. This preparation was a cloudy suspension which was not examined by analytical ultracentrifugation. c, E2-E3 subcomplex, prepared by treatment of intact complex at pH 10 according to refs 24,25; 57 mg ml⁻¹; $s_{20,w}$ at 3.0 mg ml⁻¹ = 30.9 \$.

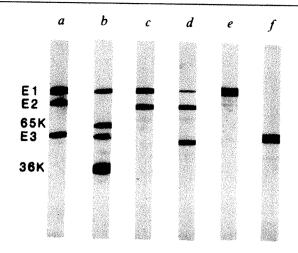


Fig. 3 Analysis by SDS-polyacrylamide gel electrophoresis of $E.\ coli$ PDH complexes and subcomplexes whose NMR spectra are shown in Figs 2 and 4. All samples were run on standard 7.5% gels in phosphate buffer 16. a, Intact enzyme complex; b, enzyme complex after limited digestion with trypsin and gel filtration on Sepharose 6B-CL 19; c, E1-E2 subcomplex prepared by removal of E3 subunits in the presence of 6M urea 24; d, E2-E3 subcomplex prepared by removal of E1 subunits at pH 10 (refs 24, 25); e, free E1 (dimers); f, free E3 (dimers). Fragments with apparent M, of 65,000 and 36,000 in e are tryptic products of the E1 and E2 chains, respectively 29.

regions of the lipoate acetyltransferase core protrude physically in the complex^{19,20,29}, and may form a 'fuzz' of lipoyl domains surrounding the E2 core in favourably oriented electron micrographs²⁰. The largest identifiable fragment containing lipoic acid residues has an apparent M_r of $45,000^{20}$ but this is rapidly broken down further by trypsin. Some degradation of the E1 chains from an apparent M_r of 100,000 to 65,000 is usually found and the E2 core is then composed of fragments of the E2 chain with an apparent M_r of 36,000, compared with an apparent M_r of 80,000 for the intact E2 polypeptide^{20,29} (see Fig. 3). Figure 4d shows the proton NMR spectrum of a trypsintreated complex, gel-filtered on Sepharose 6B to remove the lipoic acid-containing peptides¹⁹. All the sharpest peaks in the spectrum of the intact complex have disappeared, indicating that the proteolysis has removed the bulk of the highly mobile region or regions of the E2 chain. The spectrum of the tryptic core still contains resonances in the aliphatic region which are too narrow to arise from a rigid complex of this size, although they are much broader than the sharp components of the spectrum of the intact complex. They indicate the presence of regions of moderate internal mobility in the tryptic core.

The almost unfettered motion of the lipoyl groups revealed by ESR spectroscopy^{14,15} is accompanied by much conformational mobility of polypeptide regions of the lipoate acetyltransferase core that encompass the lipoyl-lysine residues. These are intrinsic properties of the enzyme protein and must not be confused with substrate-induced conformational changes. The chemical shifts of the sharp resonances in the NMR spectra are consistent with a random coil-like structure for the mobile regions, although some parts of these regions may have defined three-dimensional structures. Such structured regions near the lipoic acid residues might facilitate recognition by the enzyme active sites through protein-protein interaction.

Conformational mobility may explain some puzzling features of the enzyme complex. It might help to span the gaps between E1, E2 and E3 active sites¹⁹, which may be larger than a single lipoyl-lysine swinging arm could cover in a rigid protein³⁰⁻³², and it is presumably important in active-site coupling¹⁶⁻¹⁸. Several lines of evidence indicate that up to about half of the

lipoic acid residues of the *E. coli* PDH complex can be removed by limited proteolysis with trypsin²⁰ or inactivated by treatment with *N*-ethylmaleimide³³ without much loss of overall catalytic activity. The overall catalytic activity of the complex is directly proportional to its E1 content^{16,18,34} and the rate-limiting step in the mechanism must involve E1^{18,35}. Loss of catalytic activity would lag behind the loss or modification of lipoic acid residues if one lipoic acid residue could take over the role of another^{20,33,36,37}. Conformational mobility in the E2 core might facilitate this by allowing a given E1 active site to be visited by more than one lipoic acid residue, even a lipoic acid residue bound to a different E2 chain^{16,33,36}. The dihydrolipoyl group could in turn have a limited choice of E3 active sites at which to be reoxidized³⁸. That this mobility is vital in the functioning of the complex is reinforced by the observation (H.W.D., R. Jaenicke, R.N.P., G.C.K.R. and E. Wawrzynczak, unpublished)

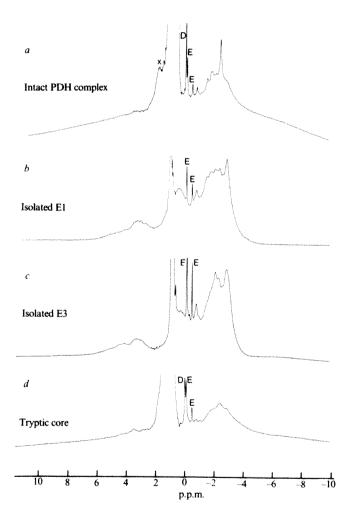


Fig. 4 The proton NMR spectra of some derivatives of $E.\ colin PDH$ complex. Protein samples were concentrated by ultracentrifugation (a,d) or ultrafiltration (b,c), and were then dialysed against the D_2O buffer and examined by NMR spectroscopy as described in Fig. 2 legend. The signal marked D in a and d is due to dioxan, ~ 1 mM, added as a reference; peaks E arise from EDTA; x is an instrumental artefact. a, Intact complex from Fig. 1. b, E1 component, obtained by the resolution procedure that yielded E2–E3 subcomplex (Fig. 2c); 51 mg ml $^{-1}$; $s_{20,w}$ at 3.0 mg ml $^{-1}$ = 9.65 S, as expected for the dimer, $M_r = 200,000$ (ref. 10). c, E3 component, obtained by the resolution procedure that yielded E1–E2 subcomplex (Fig. 2b); 12 mg ml $^{-1}$; $s_{20,w}$ at 3.0 mg ml $^{-1}$ = 6.03 S, as expected for the dimer, $M_r = 112,000$ (ref. 10). d, Core complex obtained by limited proteolysis of native PDH complex with trypsin and gel filtration on Sepharose 6B-CL, as in ref. 19; 66 mg ml^{-1} ; $s_{20,w}$ at 3.0 mg ml $^{-1}$ = 41.2 S.

that the PDH complexes of Bacillus stearothermophilus and ox heart and the 2-oxoglutarate dehydrogenase complexes of E. coli and ox heart all display very similar NMR spectra, indicating similar conformational mobility.

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- Gurd, F. R. N. & Rothgeb, T. M. Adv. Protein Chem. 33, 73-165 (1979).
 Williams, R. J. P. Biol. Rev. 54, 389-437 (1979).
 Frauenfelder, H., Petsko, G. A. & Tsemoglou, D. Nature 280, 558-563 (1979).
 Artymuik, P. I. et al. Nature 280, 563-568 (1979).
- 5. Campbell, I. D., Dobson, C. M., Moore, G. R., Perkins, S. J. & Williams, R. J. P. FEBS Lett. 70, 96-100 (1976).
- Wagner, G., De Marco, A. & Wüthrich, K. Biophys. Struct. Mech. 2, 139-158 (1976). Ribeiro, A. A., King, R., Restivo, C. & Jardetzky, O. J. Am. chem. Soc. 102, 4040-4051
- 8. Jardetzky, O., Adasaka, K., Vogel, D., Morris, S. & Holmes, K. C. Nature 273, 564-566
- 9. Highsmith, S. et al. Biochemistry 18, 4238-4244.
- Reed, L. J. Acc. chem. Res. 7, 40-46 (1974). Danson, M. J. et al. J. molec. Biol. 129, 603-617 (1979).
- 12. Green, D. E. & Oda, T. J. Biochem., Tokyo 49, 742-757 (1961).

- Koike, M., Reed, L. J. & Carroll, W. R. J. biol. Chem. 238, 30-39 (1963).
 Ambrose, M. C. & Perham, R. N. Biochem. J. 159, 429-432 (1976).
 Grande, H. J., Van Telgen, H. J. & Veeger, C. Eur. J. Biochem. 71, 509-518 (1976).
- Bates, D. L., Danson, M. J., Hale, G., Hooper, E. A. & Perham, R. N. Nature 268, 313-316
- Collins, J. H. & Reed, L. J. Proc. natn. Acad. Sci. U.S.A. 74, 4223-4227 (1977).
 Danson, M. J., Fersht, A. R. & Perham, R. N. Proc. natn. Acad. Sci. U.S.A. 75, 5386-5390
- 19. Hale, G. & Perham, R. N. FEBS Lett. 105, 263-266 (1979).
- 20. Bleile, D. M., Munk, P., Oliver, R. M. & Reed, L. J. Proc. natn. Acad. Sci. U.S.A. 76, 4385-4389 (1979).
- Woessner, D. E., Snowenden, B. S. Jr & Meyer, G. H. J. chem. Phys. 50, 719-721 (1969), Werbelow, L. G. & Marshall, A. G. J. Am. chem. Soc. 95, 5132-5134 (1973). Bates, D. L., Harrison, R. A. & Perham, R. N. FEBS Lett. 60, 427-430 (1975).

- Reed, L. J. & Willms, C. R. Meth. Enzym. 9, 247-265 (1966) Coggins, J. R., Hooper, E. A. & Perham, R. N. Biochemistry 15, 2527-2533 (1976). Wüthrich, K. NMR in Biological Research (Elsevier, Amsterdam, 1976).
- 27. Jardetzky, O. & Roberts, G. C. K. NMR in Molecular Biology (Academic, New York,
- 1981).
 Danson, M. J. & Perham, R. N. *Biochem. J.* **159**, 677–682 (1976).
 Hale, G. & Perham, R. N. *Eur. J. Biochem.* **94**, 119–126 (1979).

- Haie, G. & Pernam, K. N. Eur. J. Biochem. 94, 119-120 (1979).
 Shepherd, G. B. & Hammes, G. G. Biochemistry 15, 311-317 (1976).
 Angelides, K. J. & Hammes, G. G. Biochemistry 18, 1223-1229 (1979).
 Scouten, W. H. et al. Eur. J. Biochem. 112, 9-16 (1980).
 Ambrose-Griffin, M. C., Danson, M. J., Griffin, W. G., Hale, G. & Perham, R. N. Biochem.
- Angelides, K. J. & Hammes, G. G. Proc. natn. Acad. Sci. U.S.A. 75,4877-4880 (1978).
- Akiyama, S. K. & Hammes, G. G. Biochemistry 19, 4208-4213 (1980). Cate, R. L., Roche, T. E. & Davis, L. C. J. biol. Chem. 255, 7556-7562 (1980).
- Berman, J. N., Chen, G.-X., Hale, G. & Perham, R. N. Biochem. J. (submitted).
- Reed, L. J. et al. Proc. natn. Acad. Sci. U.S.A. 72, 3068-3072 (1975).

Delayed recruitment of maternal histone H3 mRNA in sea urchin embryos

Dan E. Wells, Richard M. Showman, William H. Klein & Rudolf A. Raff

Program in Molecular, Cellular and Developmental Biology, Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

During the early stages of embryogenesis, the sea urchin embryo uses maternally synthesized mRNA stored in the egg as inactive messenger ribonucleoproteins (mRNPs)1. Release of this mRNA for translation allows the embryo to develop even in the absence of new mRNA synthesis. Comparison using twodimensional gel electrophoresis shows that the same spectrum of prevalent proteins are synthesized by eggs and zygotes 30-60 min after fertilization². Thus, unlike the case of the clam Spisula in which there are several prominent changes in translation associated with fertilization3, sea urchins have been thought to show little or no regulation of translation of specific mRNA sequences. Histone mRNAs are major components of the mRNA pool, comprising as much as 4-8% of the total mRNA of eggs4, yet their products, histones, would not normally be detectable on two-dimensional gels. We report here that mRNA complementary to a histone H3 cloned probe remains in the inactive mRNA pool for 90 min after fertilization before it begins to be translated. This is long after the rapid increase in overall protein synthesis, using stored mRNAs, has begun. As we can demonstrate no significant synthesis of histone H3 mRNA during this period, stored histone H3 mRNA must be subject to sequence-specific translational regulation.

Patterns of protein synthesis during early sea urchin embryogenesis have supported the idea of a rapid linear release of stored maternal mRNA from the mRNA pool. The kinetics of protein synthesis require that the release of stored mRNA for translation starts 2 min after fertilization⁵. This rapid initiation of the release of stored mRNAs is further indicated by the kinetics of recruitment of free ribosomes into polysomes (Fig. 1). Whether measured by optical density or by use of a ribosomal RNA-specific cloned probe, recruitment starts immediately after fertilization and proceeds in a linear manner for ~90 min before reaching a plateau at 20-25% of the available rRNA. In

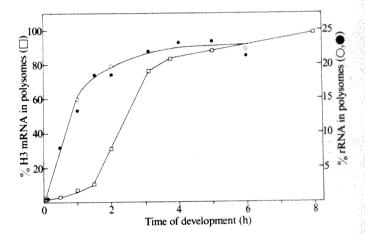


Fig. 1 Time of appearance of rRNA and histone H3 mRNA in the polysomes of S. purpuratus embryos. Embryos were cultured at 13.5 °C for the appropriate lengths of time and polysomes were prepared by the procedure of Wold et al. 10, omitting the polysinyl sulphate. Material was layered on to 5-40% exponential sucrose gradients and centrifuged for 2.5 h, 26,000 r.p.m. in an SW27 rotor. Individual fractions were collected, deproteinized with phenol/chloroform/isoamyl alcohol (25:24:1) and RNA precipated with ethanol. RNA samples were taken up in 1×MOPS buffer (20 mM morpholinopropane sulphonic acid, 5 mM sodium acetate, 1 mM EDTA) at pH 7.0, 6% formaldehyde and 50% formamide, heated at 65 °C for 5 min, cooled rapidly and electrophoresed on a 1% agarose gel in 1 × MOPS buffer containing 6% formaldehyde. After electrophoresis the gel was soaked for 30 min in 20 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4), and blotted on to a nitrocellulose filter in 10×SSC. The filter was rinsed with 3×SSC and baked for 2 h at 80 °C in a vacuum oven. Probes for histone H3 (p3H1, provided by L. Kedes) and rRNA (pLv 60, provided by D. Stafford) were nick-translated to $1-5 \times 10^7$ c.p.m. μg [α-32P]dCTP. Filters were prewashed in 10×Denhardt's solution (0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinyl pyrolidone), 0.1% SDS, 0.02 M phosphate buffer, $5 \times SET$ ($1 \times SET = 0.15$ M NaCl, 0.025 M Tris, pH 8.0, 0.002 M EDTA) at 68°C for 1-2 h. The filters were prehybridized at 45°C in 10 ml of 10% dextran sulphate, 5×SET, 0.02 M phosphate buffer, 1×Denhardt's, 100 µg ml⁻¹ sheared calf thymus DNA. 100 μ g ml⁻¹ poly (rA) and 50% formamide. After 1-2 h, 1-5 × 10⁷ c.p.m. of nick-translated plasmid was added and hybridized at 45 °C for 12-16 h. After hybridization, the filters were washed at 68 °C for 1 h in each of the following: (1) $5 \times SET$, 0.1% SDS, 0.1% sodium pyrophosphate and 0.025 M phosphate buffer; (2) $1 \times SET$, $1 \times Denhardt's$, 0.1% sodium pyrophosphate phate and 0.025 M phosphate buffer; (3) 0.3 × SET, 0.025 M phosphate buffer, 0.1% sodium pyrophosphate, 0.1% SDS. The filters were air dried and exposed to preflashed 11 Kodak X-Omat RPfilm at -70 °C using Dupont Cronex lighting plus intensifier screens. Exposure times ranged from 2 to 96 •, % rRNA in polysomes as determined by A253; O, % rRNA in polysomes as determined by hybridization with rDNA probe pLv60; □, % of histone H3 mRNA in polysomes

addition, titrations with labelled poly(U) show that >70% of the total cytoplasmic poly(A) is present in the polysomes of 2-h-old embryos, while <5% is found in polysomes of unfertilized eggs⁶.

To determine whether H3 mRNA is recruited with the same kinetics as total mRNA, we have measured the relative amount of H3 mRNA present in the mRNA and polysome compartments of the embryo from fertilization through 8 h of development. Strongylocentrotus purpuratus embryos were cultured for specific times and the polysomes displayed on 5-40% exponential sucrose gradients. After fractionation the RNA was extracted, electrophoresed on agarose gels and blotted on to nitrocellulose filters. The filter-bound RNA was then hybridized to a ³²P-labelled nick-translated clone containing only the coding sequence for histone H3. The amount of probe hybridizing to each region of the gradient gave a direct measure of the percent of H3 mRNA found in the polysomal and mRNA compartments of the embryo at a given time (Fig. 2a, c). Quantification was done by densitometric scanning of autoradiographs exposed within the linear range of the film. To ensure that the detected RNA was polysomal, parallel experiments were performed using EDTA to dissociate polysomes (Fig. 2b, d).

At 90 min after fertilization, <10% of the total hybridizable H3 mRNA is found in the polysomal region of the gradient (Fig. 1). Between 90 min and 4 h there is a rapid linear rise in the per cent of the total H3 mRNA in the polysomes until, after 4 h, >85% of the H3 mRNA is detected in the polysome region of the gradient. Whether embryos are cultured in the presence or absence of actinomycin D, we can detect no significant increase in the amount (per µg of total RNA) of H3 mRNA during the first 5 h after fertilization (Fig. 3). This, in conjunction with the recruitment data in Fig. 1, suggests that little of the H3 mRNA initially present in the egg remains in the masked state. Newly synthesized H3 mRNA begins to accumulate measurably 6-8 h after fertilization, and seems to move rapidly into the polysomal fraction, as by 8 h >95% of the H3 mRNA is present in polysomes. In contrast to H3 mRNA, α-tubulin mRNA does not exhibit the 90-min delay, but is significantly loaded by 30 min after fertilization (data not shown).

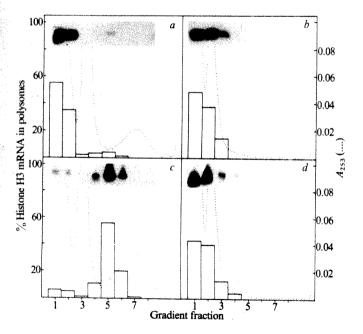


Fig. 2 Autoradiographs and quantification of representative H3 mRNA titration from 1-h and 3.75-h embryos. The amount of probe hybridizing to each fraction of the gradient is expressed as the percentage of the sum of the probe hybridized in all fractions. A_{253} (.....) was obtained using an Isco UA5 absorbance monitor. Fractions were collected from the top. a, 1-h embryos; b, 1-h embryos, EDTA released; c, 3.75-h embryos; d, 3.75-h embryos, EDTA released.

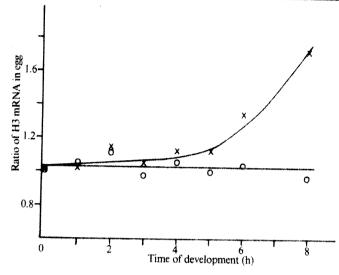


Fig. 3 Titration of total H3 mRNA from unfertilized egg through 8 h of development in the presence or absence of actinomycin D. Embryos were cultured in the presence of 25 µg ml⁻¹ actinomycin D. At each time point, identical aliquots were removed and the total RNA extracted, electrophoresed, blotted and hybridized as described in Fig. 1 legend. Data are given as the ratio of the amount of H3 at each time point to the amount in the unfertilized egg. x, Control; O, actinomycin D.

Consistent with these results is a recent study of in vitro translation of sea urchin mRNA using a cell-free system derived from sea urchin eggs. This study shows that histone H3, as well as other histones, are synthesized in vitro in significant amounts; however, very little histone synthesis was observed in vivo until at least 1 h after fertilization7. Our data show that this delay in appearance of histone H3 is to be expected from the behaviour of the H3 mRNA. Because most mRNAs in the egg must be unmasked before translation can occur8, the delay in H3 mRNA recruitment may be due to a specific delay in unmasking of histone mRNAs. Alternatively, the delay may be due to a change in initiation properties of ribosomes or the lack of a histone mRNA-specific translation factor. We cannot yet discriminate between these possibilities. Woods and Fitschen⁹ have observed changes in histone mRNA distribution in sea urchin polysomes during telophase of the first cleavage cycle and suggest that it may be associated with the onset of DNA synthesis. However, the absence of any further association of use of histone mRNA with the cell cycle argues against a direct causal association between DNA synthesis and histone mRNA recruitment. The marked contrast between the recruitment of H3 mRNA and that of most maternal mRNAs clearly demonstrates translational regulation of early embryonic protein synthesis in the sea urchin embryo.

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- Raff, R. A. in Cell Biology: A Comprehensive Treatise Vol 5, 107-136 (Academic, 1980).
 Brandhorst, B. Devl Biol. 52, 310-317 (1976).
 Rosenthal, E. T., Hunt, T. & Ruderman, J. V. Cell 20, 487-494 (1980).
 Davidson, E. H. Gene Activity in Early Development, 2nd edn (Academic, New York, 1972).
- 5. Raff, R. A., Brandis, J. W., Huffman, C. J., Koch, A. L. & Leister, D. E. Devl Biol. (in the
- Dolecki, G. J., Duncan, R. F. & Humphreys, T. Cell 11, 339-344 (1977)
- Winkler, M. & Steinhardt, R. Devl Biol. (in the press).

 Jenkins, N. A., Kaumeyer, J. F., Young, E. M. & Raff, R. A. Devl Biol. 63, 279-298 (1978).

 Woods, D. E. & Fitschen, W. Cell Diff. 7, 103-114 (1978).
- Wold, B. J., Klein, W. H., Hough-Evans, B. R., Britten, R. J. & Davidson, E. H. Cell 14, 941-950 (1978).
- 11. Laskey, R. A. & Mills, A. D. Eur. J. Biochem. 56, 335-341 (1975).

BOOK REVIEWS

In pursuit of a self-conscious science

J. O'Keefe and P.D. Wall

FOR the half-century between 1920 and 1970, most neurophysiologists and experimental psychologists were content to leave "consciousness" outside the boundaries of their disciplines. The properties traditionally attributed to consciousness—freedom of the will, creativity, self-knowledge—seemed difficult, if not impossible, to capture within the accepted notions of deterministic science. Furthermore there was a strong reaction to the use of consciousness as an explanatory device: two actions were different because one was voluntary; two stimuli differed because one was attended to.

In recent years this radical behaviourist approach has been modified and recent work has thrust the problem of consciousness to the forefront again. One scientist who deserves considerable credit for insisting on a place for consciousness in neurophysiology during this scientific dark night of the soul is the author of the book under review. In 1953, Sir John Eccles published a book entitled Neurophysiological Basis of Mind (Oxford University Press) which was quite the best summary up to that time of how nerve impulses pass from one cell to another. Having been for many years one of the main proponents of the electrical synapse theory. Eccles accepted the new evidence for the chemical synapse and became one of the architects of our modern understanding of neuronal transmission, for which work he received the Nobel prize in

In 1953 virtually no aspect of brain research had a direct bearing on the question of consciousness and Professor Eccles's inclusion of a final chapter on this topic was regarded as a mild eccentricity. Consciousness entered the scientific picture as a subtle force operating at the level of the indeterminate synapse. Eccles's preoccupation with the brain-mind problem has increased over the years: the last chapter of the 1953 book has become the first chapter of the present book. The book is divided into three sections. The first section (Lectures 1 and 2) sets out Eccles's current theory of the mind which he has developed in association with the philosopher Sir Karl Popper. The second (Lectures 3-6) describes recent experiments in neurophysiology and psychology which bear on the question of consciousness, and the third (Lectures 7-10) marks a shift to a consideration of

The Human Psyche. The Gifford Lectures, University of Edinburgh 1978-1979. By John C. Eccles. Pp.279. ISBN 3-540-09954-9. (Springer-Verlag: 1980.) DM44, \$26.

the broader issues of the relation between consciousness and social values.

Eccles's theory of consciousness views it as an entity which exists independently of the brain but which interacts strongly with it. He rejects materialist theories which identify consciousness with the activity of a part or all of the brain. The brainconsciousness interaction takes place at the level of the cerebral cortex which unfortunately does not have the appropriate anatomical structure. Whereas consciousness appears to be a unity which maintains its integrity and continuity over time, the cerebral cortex consists of numerous, relatively independent modules each coding for a different aspect of the sensory environment or motor behaviour. Eccles concludes that "... the unity of conscious experience is provided by the self-conscious mind and not by the neural machinery of the liaison areas of the cerebral hemisphere" (p.50). How is this accomplished?

... the self-conscious mind can scan the activity of each module of the liaison brain or at least those modules tuned in to its present interest... the self-conscious mind has the function of integrating its selections from the immense patterned input it receives from the liaison brain... in order to build its experiences from moment-to-moment [p.46].

It would appear that on this formulation the problem of consciousness is inaccessible to neurophysiological analysis and perhaps to any scientific analysis. It is a non-neural entity with its own structure, interests, expectations and creative impulses, attributes which are never discussed but which are constantly invoked to "explain" psychological and physiological findings.

Much of the middle section of the book is an excellent description of recent findings. As might be expected from the author's preoccupation with the cerebral cortex as the part of the brain which liaises with the self-conscious mind, much (and by far the most satisfactory part) of this section deals with effects ascribable to the cortex. Visual illusions show that much of the sensory world is created by the brain

from inadequate data; EEG recordings of the brain potentials from the scalp show that cortical activity precedes voluntary movements and accompanies anticipated stimuli. Unfortunately, these fascinating findings are "explained" by hand-waving references to the self-conscious brain. Since the "explanation" is given in terms of a world with normal scientific rules, one may reasonably ask for experiments to investigate this world, but as we approach it retreats as a will'o the wisp.

Subsequent chapters give a cursory but adequate account of the limbic system, the pain system and the reticular activating system of the brainstem. Professor Eccles acknowledges that these may have some bearing on the problem of consciousness but keeps his sights firmly fixed on the cerebral cortex. A surmise in the section on the limbic mechanisms of emotion "that Gary Gilmour, the last criminal to be executed in the United States, may have been suffering from amygdaloid seizures" (p.116-117) warms us of what to expect in the third and final section of the book: the relation between brain, consciousness and social values.

This last section contains a wide ranging set of comments and quotations on topics centred around the twilight area where scientific ideas impinge upon social values. Professor Eccles is particularly severe on scientists who would reduce human action or human values to mechanistic explanations. The sociobiologist E.O. Wilson who seeks to explain altruistic behaviour on the basis of selfish genes and the molecular biologist Jacques Monod who seeks to emphasize the chance elements in human existence come in for special opprobrium. Although some cogent arguments are advanced, much of the discussion takes the form of poetic quotations from suitably authoritative sources: Sherrington, Planck, Heisenberg. Eddington and Einstein. We suggest that most readers will take these last chapters for what they are: the personal prejudices of a great neurobiologist who has strayed outside the bounds of his area of expertise. We hope it won't deter them from giving serious consideration to the earlier chapters.

J. O'Keefe and P.D. Wall are members of the Cerebral Functions Research Group at University College, University of London.

A tool of elegance and great power for physicists...

Peter McClintock

Green's Functions and Condensed Matter. By G. Rickayzen. Pp.357. ISBN 0-12-587950-4. (Academic: 1980.) £22.80, \$55. Many-Particle Physics. By G.D. Mahan. Pp.1,003. ISBN 0-306-40411-7. (Plenum: 1981.) \$85, £53.55.

WHEN George Green, miller and part-time theoretical physicist, died in 1841 at the early age of 47 the Nottingham Review commented regretfully that "... had his life been prolonged, he might have stood eminently high as a mathematician". Little did they realize. During his brief but remarkably productive twelve-year spell of scientific activity, starting with An Essay on the Application of Mathematical Analysis to the Theories of Electricity and Magnetism (published privately in 1828), Green had introduced the concept of electric potential, had carried out work of fundamental importance in wave theory and hydrodynamics and had laid the foundations for modern theories of elasticity. Notwithstanding this substantial contribution, however, and despite his acknowledged influence on both Stokes and Kelvin, Green's memory remained relatively obscure until more than a century after his death, when R.P. Feynman took up some of the techniques introduced by Green, and developed them for application to nuclear physics and the theory of elementary particles. The use of Green's function methods has subsequently spread through the rest of physics including, particularly, solid state physics.

Thus it has come about that no physicist in the 1980s can remain indifferent to Green's functions. For those who love them including, probably, the majority of theorists, Green's functions constitute a tool of elegance and great power, indispensable in some areas of physics, which can with advantage be applied to almost any problem with which they may be confronted. Others regard them with profound suspicion. They feel that devotees frequently employ Green's function techniques for their own sake, for the sheer joy of using them, and often at the expense of physical insights which can more readily be gained through simpler approaches. Their mistrust probably relates, at least in part, to a lack of understanding. The publication of two new advanced textbooks on the subject, each aimed at filling what has been an unfortunate lacuna in the literature, is therefore to be applauded.

The similarities between G. Rickayzen's Green's Functions and Condensed Matter and G.D. Mahan's Many-Particle Physics are greater than their differences. Both books are aimed at advanced graduate students, or academic staff, and both assume a good prior knowledge of quantum mechanics at the level provided.

say, by the classic texts of Schiff or Landau and Lifshitz. They are both most definitely books of physics, as opposed to mathematics, each consisting very largely of an account of how Green's function and Fevnman diagram techniques can be applied in practice to a selection of topics in solid state physics. Both books cover, for example, phonons, the Coulomb gas, electron-phonon interactions, transport theory, linear response, superconductivity and liquid helium (which by tradition is treated as solid state physics). Both provide, for each chapter, references to the original literature (Mahan's being the more extensive) and a set of problems, without solutions. In each case, the standard of production is high. The most obvious difference between the two books lies in their respective lengths: Rickayzen's has 357 pages in total whereas Mahan's, with 1,003, is almost three times as long. Much of this difference can be accounted for in terms of Mahan's detailed treatment of certain topics - for example, optical properties or polarons — which Rickayzen either omits or treats less fully; but, to a considerable extent, it also relates to their very different styles of presentation.

Mahan's stated objective was

... to take standard subjects ... and to summarize what is generally known. All the steps are retained in the derivation, so that the answers are obtained by starting from the beginning and working through to the end.

This latter feature is quite unusual in advanced texts, which commonly leave the reader to fill in a lot of the intermediate steps for himself, and it is a reflection of Mahan's declared intention of producing a book which graduate students will be able to use on their own, if necessary, in the

absence of a formally taught course. It will also be a considerable help and encouragement to the less theoretically biased readers at all ages and stages. Mahan writes in a style which is relaxed without being imprecise, and which often displays refreshing candour as, for example, in relation to the ground state properties of quantum fluids where he comments that, unusually, "... the Green's function method gives awful results".

Rickayzen's book, which starts disarmingly with the truism that "Anyone who has used the Coulomb potential due to a point charge has used a Green's function", is by contrast a model of brevity and succintness. The most immediate benefit lies in the remarkable range of topics which he has managed to treat at a useful level in the space available: in addition to the items mentioned above. the book also includes chapters on magnetism, disordered systems and critical behaviour. The relatively condensed presentation should be well suited to the graduate student in theoretical physics at whom the book is primarily aimed, particularly if used to support and supplement the material provided in a formal course of lectures on the subject.

Each of these books is excellent in its own way. They should be a real help both in educating the next generation of solid state theorists and also in rendering "Greenery" just a little bit less daunting to those professional physicists who have not yet fully come to terms with the power and utility of these techniques.

Peter McClintock is a Senior Lecturer in the Department of Physics, University of Lancaster.

... and another for molecular biologists

Ueli Schibler

Genetic Engineering 1. Edited by Robert Williamson. Pp.168. ISBN 0-12-270301-4. (Academic: 1981.) £9.80, \$24.

GENETIC engineering has not turned dull molecular biologists into imaginative ones. There is, however, little doubt that both could profit immensely from this elegant and powerful technique. This is enough justification for a new series entitled *Genetic Engineering*. According to the editor, the purpose of the new periodical is to rapidly publish comprehensive reviews about different aspects of recombinant DNA technology. This first issue addresses a very heterogeneous readership, including students, experienced researchers and physicians. It brings together a rather technical article about cDNA cloning, an

article about prenatal diagnosis of abnormal haemoglobin genes and a review on transcription of cloned genes in different experimental systems.

J. E. Williams provides a meticulous account of every aspect of the applicability and feasibility of cDNA cloning, and evaluates most of the tricks used in the enzymatic construction of recombinant molecules containing cDNA and their propagation in bacterial host cells. Particularly helpful are the discussions of mRNA complexity and the size of a cDNA library. Using this information one can easily calculate how many bacterial colonies should be screened to obtain, with a certain probability, an mRNA sequence of a given abundance. It is a pity that Williams did not include a short protocol

which he has found to be generally applicable. This might have reduced the beginner's confusion over choosing the most appropriate procedures, since he has just read 55 pages describing alternative methods, loop-holes and pitfalls.

The second article deals with a medical application of recombinant DNA technology. P. F. R. Little summarizes the lesions of haemoglobin genes associated with different haemoglobin deficiencies and how they can be detected by Southern blotting of DNA from fetal cells obtained by amniocentesis. Two major principles are discussed: the first is direct demonstration of deletions within a globin gene itself; this approach is particularly valuable for certain thalassaemias. The second is based on the fact that deficient genes are often linked to polymorphism of nearby restriction endonuclease sites, which in turn is readily detectable. This approach can be applied to any disfunctional gene where linkage exists, and does not depend on the nature of the lesion in the DNA. As, thanks to molecular cloning technology, our knowledge about genes increases rapidly, the antenatal diagnosis of lesions at the DNA level will certainly have an important future in preventive medicine.

Several systems to study the expression of cloned genes either in vitro or in vivo have recently become available. Two of them, the transcription of isolated genes in microiniected oocytes, and in cell-free extracts, are reviewed in an authoritative article by M. P. Wickens and R. A. Lasky. Most of the important published work carried out on the expression of eucaryotic genes in these systems is critically reviewed and put into perspective. In vitro transcription of genes, both wild type and after mutation in vitro, has been important in recognizing signals on DNA. This strategy succeeded in defining promotors for various genes transcribed by RNA polymerase III, and sequence elements in genes transcribed by RNA polymerase II. which are required for correct initiation and modulation of transcription.

All three articles of the first issue of Genetic Engineering are well written and certainly worth reading. It seems to me that the articles which are published in this issue and are planned for publication in further issues could have been grouped more logically, for example by putting the construction of cDNA (this issue) and genomic DNA recombinants (planned for a future issue) into the same volume. The same holds true for Wickens and Lasky's article and the planned article on expression of cloned genes in transfected cells. However, if the promised publishing strategy is adhered to, the series should quickly build into a useful reference work on recombinant DNA technology.

Ueli Schibler is a Junior Staff Member at the Swiss Institute for Experimental Cancer Research, Lausanne.

Isolation and assay of insect hormones

H. F. Nijhout

Neurohormonal Techniques in Insects. Edited by Thomas A. Miller. Pp.282. ISBN 3-540-90451-4. (Springer-Verlag: 1981.) DM79, \$46.70.

If it is possible to pinpoint the birth of a biological discipline, then neuroendocrinology was born in 1917 when a Polish biologist, Stefan Kopeč, reported that the brain of gypsy moth caterpillars produced a hormone that stimulated pupation. The hormone he discovered we now call prothoracicotropic hormone (PTTH), because it stimulates the prothoracic glands to secrete the moulting hormone, ecdysone. In the decades that have passed since this landmark discovery, the recognition, isolation and identification of additional insect neurohormones has proceeded at a slow but steady pace and Neurohormonal Techniques in Insects provides an account of the current status of these endeavours.

The purpose of this volume is to provide a compendium of the methods that are currently used by various laboratories to collect, assay and purify the neuroendocrines of insects. The book is divided into 11 chapters dealing with as many neuroendocrine factors, to wit: proctolin; adipokinetic hormone; diuretic hormone; insulin- and glucagon-like hormones; bursicon; pupariation factors; cuticle plasticizing factors; eclosion hormone; diapause hormone and PTTH. Each chapter has a brief introduction establishing the biological properties of the hormone in question, followed by a review of various bioassay methods and finally a detailed description of the methods used in its isolation and characterization.

The first two chapters by Staratt and Steele and by Stone and Mordue on the isolation and identification of proctolin and adipokinetic hormone, respectively, are by far the best methods papers I have ever seen. The quality of the remaining contributions is, predictably, variable both in method of exposition as well as in the skill with which various problems are handled. Some of the defects become understandable, though, when one considers the enormous obstacles that must be overcome by those who discover a new hormone and venture to identify it. The numbers of insects that need to be worked up to extract a sufficient quantity of hormone are truly prodigious. For example, 125,000 cockroaches yielded 1.12 mg of proctolin, and fractionation of 96,000 silkworm heads yielded 3 mg of a heterogeneous residue possessing high PTTH activity. To make matters worse, the bioassays that must be used are cumbersome and consume vast quantities of time, manpower and hard-won hormone. The biggest problem, though, is that the specificity of a bioassay for a given

hormone is often questionable, and I found a critical consideration of what constitutes an optimal assay method and scoring procedure lacking in most chapters.

A budding biochemist should have no difficulty in following the rationale as well as the procedures employed by the various authors and it should be possible for any well equipped laboratory to employ the methods described to attempt their own hormone isolations. However, it is evident from the accounts in this volume that the development of reliable, and preferably biochemical, assays should be the first order of business for the future.

H. F. Nijhout is Associate Professor of Zoology at Duke University, Durham, North Carolina.

Creative catalysis

G.W. Parshall

Homogeneous Transition-metal Catalysis: A Gentle Art. By Christopher Masters. ISBN hbk 0-412-22110-1; ISBN pbk 0-412-22120-9. (Chapman & Hall: 1980.) Hbk £20, \$25; pbk £9.50.

THE gentle art described in this book is the synthesis of organic compounds through the skilful application of transition metal complexes as catalysts. Dr Masters' purpose in writing this book was to stimulate enthusiasm for this art. He succeeds admirably because he has produced not just a readable description of the subject as it exists today but also a perceptive analysis of developing research areas

In the introductory chapter, Dr Masters describes the basic principles of bonding and reactivity in transition metal complexes in simple terms for the non-specialist. Phenomena such as oxidative addition, β -elimination and steric and electronic effects are illustrated with significant examples. The fundamentally kinetic nature of catalysis is stressed.

The heart of the book is a description of homogeneous catalysis as it is practised today. Fourteen of the two dozen or more major commercial applications of soluble transition metal catalysts are discussed in some detail. The processes described include the reactions of carbon monoxide with acetylenes, olefins and alcohols, the dimerizations of olefins and dienes, and the oxidations of several different types of hydrocarbons. Fundamentally organometallic processes such as the hydrogenation, isomerization, polymerization and metathesis of olefins are also included, even though industry usually chooses heterogeneous catalysts for these

operations. Some significant reactions like the hydrocyanation and hydrosilylation of olefins are omitted and others are simply mentioned. However, the reactions that are included are discussed expertly and with sufficient detail to satisfy the reader who is just becoming acquainted with the field.

The book closes with analysis of research trends in homogeneous catalysis. Nitrogen fixation, C-H bond activation and CO hydrogenation are cited as well-studied areas in which the crucial discoveries have yet to be made. Other topics such as bimetallic catalysis are suggested as profitable areas for research.

Homogeneous Transition-metal Catalysis will probably be used largely as a reference or introductory text for the

industrial chemist working in this field. It may also be used in teaching graduate-level courses in catalysis or organometallic chemistry. The emphasis on understanding and on research should appeal to the student.

Dr Masters has succeeded nicely in his objective to convey enthusiasm for homogeneous catalysis. It is a dynamic subject from both scientific and practical viewpoints and this emerges clearly from the text. The reader should be both stimulated and informed by perusal of this book.

G.W. Parshall is Director of Chemical Science in the Central Research and Development Department of E.I. du Pont de Nemours & Co., Wilmington, Delaware. His most recent book is Homogeneous Catalysis (Wiley, 1980).

Illumination on the light reactions

J. Barber

Photosynthesis: Physical Mechanisms and Chemical Patterns. By Roderick K. Clayton. Pp.275. ISBN hbk 0-521-22300-8; pbk ISBN 0-521-29443-6. (Cambridge University Press: 1981.) Hbk £17.50, \$32.50; pbk £6.95, \$11.95.

CONSISTENT with Roderick Clayton's outstanding talents as a scientist and author, I judge this, his latest book, as being first class. He has written a lucid and well-structured text which makes marvellous reading for the expert and also will satisfy the requirements of nonspecialists and students. He achieves this. in part, by "digressions" and by giving background "notes" in an appendix. The overall effect is to pass on the excitement of an active scientist who, with the qualities of a talented teacher, instinctively takes the reader through logical developments calling on the basic laws of photophysics, photochemistry and thermodynamics wherever necessary.

From the title it is clear that Clayton has concentrated on the "light reactions" of photosynthesis and consequently has restricted his discussion of carbon fixation (the "dark reactions") to one short chapter at the end of the book. It is inevitable that in producing a modern text on the light reactions of photosynthesis he should not only deal with oxygen-evolving organisms, but also with photosynthetic bacteria. The book has been divided into four distinct parts, each consisting of several chapters. Part I gives a historical account which introduces the reader to the basic concepts of the light reactions, including the nature and properties of the pigments and the existence of different types of photosystems. Part II then concentrates on the organizational aspects, while Part III deals with the photochemistry and associated transfer of electrons and protons. Part IV

is concerned mainly with photophosphorylation. At the end of each chapter the author has listed a few selected references for further reading. Also, the background notes in the appendix cite other key work.

Without any hesitation, I thoroughly recommend this book to specialists and non-specialists alike who have an interest in the more biophysical aspects of photosynthesis, whether it be from the research or the teaching point of view. I also think that those working in other areas of photobiology may find much of the basic substance of the book useful.

J. Barber is Professor of Plant Physiology in the Department of Pure and Applied Biology, Imperial College, University of London.

Medical malacology

Frank J. Etges

Freshwater Snails of Africa and their Medical Importance. By David S. Brown. Pp.487. ISBN 0-85066-145-5. (Taylor & Francis, London/American Malacologists Inc., Melbourne, Florida: 1980.) £25, \$55.

DAVID Brown is to be congratulated on writing an exceptionally fine treatise on the gastropod fauna of Africa, a reference book which will be set a standard of quality and content for many years. His stated goal of presenting a "comprehensive" account of this subject which will be of value to specialists and students of taxonomy, medical malacology and freshwater biology has surely been attained. This achievement is a tribute both to the broad experience of the author, and to the abilities of his many acknowledged

colleagues and consultants. Written by a single author, the book's structure and style are unified and integrated to a degree that multi-author publications rarely achieve.

Brown divides his subject matter into 12 chapters, two of which form essentially the first half of the book and provide a systematic synopsis of African freshwater snails; two short chapters cover snail-borne parasites of medical and veterinary importance in Africa, and the remainder deal with ecological, physiological and biogeographical aspects of snail biology, stressing snail hosts of schistosomes and approaches to snail population control.

The synopsis of more than 300 gastropod species is efficiently and accurately presented, including carefully documented descriptions of morphological and ecological characteristics, plus (usefully) a brief statement of their parasitological importance as real or potential intermediate hosts. The text is thoroughly illustrated with 128 photographic plates, line drawings and distribution maps, all arranged to maximum advantage. Here, as in all chapters, a comprehensive, wellselected citation of literature stresses work done in Africa or on material of African origin; inclusion of a large number of contributions from publications in southern Africa will be of special interest to readers unfamiliar with the volume and quality of this body of literature.

The two chapters on snail-borne parasites of medical and veterinary importance concentrate on snail-parasite interrelationships. The account of snails and schistosomes is, justifiably, given prominence since the impact of schistosomiasis on human beings is so great, and because so much more data is available for discussion of this complex. One deficiency in this section is the omission of the human disease, heterophyosis (admittedly referred to in the systematic description of its snail host, Pirenella), in favour of veterinary parasites such as paramphistomes, and a potentially occurring human disease, angiostrongylosis. Overall, I felt that the parasitological section was disappointingly brief, seeming as it did to be included as a kind of (unnecessary) justification for the rest of the book.

The remaining six chapters on ecological, physiological and biogeographical aspects of African snails are excellent; particularly outstanding is that on the biology of the genus *Bulinus*, a subject of great complexity and interest to biomedical scientists. Completing the book are a unique appendix, in which major methods of field and laboratory studies of snails and parasites are presented, and taxonomic and general subject indexes; I found the latter extremely useful and accurate, and reflective of the general editorial excellence of the book.

Frank, J. Etges is Professor of Parasitology at the University of Cincinnati.

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UNIVERSITY OF OXFORD

NUFFIELD DEPARTMENT OF PATHOLOGY AND BACTEROLOGY

TECHNICIAN (MICROBIOLOGY)

Technician required for preparation of class material for course in clinical microbiology. Duties will include assistance in research projects. The successful applicant will be offered training in diagnostic microbiology. Candidates should possess a degree in biological sciences or qualifications suitable for admission to courses leading to the special examination of the Institute of Medical Laboratory Sciences. Preference will be given to persons with training or experience in medical microbiology. Salary will be on the Whitley Council scale for Medical Laboratory Scientific Officers (£4,677 to £6,597) depending on qualifications and experience.

Applications, including curriculum vitae and the names of two referees to Dr M P E Slack, Department of Bacteriology, Level 7, John Radcliffe Hospital, Headington, Oxford OX3 9DU, from whom further details may be obtained. Tel: Oxford (0865) 64711 Ext 7617.

(9185)A

WE are seeking an independent investigator with skills and demonstrated competence in the broad field of cell biology. successful candidate is likely to have had five years post-doctoral experience and should be a Canadian citizen or a landed immigrant. It is expected that the appointee would have a strong research program, develop appropriate collaboration with other faculty, and provide an educational resource to colleagues on the nature of the functioning of eukaryotic cells. It is hoped that this appointment will provide a new dimension in scientific endeavour at McMaster and, at the same time, capitalize on some of the existing strengths within the faculty. Salary, academic rank and departmental affiliation will be negotiated. During the Canadian postal strike, interested candidates are invited to send curriculum vitae and three current letters of reference to: Dr D R McCalla, Faculty of Health Sciences, McMaster University, Dept of Biochemistry, PO Box 1406, Niagara 14302. Otherwise, Falls, NY applications should be sent to: Dr D R McCalla, McMaster University, Dept of Biochemistry, 1200 Main Street West, Hamilton, Ontario L8N (NW778)A

POST DOCTORAL — RESEARCH POSITION

for Biochemist-Cell Biologist available September 1, for two to three years, to identify, isolate and characterize insulin-like growth factor and α-thrombin receptors. (J. Biol. Chem. Vol 256: 2767, 1981) from cultured cells and tissues.

Experience in membrane protein chemistry, monoclonal antibody production, and/or ligand receptor characterization is desirable but not essential. \$15,000 to \$20,000 yearly depending on qualifications.

Send curriculum vitae and names of three references to Dr James F Perdue, Lady Davis Institute for Medical Research — Sir Mortimer B Davis — Jewish General Hospital, 3755 Cote St Catherine Road, Montreal, Que Canada H3T 1E2.

(NW760)A

FACULTY Position - Tenure track Assistant Professorship Eukaryctic Molecular Cell Biologist with interests in some facet of immunclogy. Preference will be given to an individual who has potential for and commitment to developing an independent research program and who has an interest in and an aptitude for teaching graduate, medical and dental students Position available during 1981-82 academic year. Salary commensurate with experience but not less than \$26,000 for a twelve-month appointment. Applicants should submit a letter outlining current and future research interests, curriculum vitae and bibliography and should arrange for three letters of reference to be sent to: Dr Roy Curtiss III, Department of Microbiology, Box 11 SDB, The University of Alabama in Birmingham, Birmingham, Alabama 35294. An Equal Opportunity/ Affirmative Action Employer

(NW776)A



Coláiste na hOllscoile Corcaigh
University College Cork

RESEARCH SCIENTISTS IN MICROBIOLOGY

required to work on the molecular Biology of Nitrogen Fixation in Root Nodule Bacteria

1 Geneticist/Molecular Biologist

preferably with a PhD to work on a project involving the genetic engineering of root nodule bacteria. Capabilities in recombinant DNA technology would be an advantage but is not essential as training will be provided.

2 Microbiologist/Microbial Physiologist

preferably with a PhD degree, to work on the physiology and regulation of CO₂ metabolism in rhizobium. This position is tenable in the first instance for one year with the possibility of renewal for a further year.

These positions are supported by the NBS and available from the 1st October 1981. Salaries will be on the appropriate national scales (IR £6,234 — post-doctoral max, including PRSI) with the point of entry negotiable.

Applications, containing full career details and the names of two referees should be sent as soon as possible to Dr. F. O'Gara, Microbiology Department, University College, Cork, Ireland, from whom further particulars are available.



Coláiste na hOllscoile Corcaigh University College Cork

Ireland

RESEARCH ASSISTANTSHIP GENETICS OF LACTIC STREPTOCOCCI

Applications are invited for a

RESEARCH ASSISTANTSHIP

(supported by the national Board for Science and Technology) to develop a transformation system in the lactic streptococci. Applicants should preferably have a PhD degree and research experience in genetics/microbiology. The appointment is initially for one year from 1st October 1981 and is renewable.

Salary scale:

PhD degree, MSc degree, IRE5,370 — IRE5,798; IRE4,417 — IRE5,073.

Applications with Curriculum Vitae and names and a addresses of two referees should be sent as soon as possible to Dr. C. Daly, Dairy and Food Microbiology Dept., University College, Cork, Ireland.

MIDLAND CENTRE FOR **NEUROSURGERY AND NEUROLOGY**

Electron **Microscopist**

(Basic/Senior Science Graduate)

Applications are invited from experienced graduate Electron Microscopists for this newly established post based in the Pathology Department of this specialist hospital.

The Department, located in new accommodation, is well equipped with several microtomes (LKB, Reichert, Sorvall) and two electron microscopes (Siemens).

The successful applicant will be expected to continue and develop the service, and research elements of the work in CNS. PNS and muscle pathology. He/she will be expected, and encouraged, to initiate and collaborate in research on an aspect of his/her choice.

Whitley Council Conditions of Service applicable, and salary will be in accordance with previous experience/qualifications.

Intending applicants are welcome to visit the laboratory by appointment, please contact Dr M V Salmon, Neuropathologist telephone 021-558 3232 Ext. 314.

Application forms and job descriptions may be obtained by writing to the Hospital Secretary, Midland Centre for Nuerosurgery and Nuerology, Holly Lane, Smethwick, Warley, West Midlands.

Closing date for receipt of applications 28th August 1981.



UNIVERSITY COLLEGE GALWAY **IRELAND**

> **DEPARTMENT OF MICROBIOLOGY**

POSTDOCTORAL RESEARCH **FELLOW**

Applications are invited for the post of Postdoctoral Research Fellow to work on an EEC-supported project on the effects of radiation on the blue-green algae (cyanobacteria). Applications from microbiologists, biochemists, geneticists, etc. should be sent within 2 weeks to: Professor James A. Houghton, Department of Microbiology, University College, Galway, (9193)A Ireland.

FREIE UNIVERSITÄT BERLIN

Klinikum Steglitz IMMUNOLOGY RESEARCH **UNIT WE05**

Position open for MD or PhD with experience in Immunology. Available for 3 years but can be extended. The appointee will have to work on mechanisms of lymphocyte activation and regulation of immune response by activated cells. Salary approximately £10,000 per annum (minus tax), depending on experience, age and family status.

Applications to Professor Diamantstein, Immunology Research Unit, Klinikum Steglitz, Freie Universität Berlin, Hinderburg-damm 30, D 1000 Berlin 45/ (W389)A Germany.

Leeds Area Health Authority (Teaching) **Eastern District** St James's University Hospital

MEDICAL LABORATORY SCIENTIFIC OFFICER

Required to work full time on the regional neonatal hypothyroid screening programme, based in the Department of Chemical Pathology at St James's University Hospital.

Applicants should preferably be State Registered, but other candidates, especially those with degrees in the biological sciences will be considered.

Further details are available from Dr R T Evans, tel. (0532) 33144 ext. 5562, under whose direction the successful candidate will work; or from Dr. R. B. Payne on ext. 5560.

Application forms (to be returned no later than Monday 24th August 1981) available from the Personnel Department, Gledhow Wing, St James's University Hospital, Beckett Street, Leeds LS9 7TF. Tel: (0532) 33144 (9228)A ext. 5103/5851.

MEDICAL RESEARCH COUNCIL CLINICAL AND POPULATION CYTOGENETICS UNIT (MOLECULAR GENETICS SECTION)

AND **MAMMALIAN GENOME UNIT**

POSTDOCTORAL SCIENTIST

Applications are invited for a SHORT-TERM NON-CLINICAL SCIENTIFIC post in this MRC Unit, tenable for 3 years. Candidates of immediately post-doctoral or equivalent status will be preferred.

The appointee will join a group that is using new methods of analysis to map the human genome. Candidates should preferably have a knowledge of genetics, including somatic hybrid cell genetics, and DNA analysis, though this is not essential. Experience of leading a small research group would be an advantage.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Further information may be obtained from Mr. Alasdair Douglas, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH42XU Tel: 031-332 2471, with whom applications — including a full CV and the names and addresses of two professional referees — should be lodged by Monday, 12th October, 1981.

UNIVERSITY OF LEICESTER DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSISTANT

Applications are invited for the post of research assistant (MRC funded) to work on the relationship between fibronectin, laminin, and collagen and the metastases of human carcinomas. The project will involve protein purification and immunochemical techniques.

Candidates should have a degree (at least 2.ii) in Biochemistry or related disciplines and be available to start as soon as possible.

Preference may be given to candidates with one or more years relevant postgraduate experience. Salary will be on the range £5,285 — £6,880, dependent upon qualifica-

Applications, including CV and the names of two referees, should be sent to Dr DR Critchley, Department of Biochemistry, University of Leicester, University Road, Leicester LE17RH. (9219)A

TUFTS UNIVERSITY SCHOOLS OF MEDICINE

DEPARTMENT OF PHYSIOLOGY Tufts University has instituted a search for

CHAIRPERSON

Department of Physiology. The Department of Physiology is expected to be a center of research and scholarly excellence, as well as providing leadership in physiology instruction for the School of Medicine, School of Dentistry, School of Veterinary Medicine, and the Sackler School of Graduate Medical Science.

Individuals interested in being considered for this position should direct their letters to Dr Seymour Reichlin, Chairman, Physiology Search Committee, Box 275, New England Medical Center Hospital, 171 Harrison Avenue, Boston MA 02111. Tufts has an affirmative action program, and encourages applications from women and members of minority groups.

(NW771)A

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS **HEAVY ION FUSION**

Applications are invited for a POSTDOCTORAL SENIOR RESEARCH

ASSISTANTSHIP

tenable for a period of three years from 1st October 1981. The successful candidate will participate in a programme of research devoted to semi-classical calculations of charge transfer processes, making use of the ALCHEMY quantum chemistry package on the Daresbury Laboratory computer. The research is to be directed towards the realisation of fusion by inertial confinement, an integral part of the Energy Programme.

Initial salary in the range £6,070 — £7,700 plus USS.

Applications (3 copies) naming three referees should be sent by 31 August 1981 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars are available. (9211)A

POSTODOCTORAL Fellow to conduct research in the area of peptide synthesis. Selected sequences and analogs of gastrointestinal polypeptide hormones and plasma apolipoproteins are to be synthesized using an automated peptide synthesizer. Synthesized peptides will be used for structure-function studies and for raising and characterizing antibodies used in immunochemical studies. Appointee will be expected to collaborate with research biochemists and physiologists in Divisions of GI and Endocrine surgery and Lipid Research. Substantial postgraduate experience in peptide synthesis is required. Applicants should send curriculum vitae and references to: Dr R F Murphy, Department of Surgery, University of Cincinnati Medical Center, 231 Bethesda Avenue, Cincinnati, Ohio 45267.

(NW774)A

Head of Microbial Biochemistry

Greenford, Middx.

An experienced research scientist is required to manage a multi-disciplinary department of about 40 scientists and technical staff. The major disciplines in the department are biochemistry and microbiology. Research is at present concentrated on the search for selective techniques, often involving target enzymes, aimed at discovering novel biological activity of natural or synthetic origin. Current projects include work with bacteria, fungi and viruses, the design of automated assays and the study of biosynthetic pathways to identify pharmacologically active agents. Planned further development of ecology and fermentation groups will aid the search, in a diversity of habitats, for micro-organisms of possible biological or medicinal application and will optimise the production of any activity of interest.

The successful candidate is likely to be aged 35-45 years and should have a proven innovative research record, together with administrative and communication skills, and the ability to motivate staff to a high standard of scientific achievement.

Major company benefits include a company car, profit sharing bonus scheme, noncontributory pension scheme and relocation assistance if appropriate.

Please write or telephone for an application form, or initially forward a full C.V. to:

The Personnel Manager, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex. Tel: 01-422 3434, ext. 2568, quoting ref. U/381.

Glax0 Group Research Ltd.

(9227)A

MICROBIOLOGIST

A vacancy has arisen for a graduate microbiologist to work in a small group developing mainly antimicrobial products for use in veterinary medicine.

The work will involve antimicrobial testing of existing and novel compounds, kinetic studies on antimicrobials *in vitro* and *in vivo*, determinative microbiology and field and experimental study support.

A minimum of a good honours degree is essential and preference will be given to candidates (male or female) who can offer relevant experience. Consideration may also be given to candidates with related experience in other fields.

Walton Oaks is pleasantly situated in the Surrey countryside. Conditions of Employment include flexible working hours, a non-contributory pension and life assurance scheme.

For application form (to the returned by 14 August) please telephone Tadworth 4444 Ext. 250 or write to Mrs. D. Pledge, Site Personnel Officer, Beecham Pharmaceuticals Research Division, Walton Oaks, Dorking Road, Tadworth, Surrey. (9186)A

Beecham Pharmaceuticals

MOLECULAR GENETICS Ph.D's

THE INTERNATIONAL PLANT RE-SEARCH INSTITUTE (IPRI) invites applicants for its rapidly expanding programs in advanced molecular genetics. Applicants with significant records of frontier achievements are invited to join a strong team of innovative scientific leaders in the pleasant San Francisco area.

Send résumé with recent publications to:

DIVISION OF MOLECULAR GENETICS, IPRI, 853 Industrial Road, San Carlos, Calif. 94070.

Equal Opportunity Employer, M/F

(NW780)A

WHY NOT JOIN US AT THE FRONTIER OF TOXICOLOGICAL RESEARCH!

The Food and Drug Administration's National Center for Toxicological Research located in Jefferson, Arkansas has two exciting and challenging positions available. Both positions are in the Senior Executive Service with maximum salaries of \$50,112.

One position, the Associate Director for Research, involves serving as key advisor to the Center Director and directing a multidisciplinary scientific and professional staff engaged in research programs in the areas of chemistry, carcinogenesis, mutagenesis, teratogenesis, microbiology, immunology and biometry. The other position, the Associate Director for Chemical Evaluation, also involves serving as key advisor to the Center Director but requires the direction of a multidisciplinary scientific and professional staff engaged in coordinating the comprehensive toxicological evaluation of chemicals and related activities in the areas of pathology, nutrition and diet preparation, and animal husbandry.

Both positions need individuals who have professional experience in directing broad scientific programs; ability to deal effectively with high level governmental officials, the scientific/academic communities, national/international medical and health-related organizations and others; and the ability to implement equal employment opportunity programs. Applicants should have a doctorate in chemistry, biochemistry, physiology or related biological sciences and an international reputation in toxicology, chemistry, comparative metabolism, biochemistry, molecular biology, or related biological/toxicological fields.

If you feel you are qualified send a curriculum vitae to Jim Keady, Division of Personnel Management (HFA-440), Food and Drug Administration, Parklawn Building, Room 9-90, 5600 Fishers Lane, Rockville, Maryland 20857. If you need more information call Mr. Keady collect, on 301-443 3634.

These positions can also be filled by current commissioned officers of the US Public Health Service, or by former officers willing to rejoin the Corps.

An Equal Opportunity Employer.

(NW785)A

INSTITUTE OF CHILD HEALTH University of London POST DOCTORAL RESEARCH SCIENTIST

required for three year project (commencing October 1981 or as soon as possible thereafter) to investigate the immunological factors controlling tolerance to food antigens and their disturbance in diseases caused by food allergy. Initial salary within the range £7,442 — £8,257 pa (inc. London Weighting) on scale 1A for Research and Analogous Staff.

Further information: Dr R J Levinsky or Dr M W Turner, Department of Immunology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. Tel 01-242 9789 Ext. 133/30/N. (9225)A

UNIVERSITY OF LIVERPOOL CENTRE FOR

BIOMATERIALS RESEARCH

Applications are invited for the post of Senior Research Assistant in the Centre for Biomaterials Research. Candidates should hold or expect to receive their PhD. Work concerns the interaction between tissue and implanted Carbonaceous material.

Initial salary within the range £6,070 — £6,880 per annum.

Application forms and further particulars may be obtained from The Registrar, The University, PO Box 147, Liverpool L69 3BX, by whom completed forms should be received not later than 28th August, 1981. Quote Ref. RV/856.

(9202)A

EUROPEAN MOLECULAR BIOLOGY LABORATORY

Applications are invited for a position at the European Molecular Biology Laboratory Outstation on the Deutsches Elektronensynchrotron (DESY) site at Hamburg as

HEAD OF OUTSTATION

The EMBL Outstation has been established to facilitate the use for biological research of the very high-intensity X-ray beams that emerge from the synchrotron and its storage ring DORIS. The Outstation, whose activities the Head will direct, has a staff of 15 and two functions, namely to provide services to visiting biologists wishing to use cynchrotron radiation and to carry out research in some important field of molecular biology to which techniques using high-intensity X-ray beams can make major contributions.

Applicants should be molecular biologists who have extensive experience of, and have made substantial contributions to, research in biological applications of diffraction or scattering methods, or other X-ray techniques. Experience of synchrotron radiation research and the design and construction of physical instruments will be an advantage. Applicants should have experience of leading research teams and of the administration and managerial problems involved. Knowledge of German will be helpful.

The salary offered to the successful candidate will be between DM 6000 and DM 7500 monthly, after deduction of tax, in addition to which certain allowances are payable, depending on personal circumstances.

Please write for an application form, quoting reference no. 81/13, to: The Director General, EMBL, Postfach 10.2209, D — 6900 Heidelberg, Germany. (W391)A

KING'S COLLEGE LONDON DEPARTMENT OF BIOPHYSICS POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship to study the regulation of globin gene expression during the development of the frog, *Xenopus laevis*. Expertise in Molecular biology and a knowledge of vertebrate development would be beneficial. Post tenable for up to 3 years from 1 October 1981. Salary in the range £6,475 — £7,700 pa plus £967 London allowance. USS.

Apply in writing as soon as possible with a curriculum vitae and the names and addresses of two referees, to Dr R K Patient, Imperial Cancer Research Fund, Mill Hill, London NW7 1AD. (9205)A

PHYSICIST OR PHYSICAL CHEMIST

for developments of sensitive, optical methods for trace gas measurements in the atmosphere.

Permanent position possible. Annual salary, depending on age or qualifications, in the range of 45,000 - 55,000 DM.

Curriculum vitae, summary of research interests, publication list and at least 3 references or letters of recommendations to be sent to Dr Paul Crutzen, Max-Planck-Institut fuer Chemie, Postfach 3060, D-6500 Mains/West-Germany before September 12, 1981. (W390)A

THE ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen AB2 9SB

RESEARCH WORKER TO STUDY RED DEER

Applications for an appointment in the Applied Nutrition Department of the Institute are invited. The work will be concerned with the elucidation of the nutrient requirement of red deer kept under lowland farm conditions and their ability to meet these requirements from grazed pasture, or from conserved and other feeds.

Applicants should hold a First or Upper Second Class Honours degree in Agriculture or a Biological Science. Previous experience of nutritional research, particularly with farm livestock or related to pasture utilisation, would be an advantage.

The appointment will be as Scientific Officer (salary £4,809 to £6,480 per annum) or Higher Scientific Officer (£6,075 to £7,999 per annum), grade and starting salary depends on experience. At least two years' relevant post graduate experience is required for Higher Scientific Officer grading.

There is a non-contributory pension scheme.

Further details of the post may be obtained from the Secretary of the Institute, to whom applications should be submitted together with the names and addresses of two referees. Closing date 31 August 1981.

(9233)A

MEDICAL RESEARCH COUNCIL

Virology Unit Institute of Virology University of Glasgow

CAREER (TENURE) OR SHORT TERM (3-5 YEARS) APPOINTMENT **VIRUS**

GENETICIST/MOLECULAR **GENETICIST**

Applications are invited for this Non-Clinical Scientific post which may be filled by either an MRC Career appointment (tenure to normal retiring age) or a Short-Term appointment of three (to five) years according to the age and experience of the successful candidate. A career appointment will be tenable at the MRC Virology Unit in the first

The Institute of Virology is seeking a senior scientist or alternatively a post-doctoral scientist with experience in Molecular Genetics, preferably (but not exclusively) with interests in herpesvirus and/or virusspecified enzymes. The appointment provides a unique opportunity to participate in a comprehensive and multi-disciplinary research programme centred on the Genetics and Molecular Biology of Herpesviruses. The Institute provides excellent facilities and technical support for all types of biological, biochemical and recombinant DNA research. The successful applicant will be expected to collaborate with other members of the Institute whilst pursuing a vigorous and independent research programme within the general framework of research on erpesviruses as agreed with the Director.

Remuneration will be at an appropriate point on the scales for University non-clinical Academic staff. Applicants should send a Curriculum Vitae giving full details of qualifications and experience and names and addresses of three professional referees, to the MRC Unit Director, Professor J H Subak-Sharpe, Institute of Virology, University of Glasgow, Church Street, Glasgow G11 5JR. Applications should be received by Friday, 11th September, 1981. (9240)A

KING'S COLLEGE LONDON (University of London) DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

POST-DOCTORAL RESEARCH ASSISTANT

financed by the Medical Research Council and tenable for up to 3 years commencing on 1st October, 1981 or as soon as possible thereafter.

The starting salary will be £6,070 plus £967 London Allowance.

Research work will involve studies on Structure, Function and Biogenesis of Ribosomes from Yeast Mutants and Mechanisms of Antibiotic Resistance.

Applications including a full curriculum vitae and the names and addresses of 3 referees to Dr Michael Cannon, Department of Bio-chemistry, King's College London, Strand, London WC2R 2LS by 17th (9184)AAugust, 1981.

ENTOMOLOGIST

For National Collection of Insects

The British Museum (Natural History) is primarily an institution for taxonomic research and the Department of Entomology contains the largest and most comprehensive collection of insects in the world.

The successful candidate will carry out taxonomic research. particularly on parasitic wasps of the family Ichneumonidae and will be concerned with the improvement of the National Collection of Insects through collecting, rearing and exchange. The work includes curatorial and supervisory duties.

Candidates should have a 1st or 2nd degree, preferably specialising in Entomology at least 2 years postgraduate experience, including experience of current trends in systematics, such as mathematical and phylogenetic approaches, plus field experience.

> Appointment as Senior Scientific Officer £8,660 -£10,635 or Higher Scientific Officer £7,090 - £9,015. Level of appointment according to qualifications and experience. Salaries under review.

For futher details and application form (to be returned by 21 August 1981) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/70/DK.

(9223)A

British Museum (Natural History)

UNIVERSITY OF THE WITWATERSRAND Johannesburg

DEPARTMENT OF GEOLOGY

LECTURER/SENIOR LECTURER IN STRUCTURAL GEOLOGY

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin, for the above post in the field of structural geology. Applicants should have a PhD degree and be fully conversant with modern developments in structural geology, and methods of structural analysis and their application. Although not a prerequisite for appointment, an interest in the application of these techniques to mineralised terranes would be an advantage in view of the Department's interest in mineralisation in Archaean terranes and other mineralised provinces.

The starting salary will be determined according to quali-fications and experience within the following ranges: Lecturer: R10,995

— R19,230 pa. Senior Lecturer:
R14,370 — R20,850 pa.

Further details relating to this post and the attractive fringe benefits are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WClV 7HE, or from the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa 2001, with whom applications, including the names of three referees, should be lodged not later than 1st September 1981. (9214)A

The Royal Marsden Hospital, Fulham Road, London SW3

Cytogeneticist

required immediately to work at the above postgraduate teaching hospital on a research project designed to study the relationships between chromosomes and prognosis in acute leukaemia. The post is funded by a grant from the Medical Research Council, and is for a period of three years. The salary is on the Basic Biochemical grade scale: £5,366 to £7,637 p.a. according to qualifications and experience.

Application form from the Personnel Officer — Tel: 01-352 8171 Ext: 446.

Closing date 20th August 1981.

CHURCHILL HOSPITAL **DEPARTMENT OF MEDICAL GENETICS** SCIENTIFIC OFFICER

required in the Cytogenetics Laboratory, which provides a full range of cytogenetic and cell culture techniques, and is responsible for all diagnostic cytogenetics in the Oxford Region. Training could be given to a suitably qualified but inexperienced applicant.

Applications, including the names and addresses of two referees, should be sent to Dr. Dick Lindenbaum, Department of Medical Genetics, Old Road, Headington, Oxford OX3 7LE, within two weeks of the publication of this advertisement.





Yale University

DIRECTOR

PEABODY MUSEUM OF NATURAL HISTORY

Applications are invited for the position of Director of the Museum at the rank of full professor, and with appointment (according to field) to the Departments of Biology, Geology and Geophysics or Anthropology. Yale University is seeking a scholar of international reputation who will develop interdisciplinary research and teaching programs in the natural sciences through the Peabody Museum and the affiliated academic departments.

Applications, including a curriculum vitae and the names of three references, should be sent to Professor Keith S. Thomson, Dean of the Graduate School of Arts and Sciences, Yale University, P.O. Box 1504A, Yale Station, New Haven CT 06520. Closing dates for applications — September 30, 1981.

Yale University is an equal opportunity, affirmative action employer.

(NW773)A

Research Institute Director

The University of Nebraska Medical Center invites nominations and applications for the position of Director, Eppley Institute for Research in Cancer and Allied Diseases. The Institute is federally funded for research in chemical and environmental carcinogenesis.

Recently new research support has been provided by the state. Qualifications include an earned doctorate, outstanding research accomplishments, and administrative skills, Responsibilities include administration and leadership of the Institute in developing and administering research programs.

Applications and nominations, accompanied by a curriculum vitae and the names of three references, should be received by October 1, 1981. Direct communications to: Dr. David T. Purtilo, Chairman of the Search Committee, University of Nebraska Medical Center, 5001 Wittson Hall, 42nd and Dewey Avenue, Omaha, Nebraska 68105.

THE UNIVERSITY OF NEBRASKA
IS AN AFFIRMATIVE ACTION/EQUAL
OPPORTUNITY EMPLOYER

(NW779)A

LA TROBE UNIVERSITY Melbourne, Australia

SCHOOL OF BIOLOGICAL SCIENCE DEPARTMENT OF GENETICS AND HUMAN VARIATION

LECTURER IN GENETICS (FIXED TERM)

A position is available for a lecturer with research experience in eukaryote gene structure and expression, possibly with an interest in development. Applicants should have a strong training in general genetics, and teaching in prokaryote genetics will be required.

The appointee will be actively involved in research, including the supervision of honours and postgraduate students. The facilities of the Department include glasshouses, growth cabinets, constant temperature rooms, a Cl containment room, analytical and other centrifuges, and routine items of potential assistance for a molecular geneticist.

Although negotiable, it is hoped that the appointee will commence duties early in 1982.

This position is for a fixed term of four years. The University reserves the right not to make an appointment to the position.

Salary: \$A19,132 — \$A25,132.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 9th August 1981. (9183)A

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH

SERUM & VACCINE DEPARTMENT DEPUTY HEAD

Applications are invited from suitably qualified Medical or Science graduates for the above post in our laboratories situated on the outskirts of Johannesburg.

The incumbent will be responsible to the Head for the day to day running of the Serum and Vaccine Production units and must assume responsibility for the running of the department in the absence of the Head.

Duties will, inter alia, include supervision of the administration of the department, which includes aerobic and anaerobic vaccine production units, a serum production unit, dispensing, blending and bottling unit, labelling and an animal house for small laboratory animals, research and development of new vaccines and equine antigens and the improvement of existing processes.

In addition to a competitive salary, the following fringe benefits apply, namely, staff pension fund, subsidised medium aid scheme, annual service bonus (13th cheque), 30 days' annual leave and housing bond allowance, where applicable.

A member of the Institute staff will interview applicants in London in the latter part of September or early October. All applications, including detailed curriculum vitae should reach the undermentioned by 31 August 1981, in order that an interview may be arranged.

Head: Personnel Services, SAIMR, PO Box 1038, Johannesburg, Republic of South Africa. Telephone: 011-725 0511 (W388)A

UNIVERSITY OF LONDON British Postgraduate Medical Federation

CARDIOTHORACIC INSTITUTE CARDIAC MUSCLE RESEARCH UNIT

PHYSIOLOGIST — POST-DOCTORAL

A vacancy exists for a post-doctoral physiologist to work on a project utilising intracellular ion-selective microelectrodes in cardiac muscle. Previous experience in electrophysiology would be an advantage. The appointee will work in a multidisciplinary team with extensive and varied research interests relating to the heart.

Salary on appropriate point of postdoctoral Research Assistant scale 1A, currently £6,070 — £10,575 + £967 London Weighting Allowance. The post is superannuable in the Universities' Superannuation Scheme

Further details can be obtained from Dr Poole-Wilson (01-486 3043). Applications with full curriculum vitae, list of publications and names and addresses of three referees, to the Secretary, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX. Closing date 28th August 1981. (9216)A

AGRICULTURAL RESEARCH COUNCIL A VETERINARIAN

Is required at I.R.A.D. to join a team investigating the role of virus and bacterial pathogens in the aetiology of outbreaks of diarrhoea in calves and piglets.

Candidates must be members of the Royal College of Veterinary Surgeons; some bacteriological experience is essential and a higher degree would be an advantage. Experience in organising programmes of disease investigation on farms would be useful.

The person appointed will be encouraged to collaborate with a team working on genetic and acquired resistance to E. coli enfections.

Salary scale: Veterinary Research Officer £7,999 — £12,540 p.a. depending on age and experience (under review). Noncontributory pension scheme.



Application forms from: Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, Berks RG16 0NN quoting ref: 466. Closing date: 15/8/81. (9212)A

In the Faculty of Technical and Natural Sciences (Technisch-Naturwissenschaftliche Fakultät) of the Johannes Kepler University, Linz, Austria, the position of a full PROFESSOR IN BIOPHYSICS

is vacant

The candidate for this post will be expected to devote his research activites to experimental membrane biophysics as his main effort. His teaching should be focussed on which enable graduate courses, which enable graduate students in physics, chemistry, computer sciences and mathematics to acquire knowledge in biophysics. Applicants should be proficient in German.

Applications including a curriculum and a list of publications should be sent before October 30th, 1981, to the Dean (Dekan) of the Faculty, Altenbergerstr. 69, A-4040 Linz, Austria. Dean Prof Dr Bruno (W394)A Buchberger.

RESEARCH TECHNICIAN (Grade 3)

Applications are invited from suitably qualified persons interested in working on DNA gyrase and the enzymology of DNA supercoiling in bacteria and eukaryotes. Experience in the techniques of DNA bio-chemistry, protein chemistry or enzymology an advantage. This post, funded by the Cancer Research Campaign, commences on or around 12 October and is renewable annually for up to three years. Salary in the range £4,672 — £5,473 plus £1,027 London Allowance. 37½ hour week, Monday to Friday. Five weeks annual holiday. Superannuation

Apply in writing by September 7 1981, with a curriculum vitae and the names and addresses of two referees, to Dr L M Fisher, Laboratory of Molecular Biology, Building 2, Room 322, National Institutes of Health, Bethesda, Maryland, USA 20205. (NW783)A

UNIVERSITY OF ABERDEEN POST-DOCTORAL RESEARCH FELLOW (supported by

The British Heart Foundation)

Applications are invited for the above post, tenable for a maximum of three years, from protein biochemists to work on the structure of fibrin and fibrinogen in human atherosclerotic plaques, one aspect of a long-term research programme on atherosclerosis for which the Research Fellow will take major responsibility. Experience in modern methods of protein separation essential.

Salary on range 1A scale, starting point up to a maximum of £6,985 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 21 August 1981. (9210)A

INSTITUTE OF CHILD HEALTH University of London **ELECTRON MICROSCOPY** SCIENTIFIC OFFICER

required to work with a group investigating pulmonary vascular disease in children. Applicants should have experience in fixation of human tissue and preparation and staining of ultrathin sections for examination in a Transmission Electron Microscope. Some knowledge of immunolabelling techniques for light and electron microscopy would be an advantage.

Whitley Salary Scales for Medical Laboratory Scientific Officers, according to age, qualifications and experience.

Application forms may be obtained from Miss Janet Keeble, Administrative Assistant (203906), Institute of Child Health, 30 Guilford Street, London WC1N2EH (telephone 01-242 9789) N.

(9224)A

CSIRO

AUSTRALIA

Research Scientist (Research Fellow)

Division of Mineral Chemistry Port Melbourne, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,400 employees - 2,500 of whom are research and professional scientists located in Divisions and Sections throughout Australia

Field: Hydrometallurgical Treatment of Gold Ores. General: The Division conducts research in the fields of extractive metallurgy, electrochemistry, surface chemistry, and solid-

state chemistry. With the objective of developing improved methods of treating Australian gold ores, the Division is undertaking a research project on novel leaching methods for recovery of gold and

Duties: To conduct investigations into novel gold and silver recovery processes. In particular, to study the chemical and mineralogical factors which effect the dissolution rate and extraction of these metals from ores.

Qualifications: PhD or equivalent in an appropriate branch of chemistry, chemical metallurgy, or chemical engineering

Salary: Research Scientist (Research Fellow) \$A19,662 -\$A24,100 p.a.

Tenure: Two years initially. Superannuation benefits available. Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number M9563 should reach: The Personnel Officer, Australian Scientific Liaison Office, Australia House, Strand, London WC2B 4LA by 28 August 1981. Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036.

Current vacancies in CSIRO appear on PRESTEL page 252903.

Molecular Biologists

Enzo Biochem, Inc. of New York is seeking qualified scientists to join its research program in recombinant DNA. We are interested in persons who have at least two years of specific recombinant DNA research. The growth of Enzo's activities in this area have created the need for several Ph.D. level scientists.

The recombinant DNA division is cloning and sequencing genes both from mRNA and from genomic DNA. We are also developing new cell and vector systems designed to improve the level of expression of cloned genes in several bacterial species as well as in yeast and plants.

Our new research facilities are located in New York's Soho district. This is a pleasant and superbly "urban" neighborhood. We are an equal opportunity employer M/F.

Persons interested should send a copy of their

Personnel Department, Enzo Biochem, Inc. 325 Hudson Street, New York, New York 10013

(NW792)A



AGRICULTURAL ZOOLOGIST SENIOR/HIGHER/SCIENTIFIC OFFICER £4,809 — £9,619 (under review)

Age limit: Applicants must not be more than 55 years of age on the closing date (ie born on or after 20th August 1926)

Closing date: 20 August 1981 Job Ref. SB 81/81/NN

Tel. ext 256

DEPARTMENT OF AGRICULTURE (NORTHERN IRELAND) AGRICULTURAL ENTOMOLOGY RESEARCH DIVISION

The appointment will be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level.

The successful applicant will be expected to conduct both basic and applied research in the field of Agricultural Entomology and/or Plant Nematology and may also be responsible for the provision of specialist advice.

Experience in the use of electrophoretic techniques is desirable but not essential.

The successful applicant may also be required to undertake teaching duties in the Faculty of Agriculture and Food Science, The Queens University of Belfast.

Senior Scientific Officer

Applicants must possess a first or second class honours degree in Zoology, Agricultural Zoology or a related subject and at least 4 years' appropriate post graduate experience in a relevant field.

Higher Scientific Officer

Applicants must possess an honours degree as above and at least 2 years' appropriate post graduate experience in a relevant field.

Scientific Officer

Applicants must possess an honours degree as above.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available, including level of academic attainment and relevant experience to be best qualified.

Salary Scales (under review) Senior Scientific Officer

£7,644 — £9,619 £6,075 — £7,999 £4,809 — £6,480

Higher Scientific Officer
Scientific Officer
Scientific Officer

In addition there is a non-contributory pension scheme apart from a 1 % % deduction from salary to cover widow's benefits in the case of male officers.

Grading and starting salary will be related to qualifications and experience.

The post is open to both men and women. Please write or telephone for an application form (using the extension number indicated and quoting the job reference) to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585). Completed forms must be returned to arrive not later than the closing date stated. (9187)A

NORTHERN IRELAND CIVIL SERVICE

NATIONAL BIOLOGICAL STANDARDS BOARD DEPARTMENT OF VIRAL PRODUCTS

POST DOCTORAL SCIENTIST

An opportunity has arisen for a research scientist to join a group working at the Institute on the genetic structure of polioviruses with particular reference to studies of poliovaccines used in man.

The appointment is of a three year duration only and is suitable for a candidate who has recently completed a PhD degree in a relevant field. The successful candidate should have experience in virology and/or nucleic acid biochemistry.

The salary payable is in accordance with qualifications age and experience on scale £7,037 - £10,717 p.a. inclusive.

Application forms and further details from Personnel Officer, N.I.B.S.C., Holly Hill, Hampstead, London NW3 6RB. Telephone 01-435 2232, quoting reference n.o. SG 293. Closing date 28 August 1981. (Informal enquiries please contact Dr. G. Schild).

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of POSTDOCTORAL RESEARCH FELLOW

and a post of PREDOCTORAL

RESEARCH ASSISTANT in the above Department for work on an MRC funded project on molecular organisation and expression of androgen-responsive genes.

Candidates for the post of Research Fellow should have, or shortly expect to have a PhD, together with experience in Molecular Biology, Recombinant DNA Technology or related areas. For the post of Research Assistant, a good honours degree in Biochemistry, Genetics, Microbiology or a related subject is required and laboratory experience would be an advantage.

Both posts available from 1 October 1981 for a fixed period of up to three years. Salary on the IA Scale for Research and Analogous Staff (Research Fellow) (£6,070 — £10,575), or the IB Scale (Research Assistant) (£5,285 — £7,700), according to age, qualifications and experience.

Informal enquiries may be made to Dr S J Higgins. Telephone Leeds 31751 ext. 7439.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/37/D. Closing date for applications 28th August 1981.

(9204)A

THE ROWETT RESEARCH INSTITUTE Bucksburn, Aberdeen AB2 9SB

DEPARTMENT OF MICROBIAL BIOCHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship to join a team undertaking research on the enzymatic conversion of the carbohydrates of straw into soluble sugars. Initially the work will be concerned with the isolation of mutant strains of fungi with improved capacities for producing polysaccharide-hydrolysing enzymes.

The project is funded in part by the Commission of the European Communities and this appointment will be for a period of two years in the first instance, with probable extension to three years. The post will be supported by non-graduate assistants.

Candidates should have a good Honours degree and a PhD degree and relevant experience in microbiology, preferably including isolation of mutants.

Starting salary, depending on qualifications and experience, will be in the range £6,075 to £6,683 per annum (scale currently under review). Superannuation Scheme.

Applications, including curriculum vitae and the names of two referees, should be sent to the Secretary of the Institute, as soon as possible.

(9234)A

SENIOR PROFESSORSHIP IN INORGANIC CHEMISTRY

Professor, University of Florida, inorganic chemistry, with an active program in internationally recognized inorganic chemical research. Teaching duties may include undergraduate and graduate level inorganic courses. Top-level salary with major departmental research support.

Preferred starting date: August, 1982. Direct inquiries or applications to Professor Harry H Sisler, Chairperson of Search Committee, Department of Chemistry, University of Florida, Gainesville, FL 32611.

Applications should include complete curriculum vitae, bibliographies of publications, indication of on-going and probable future research interests, and a list of three or more references who will be asked to supply letters of recommendatin (a university personnel requirement). The University of Florida is an Affirmative Action/Equal Employment Opportunity Employer. Application deadline: November 15, 1981. (NW766)A

TUMOR IMMUNOLOGY

PRE/POSTDOCTORAL

position available immediately. Monoclonal Ab, characterization for human tumor Ag.

Send CV, three ref to; Dr Daniel Eskinazi, USC, PO Box 77912, GER 323, Los Angeles, CA 90007 EOE/MFH (NW765)A

UNIVERSITY OF THE WITWATERSRAND

Johannesburg and

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH CHAIR OF CLINICAL BIOCHEMISTRY

Applications are invited for appointment to the above post on the joint staff of the University and the Institute in the School of Pathology. Although preference will be given to medically qualified persons, applicants with a non-medical background will also be considered.

The salary attached to the post is R29 190 per annum and in certain circumstances there is provision for generous remuneration for approved overtime duties.

The appointment will be made on University conditions of service but provision exists for the successful applicant (if already a member of the Institute staff) to elect to remain on Institute conditions.

Intending applicants should obtain the information sheet relating to this post from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa 2001, with whom applications should be lodged not later than 31 August 1981. (9213)A

ASSOCIATE PROFESSORSHIP (C3) WITH TENURE AVAILABLE AT THE INSTITUTE OF NEUROBIOLOGY DEPARTMENT OF BIOLOGY UNIVERSITY OF HEIDELBERG (EOAAE)

Candidates should be experienced in developmental and/or cell biological research and interested in genetic, biochemical and/or cellphysiological studies of higher nervous systems.

The position includes participation in teaching biology.

Send (until October 15, 1981) application including curriculum vitae and publication list to: Dr. Melitta Schachner, Institut Fuer Neurobiologie, Im Neuenheimer Feld 504, 6900 Heidelberg, Fed. Rep. Germany. (W393)A

THE UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSIOLOGY FACULTY OF MEDICINE AND DENTISTRY

Applications are invited for the post of Research Fellow 1A to work on the role of phospholipases in the control of membrane properties of skeletal muscle. Applicants should have experience in membrane biochemistry or enzymology.

Salary £6,070 — £10,575 with superannuation. Maximum starting salary £6,880. Post supported by the Wellcome Trust for three years.

Further information from Dr ME Smith, Physiology, 021-472 1301 ext. 244.

Applications (three copies) to Assistant Registrar, Medical School, Birmingham B15 2TJ by 22nd August 1981. Quote Ref. RF/Phys/ 581. (9222)A

UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURER IN PHYSICS

Applications are invited for the position of Lecturer in the Department of Physics. The Department has recently accepted responsibility for teaching some of the courses in Computer Science (most of which are taught by the Computing Centre staff) and will shortly increase teaching of electronics and microelectronics at postgraduate level. Consequently preference will be given to candidates with strengths and experience in these areas.

The appointee will be expected to pursue research in one of the fields currently pursued in the Department (atomic and laser physics, radio and space physics, environmental and applied physics), or in electronics. The Department has recently occupied a well-appointed, modern building and has several mini and microcomputers for teaching and

Salary: NZ\$19,140 — NZ\$23,520 per annum.

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Registrar, PO Box 56, Dunedin, New Zealand.

Applications close on 30 September 1981. (9217)A

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

PHYSICAL SCIENCES DEPARTMENT A GRADUATE

is required to work for 18 months on the effect of heat on the proteins and enzymes of human milk. The work involves a study of the kinetics of their heat denaturation to improve knowledge of the effects of pasteurisation treatments on human milk.

Qualifications: First or Upper Second Class Honours degree in biochemistry or food science.

Appointment, for a limited period of only 18 months, will be as Scientific Officer; current salary scale £4,809 to £6,480.

Application forms are obtainable from the Secretary, NIRD, Shinfield Reading RG2 9AT. Quote reference 81/22

Closing date: 14 August 1981 (9162)A



Royal Postgraduate Medical School

(University of London) CLINICAL PHARMACOLOGY TECHNICIAN

required to join a group investigating cell surface receptors that regulate mast cell degranulation. Duties will include measurement of mediator release, adenylate cyclase activation, methylation of membrane lipids, and quantitation of surface receptors by radio-ligand binding. Applicants should have a degree or HNC in biochemistry or an appropriate subject. Experience in the following an advantage: handling of radiolabelled compounds, receptor pharmacology, cell culture, immunology, but training will be given. The post is funded by the Wellcome Trust for 3 years with possible renewal for a further 2 years. Salary on the scale £5,427 — £5,914 inclusive of London

£5,914 inclusive of London Allowance a year (under review).

Application forms and further details are available from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 20/305.



ALBERTA HERITAGE MEDICAL FOUNDATION RESEARCH POSITIONS IN ENDOCRINOLOGY

The Endocrine Research Group at The University of Calgary, Faculty of Medicine, Calgary, Canada, will be seeking up to four new faculty members over the next two years. Although junior faculty positions are envisioned, applications from more senior investigators will be considered. Preference will be given to those interested in molecular or clinical endocrinology with expertise in recombinant DNA technology, peptide synthesis and/or microsequencing, membrane chemistry or in the study of hormone action in tissue culture systems.

Enquiries, including a complete curriculum vitae and at least two personal references, should be directed to:

Dr. Y. Lefebvre, Head Endocrine Research Group, Faculty of Medicine Health Sciences Centre 2961 The University of Calgary 3330 Hospital Drive N.W. Calgary, Alberta T2N 4N1

(NW786)A

PROFESSORSHIP in the Clinical Smell and Taste Research Center at the University of Pennsylvania. Established scientist working in olfactory neurophysiology in Physiology and/or Otorhinolaryngology Department. Based in VA Hospital. Preferably with an MD degree. Write to Dr James B Snow, Jr, Department of Otorhinolaryngology and Human Communication, 5th Floor Silverstein, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104 and provide three letters of reference. The University of Pennsylvania is an Equal Opportunity/ Affirmative Action Employer.

(NW751)A

UNIVERSITY of Leeds Rheumatism Research Unit. Laboratory Research Technician Grade 3 required for Clinical Pharmacology Unit, Royal Bath Hospital, Harrogate, North Yorkshire. The work will include biochemical and drug assays in biological fluids. Applicants must have at least ONC or equivalent qualification, and previous laboratory experience. The appointment will be for 2 vears initially from 1st October 1981. Salary scale £4,672 £5,473. Applications giving details of age, education and experience to Dr ME Pickup at the Harrogate address. (9198)A

TEMPORARY LECTURER GRADE II IN MICROBIOLOGY

(£6,462 — £10,431)

Applications are invited for a Temporary appointment starting on 1 September 1981 (or as soon as possible after that date), for two academic terms ending on 30 April 1982. Applicants should have a first degree and post graduate experience in Microbiology or a closely related subject.

Further details and form of application from the Assistant Director (Administration) Trent Polytechnic, Burton Street, Nottingham NG1 4BU. Closing date 10 August 1981.

TRENT POLYTECHNIC NOTTINGHAM

TRANSGENE

STRASBOURG - FRANCE

TRANSGENE was launched by leading industrial and financial French groups.

TRANSGENE carries out high level research oriented towards applications of medical and industrial importance.

TRANSGENE is now expanding its laboratory and invites applications from:

MOLECULAR GENETICISTS

EXPERTS IN RECOMBINANT DNA TECHNIQUES AND CHEMICAL SYNTHESIS OF DNA

IMMUNOLOGISTS

from the post doctoral level to group leaders to join its first team of highly qualified scientists

Expertise in the use of Bacillus subtilis, Yeast and Eucaryotic cells as cloning systems is of special interest.

Salaries will be competitive and commensurate with qualification.

Knowledge of French is not necessary.

STRASBOURG is a pleasant historical town, located in the heart of Europe, very close to GERMANY and SWITZERLAND.

The laboratory is located on the University campus and enjoys close contacts with well known groups in fundamental research.

For confidential consideration, send a CV, a list of publications and indication of salary requirements to:

> TRANSGENE S.A. attn J-P LECOCQ B.P. 146 67028 STRASBOURG CEDEX

(W392)A

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF BIOCHEMISTRY **FIXED TERM (3 YEAR) LECTURER**

Applicants with PhD invited to undertake teaching duties to science and medical students, and participate in the ongoing research programme into the biogenesis of mitochondria. Candidates should have a general background in biochemistry with research experience in technique of molecular biology such as DNA cloning and sequencing.

Applicants with experience in yeast studies preferred, but this is not essential. Enquiries to Professor A W Linnane FRS, FAA in the University. Salary: \$A19,821 — \$A26,036 pa.

Applications including Ref no 30212, curriculum vitae and 3 referees to the Registrar, Monash University, Clayton, Victoria 3168, Australia with copy to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF by 14/8/81. Conditions of appointment the can be obtained from (9201)A Association.

GEOPHYSICIST

Faculty position for 12-month, tenure-track appointment. A seagoing marine seismologist with interests in seismic reflection, refraction or microseismicity is sought. Candidates with strong backgrounds in non-marine seismology or other branches of marine geophysics will also be considered. Duties include maintaining active research programs and obtaining outside funding, teaching graduate courses and supervising graduate students. Rank is Associate Professor.

Applicants who meet all requirements, but have less required for Associate Professor rank, will be considered for appointment at the rank of Assistant Professor. Salary \$24,000 to \$37,000, commensurate with experience.

Send résumé and names of three references by 1 October 1981 to G Ross Heath, Dean, School of Oceanography, Oregon State University, Corvallis, Oregon, 97331. OSU is an Affirmative Action/ Equal Opportunity Employer

(NW789)A

LOTHIAN REGIONAL COUNCIL NAPIER COLLEGE OF **COMMERCE AND** TECHNOLOGY SENIOR LECTURER A IN CHEMISTRY

Salary on Scale: - £12,357 (Bar) - £14,019 £11.112 required in the Department of Chemistry to promote the research and consultancy interests of the Department and a knowledge of instrumental methods of analysis and the computer control of such instruments would be of particular interest. In addition, the Senior Lecturer would be expected to share the administrative load of the Department and take part in the further development of the BSc Applied Chemistry Course.

Applicants should possess an Honours Degree and/or equivalent professional qualifications, together with a Higher Research Degree and must have substantial relevant research and teaching experience. Recent Industrial experience would be an added advantage.

Application forms and further particulars from: The Administrative Officer (Personnel), Napier College of Commerce and Tech-nology, Colinton Road, Edinburgh EH10 5DT. Closing date: 28th August 1981. (9206)A

UNIVERSITY COLLEGE LONDON

Holmbury St Mary Dorking, Surrey DEPARTMENT OF PHYSICS AND ASTRONOMY

MULLARD SPACE SCIENCE LABORATORY

Three postdoctoral research assistantships financed by the Science and Engineering Research Council and commencing on or after 1 October 1981 are available to space scientists to participate either in the development of imaging detectors for use at X-ray and optical wavelengths, or in a programme of research into the physics of plasmas near the Earth, including the analysis of data from the ESA GEOS spacecraft and the preparation of instruments for the Giotto Comet Halley Flyby and Ampte spacecraft. Commencing salary in the range £6,070 — £7,700 plus USS membership.

Application, with curriculum vitae and the names of two referees, should be made to Professor R L F Boyd CBE, FRS, at the above address.

(9190)A

RESEARCH ASSOCIATE \$16,000

Effective immediately PhD in biochemistry or molecular biology. Experience necessary in area of molecular biology, particularly in recombinant DNA work. Project involves carcinogengenome interactions. Send curriculum vitae and three letters of reference to: Dr. Edward Bresnick, Department of Biochemistry, University of Vermont, Burlington, Vermont (NW787)A

UNIVERSITY OF **BIRMINGHAM**

DEPARTMENT OF BIOCHEMISTRY Applications are invited for a post of

RESEARCH ASSOCIATE

for one year (but may be extended) to ioin a group working on characterisation of muscle protein and muscle nerve interaction. The post involves producing and characterising monoclonal antibodies to myofibrillar proteins and is supported by a grant from the Muscular Dystrophy Group of Great Britain to Professor S V Perry. Research experience in protein chemistry and acquaintance with tissue culture techniques will be an advantage.

Salary within the range £5,285 -£6,070 depending on age, qualifications and experience.

Applications (2 copies) naming 2 referees should be sent by 31st August to Miss J Nelson, Senate Registry, University of Birmingham, PO Box 363, Birmingham B15 2TT, from whom further particulars may be obtained. (9220)A

RESEARCH TECHNICIAN

Position available immediately Applicants should have several years experience in recombinant DNA technology and electron microscopy of nucleic acids. This is a grant sponsored position.

Applications should be sent to: Dr R G Deeley, Department of Biochemistry, Queens University, c/o General Delivery, Watertown, New York 13601, (613) 547-2843.

(NW775)A

SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH PATHOLOGISTS/SCIENCE **GRADUATES**

Applications are invited for posts in Histopathology, Microbiology, Haematology, Clinical Biochemistry, Immunology, Tropical Pathology and Human Genetics. Some of the vacancies are within the School of Pathology, and these are joint posts on the establishment of the Institute and the University of the Witwatersrand. Others are research posts in special fields.

In addition to a competitive salary, the following fringe benefits apply, na rollowing iringe benefits apply, namely, staff pension fund, subsidised medical aid scheme, annual service bonus (13th cheque), 30 days' annual leave and housing bond allowance, where applicable.

A member of the Institute staff will interview applicants in London in the latter part of September or early October. All applications including detailed curriculum vitae should reach the undermentioned by 31 August 1981, in order that an interview may be arranged.

Head: Personnel Services, SAIMR, PO Box 1038, Johannesburg, Republic of South Africa. Telephone: 011-725-0511.

. (W387)A

RECOMBINANT DNA TECHNOLOGIST

Technicians with BA or MS with experience in:

- 1. Bacterial culture and phage growth.
- 2. Nucleic acid isolation and purification.
- Gel electrophoresis, radioactive labelling and hybridization of nucleic acids.
- 4. Molecular cloning (cDNA and/or genomic DNA cloning).



Send Résumé to:

Applied Molecular Genetics, Inc. 1892 Oak Terrace Lane, Newbury Park, CA 91320, (805) 499-3617.

Equal Opportunity Employer.

(NW777)A

UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURESHIP (SCIENCE) IN ORAL BIOLOGY

Applications are invited for appointment to a Lectureship (Science) in the Department of Oral Biology in the Faculty of Dentistry. The successful applicant will have interests and experience appropriate for research and teaching in oral microbiology and his/her principal purpose is the development of collaborative research with clinical staff in the School of Dentistry. In addition, some teaching at undergraduate and postgraduate levels is expected.

Salary: NZ\$19,140 — NZ\$23,520 per annum.

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, PO Box 56, Dunedin, New Zealand.

Applications close on 30 September 1981. (9218)A



LONDON OFFICE
Jean Neville
4 Little Essex Street
London WC2R 3LF
Tel: 01 240 1101
Telex: 262024

NEW YORK OFFICE Cathy Moore 15 East 26 Street New York, NY 10010 Tel: (212) 689 5900

TORONTO OFFICE Peter Drake Associates 32 Front Street West 201 Toronto, Ontario M5J 1C5 Tel: (416) 364 1623

HARVARD SCHOOL OF PUBLIC HEALTH

DEPARTMENT OF BIOSTATISTICS PROFESSOR

Applicants from the fields of Biostatistics, Applied Mathematics or Biomedical Computing are invited to apply. Candidates should be outstanding scholars of international reputation who have made distinguished contributions to be biomedical sciences. The individual selected for this tenured position is expected to initiate and lead a significant research program and participate in the teaching programs of the department.

Send a curriculum vitae, statement of current and future interests and the names of five individuals who would be willing to respond to a request for evaluation to: Professor Donald F Hornig, Harvard School of Public Health, 665 Huntington Avenue, Bldg. 1, Room 1308, Boston, MA 02115, USA.

Harvard University is an Equal Opportunity Employer with an Affirmative Action Program. (NW781)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF BOTANY POST-DOCTORAL RESEARCH FELLOW IN BIOTECHNOLOGY

Applications are invited from Botanists, Biochemists or Microbiologists of post-doctoral status to join a group led by Professor M M Yeoman investigating the synthetic potential of cultured plant cells. This project is funded jointly by Albright and Wilson Ltd and NRDC.

The appointment will be for three years and the initial placement will be at the third or fourth points of range 1A (£6,880 and £7,290) plus superannuation.

Applications, including a curriculum vitae and the names of two referees, should be sent by September 11th to the Secretary, University of Edinburgh, Old College, Edinburgh. Further Particulars may be obtained from Professor M M Yeoman at the Department of Botany. Please quote reference 5040. (9236)A

Recombinant DNA Technology

Armos Corporation

due to expansion of its laboratories, is seeking additional PhD's familiar with:

- * Nucleic Acids Synthesis
- * Expression of Polypeptides in Bacteria or Yeast
- * Virology
- * Immunology
- * Protein Chemistry.

Qualified candidates will play an important role in the growth of the firm while doing research in this exciting field.

ARMOS offers an outstanding salary and benefits package, plus an attractive equity participation program. Female and minority applicants are encouraged to apply.

Please send curriculum vitae, reprints of pertinent publications, and letters of recommendation to ARMOS CORPORATION, 180 Kimball Way, South San Francisco, California 94080.

THE INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS (ICARDA)

has a vacancy for the post of

LIBRARIAN

at its Research Station in Aleppo, Syria. Starting date: January 1, 1982. Qualifications and experience required: MSc or PhD degree in Library Science; fluent English and Arabic; working knowledge of agriculture; working experience in the Arab World.

Applicants should send their c.v. and write for further details to: The Personnel Officer, ICARDA, PO Box 5466, Aleppo, Syria. (W384)A

STANFORD UNIVERSITY California

Applications are invited for an opening at the

ASSISTANT PROFESSOR level in the newly-formed Program in

level in the newly-formed Program in the History of Science.

Candidates should have demonstrated ability in the area of 19th and 20th century physical science.

Curriculum vitae and the names of at least two referees should be sent before November 15, 1981 to: Professor Eric Hutchinson, Academic Secretary, Stanford University, CA 94305.

Stanford University is an equal opportunity employer. (NW762)A

ROYAL MILITARY COLLEGE OF SCIENCE Shrivenham, Swindon, Wilts

DEPARTMENT OF CHEMISTRY AND METALLURGY MAGNETIC CERAMICS

Applications are invited for the post of Research Scientist/ Higher Research Scientist to study the preparation of a range of ferrimagnetic ceramics, and to measure their permeabilities at microwave frequencies. The work will also involve some electron microscopy, X-ray diffraction and computer programming, but advice on these facilities is available if required. A suitable candidate may be able to register for a higher degree.

Applicants must possess a good degree in Ceramics, Materials Science, Physics or Chemistry, or an equivalent qualification, and for the higher post at least two years relevant post-graduate experience is required. Some experience with electrical measurements would be an advantage, but is not essential.

The appointment will be for a 3 year period in the grade of Research Scientist (£4,809 — £6,480) or Higher Research Scientist (£6,075 — £7,999) (salary scales currently under review) depending on qualifications and experience.

Application forms may be obtained from the Civilian Admin Office, Royal Military College of Science, Shrivenham, Swindon, Wilts SN6 8LA; telephone 0793-782551 Ext 421, and further information from Dr. G. B. Perter Ext 356. Please quote reference HQ 120/1/38 in all correspondence. Closing date for applications 20th August 1981. (9181)A

BRIGHTON POLYTECHNIC

DEPARTMENT OF CIVIL ENGINEERING

RESEARCH ASSISTANT

£4,386 -- £5,034

Applications are invited from graduates with good academic qualifications.

The research programme will involve the design of new rock pillars and stability of existing pillars in relation to new methods of working in the Brightling Gypsum Mines.

The successful candidate will be registered for an MPhil/PhD of the CNAA.

Application form and further details from the Deputy Head of Personnel, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4AT. Telephone Brighton 693655 Ext 2537. Closing date 14 August 1981. (9191)A

ROYAL COLLEGE OF SURGEONS OF ENGLAND RESEARCH FELLOW

A vacancy exists for a scientist to join team investigating the microbiological aspects of dental caries at the College's research establishment in Downe, near Orpington, Kent. Much of the work is concerned with the biochemical and immunochemical characterisation of Streptococous mutans, in order to understand the working of an anticaries vaccine. A wide range of bacteriological, biochemical and immunological techniques is in use and the position would suit a recent PhD or good honours graduate, although it will not be possible to enroll for a higher degree.

The initial appointment will be for 3 years. Salary on the University of London Research and Analogous Staff scale IB (£6,252 to £8,667) according to experience.

Please apply to the Personnel Officer, Royal College of Surgeons of England, 35-43 Lincoln's Inc. Fields, London WC2A 3PN

(9197)A

-WANTED-**MOLECULAR BIOLOGIST**

to carry out study of immunoglobulin gene arrangement in human leukemias and lymphomas. Background in relevant techniques (Southern blotting, nicktranslation, etc.) required; some experience in immunology helpful, but not necessary. Appointment is at the research associate level; exceptionally qualified individuals will be considered at the Assistant Member (Professor) level. Position available immediately.

Send a curriculum vitae and the names of three references to: Dr. G. R. Kitchingman, Division of Virology, St. Jude Children's Research Hospital, Memphis, TN. 38101. (NW790)A

NATIONAL BIOLOGICAL STANDARDS BOARD **PHARMACOLOGIST**

There is an immediate vacancy for a post-doctoral pharmacologist in the Division of Hormones. The work currently involves research on the mechanism of action and methods of detection of pyrogens. and in the characterization of small pharmacologically-active peptides such as gut peptides.

Applicants should have a postdoctoral degree in pharmacology or physiology and have had experience of laboratory pharmacology or physiology and have had experience of laboratory pharmacology research.

The salary payable is in accordance with qualifications, age and experience on the M.R.C. Grade II (£7,037 p.a. - £10,717 p.a. inclusive).

For further details and an application form please contact the Personnel Officer, NIBSC Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232. Please quote ref. no (HO/049. Closing date 28th August 1981. (9209) A

POSTDOCTORAL position available immediately for studies into the biochemistry of calcium transport during avian egg shell mineralization and its regulation by Vitamin D. A strong background in biochemistry required; experience in membrane transport and/or subcellular fractionation preferred. Salary starting at \$15,800 plus fringe benefits. Send résumé and letters of recommenda-tion to: Dr William A. Coty, Department of Biological Chemistry UCLA School of Medicine, Los Angeles, CA 90024. UCLA is an Equal Opportunity/Affirmative Action Employer. (NW791)A

McGILL UNIVERSITY DEPARTMENT OF MICROBIOLOGY MACDONALD CAMPUS

RESEARCH ASSOCIATE

Applications are invited for a research associate to study aspects of the molecular biology of membrane transport. The study will involve cloning of genes for transport proteins, in vitro synthesis transport proteins and DNA sequencing.

Applicants should hold a PhD degree in Molecular Biology or in Biochemistry or Microbiology and have had previous laboratory experience, preferably at the postdoctoral level in the molecular biological techniques required for this study

Salary \$22,000 per annum.

Please apply in writing giving full curriculum vitae and the names and addresses of two referees to: Dr R A MacLeod, Department of Microbiology, Macdonald Campus, McGill University, Ste Anne de Bellevue, Quebec, Canada H9X 1CO

(NW782)A

FELLOWSHIPS

UNIVERSITY OF LEICESTER SCHOOL OF BIOLOGICAL

SCIENCES

ICI/UNIVERSITY JOINT **LABORATORY** Applications are invited for a POST-DOCTORAL **FELLOWSHIP**

in a group studying the molecular genetics of methylotrophic bacteria.

The post provides an opportunity to use modern bacterial genetics and recombinant DNA techniques to study chromosomal organisation in an industrial micro-organism. Applicants should have postgraduate experience in a relevant discipline, but experience in molecular cloning is not essential.

The salary will be in the research and analogous scale £6,070 — £10,575 and the appointment will initially be for two years.

Applicants should send curriculum vitae and the names and addresses of two referees to Professor W J Brammar, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, from whom further details can be obtained.

UNIVERSITY COLLEGE **DUBLIN**

DEPARTMENT OF CHEMISTRY

Applications are invited for a POST-DOCTORAL **FELLOWSHIP IN** PHYSICAL CHEMISTRY

for a theoretical investigation of transport processes in the corrosion of metals. The appointment which is supported by the National Board for Science and Technology of Ireland, will commence as soon as possible after September 1st, 1981. It will be initially for one year with a possibility of renewal for a further two years. Salary will be in the range IR£5,370 - IR£6,234.

Applications accompanied by a Curriculum Vitae and the names of two Referees should be sent to: Dr Corish, Department of Chemistry, University College, Belfield, Dublin (9199)E 4. Ireland.

LA TROBE UNIVERSITY Melbourne, Australia SCHOOL OF AGRICULTURE

RESEARCH FELLOWSHIP IN IMMUNOLOGY

The Wool Research Trust Fund is funding a postdoctoral Fellow to work on the development of assays for sheep IgE and their application to an understanding of natural resistance to common parasites.

Salary: \$A17,083 — \$A26,037.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 C Square, London WC1H 0PF. 36 Gordon

Applications close on 14th August (9182)E

ROYAL NATIONAL HOSPITAL FOR RHEUMATIC DISEASES AND UNIVERSITY OF BATH SIDNEY ROBINSON RESEARCH FELLOWSHIP

Applications are invited for th above Fellowship tenable for two years from October 1981. successful applicant will join at active research team working on the biochemical and functiona characterisation of lymphocyte subpopulation in-patients with rheumatic diseases. Candidate should have a PhD in immunolog; and should preferably have some experience in lymphocyte culture The appointment will be made on the University Research Officer/Medica Registrar scale as appropriate.

Send SAE for application form and descriptive leaflet to the Hospita Administrator, Royal Nationa Hospital for Rheumatic Diseases Upper Borough Walls, Bath BA1

Closing date 8th September 1981. (9189)E

OUEEN MARY COLLEGE University of London CHEMISTRY DEPARTMENT

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

for ESR studies on food constituents in exploratory work supervised jointly by Professor R Bonnett and Dr K D Sales. Appointment is for 1 year from 1 October 1981. Salary in rage £7,037 — £8.257.

Please apply by letter, including c.v. and names of 2 referees, to The Secretary, (N) Queen Mary College, Mile End Road, London E1 4NS (9207)F

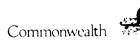
UNIVERSITY OF KENT AT CANTERBURY

ELECTRONICS LABORATORY

RESEARCH FELLOWSHIP

Applications are invited for the post Research Fellow to work on a DHSS-supported project to study the application of pattern recognition techniques to the design of a microprocessor-based speech therapy aid. Applicants should have a good Honours degree in electronics or computer engineering and should preferably hold a PhD degree in an appropriate area. The appointment is for two years and the salary will be in the range £6,070 - £7,290 on the Grade 1A scale.

Application forms and further particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH. The closing date for applications is 25 August 1981. Please quote ref. A21/81/N. (9208)E





"QUEEN'S FELLOWSHIPS IN MARINE SCIENCE"

To commemorate the Royal Visit to Australia in 1970 the Australian Government established the Queen's Fellowships Scheme. Under this Scheme up to five fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proven capacity for original work. These awards are tenable at an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS: Queen's Fellows should have a Ph.D., or equivalent qualifications, or equivalent research or professional experience in a

discipline applicable in marine science or coastal engineering.

SALARY: \$21509 (Australian) per annum – increased to \$23107 at age of 28 years (at present under review).

TRAVEL EXPENSES: Necessary travel expenses are payable.

APPLICATIONS: Persons interested in applying for the above fellowships should obtain application forms from: The Minister (Scientific), Australia House, The Strand, London WC2B4LA. The next round of awards will be announced in January 1982. Applications close in Canberra on 11 September 1981.

(9231)E

UNIVERSITY OF EXETER STRUCTURAL STUDIES OF LIQUID CRYSTALS

Applications are invited for an SERC Postdoctoral Research Fellowship to work with Professor A J Leadbetter on X-ray diffraction studies of smectic liquid crystals. A new position-sensitive X-ray detection system will be used to study novel structures and phase transitions in these systems and some neutron scattering work using the facilities at ILL Grenoble will also be involved. Applicants may be chemists or physicists and some experience of X-ray or neutron diffraction and computing would be an advantage, though not essential.

The period of the Fellowship is 1 October 1981 until 31 July 1983 with starting salary up to £6,880 per annum depending on age and experience.

Applications should be sent as soon as possible to Professor A J Leadbetter, Department of Chemistry, Stocker Road, Exeter EX44QD. (9200)E

KING'S COLLEGE Cambridge RESEARCH FELLOWSHIPS IN CHEMISTRY

Applications are invited for Junior Research Fellowships in Chemistry to commence on 1 October 1982. The normal tenure is four years, ending 30 September 1986. Arrangements have been made for successful candidates to work in either the Department of Organic and Inorganic Chemistry or the Department of Physical Chemistry.

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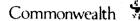
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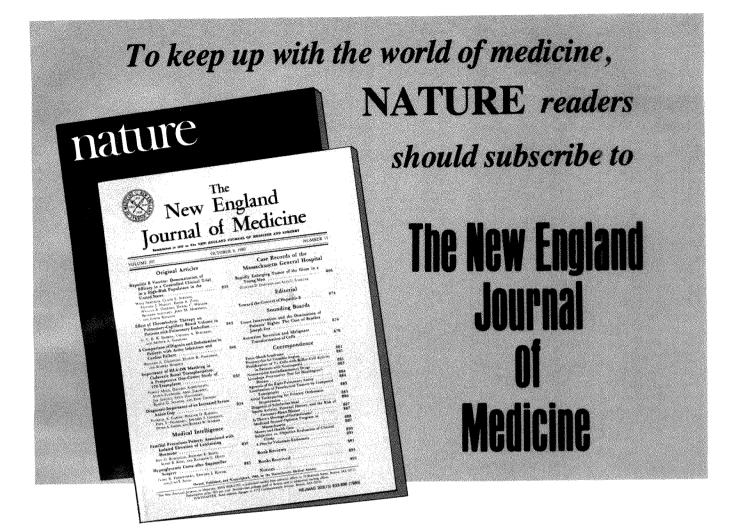
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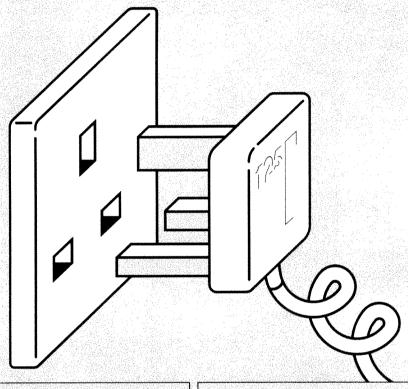
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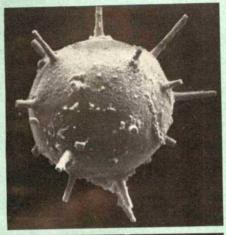
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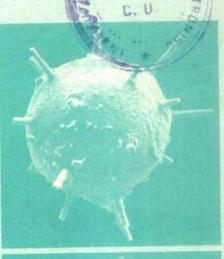
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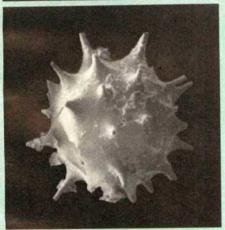


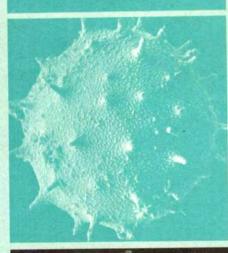
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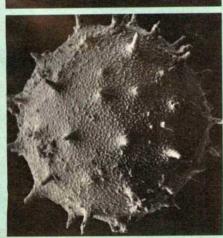


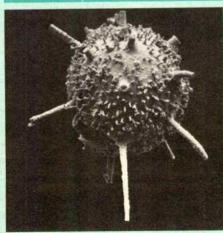


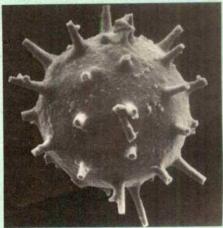












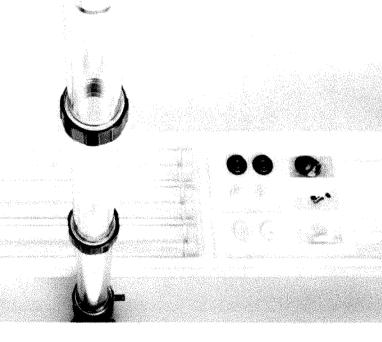
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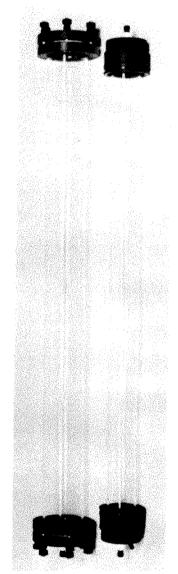
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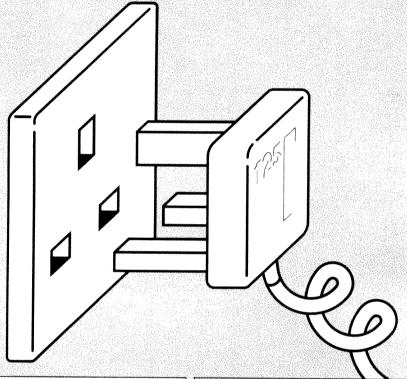
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- the time of thermodynamics and of the evolutionary sciences such as biology
- the time of conscious awareness.

The book traces the differences and similarities between these three concepts and their relative fields of application. Much attention is given to the nature of temporal ongoings in the universe. to the coming into being of genuinely new things during "the course of time" and to the linking up of time with issues in biology and with the mind/ body problem.

Although the author emphasizes the ideas of modern philosophy, a background in philosophy is not a prerequisite. The book's distinctive approach - viewing time as neither a purely physical nor a purely mental concept, but partaking of both - will enable readers to confront the strange puzzles and paradoxes of time from a much wider viewpoint than is presented in most other texts.

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EDITORIAL OFFICES

London

4 Little Essex Street, WC2R 3LF Telephone: (01) 836 6633 Telex: 262024 Telegrams: Phusis London WC2R 3LF

Editor: John Maddox

Deputy Editor: Peter Newmark

Editorial Staff

Aiun Anderson
Philip Campbell
Isobel Collins
Konrad Guard Miranda Robertson Robert Walgate Konrad Guettler Tim Lincoln Naomi Molson Charl Nigel Williams Charles Wenz

Washington News Bureau 801 National Press Building, DC 20045 Telephone: (202) 737-2355 Telex: 64280 David Dickson (Washington News Editor)

Publisher: Elizabeth Hughes Marketing Director: Ray Barker International Advertising Manager: Andy Sutherland
Features Advertising Manager: Marion Delaney Promotion Manager: Jonathan Earl

New York

15 East 26 Street, New York, NY 10010 Telephone: (212) 689-5900 American Publisher: Robert Ubell American Advertising Manager: Henry Dale Marketing Manager: Sheila Kane

> Classified Advertising Jean Neville, 4 Little Essex London WC2R 3LF Telephone: (01) 240 1101

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nature

Can cancer at work be regulated?

In the century and a half since cancer of the scrotum among chimney sweeps was first recognized as an occupational disease, interest in (and anxiety about) occupational cancer has grown steadily. Since the Second World War, there has been continuing concern for the health of those occupationally exposed to ionizing radiation, and all governments have introduced regulations intended to limit the exposure of workpeople and thus the risk of overt disease. More recently, attention has turned to the occupational risks of the exposure of workpeople (and also the general population) to industrial chemicals. In the United States, the passage of the Occupational Health and Safety at Work Act in 1970 led to the creation of two separate organizations with responsibility in the field - the National Institute for Occupational Safety and Health (whose function is research and evaluation) and the Occupational Safety and Health Administration (which can regulate industrial practice). In Britain, legislation on the American model led in 1974 to the creation of the Health and Safety Executive and its supervisory commission. For the past three years, the Health and Safety Executive has been struggling with a regulation requiring the notification of new chemical substances (see Nature 16 July, p.190) but even now is unlikely to meet the deadline of 18 September decreed by the European Commission.

Nobody should be surprised. European administrations' attempts to restrict the introduction of potentially hazardous chemicals are the analogues of the regulations enshrined in the Toxic Substances Control Act of 1976, which took the best part of six years to emerge from an embattled Congress in the United States. There are several contentious issues. Chemical companies resent the cost of subjecting all new chemicals to a battery of short and long-term toxicity tests - and, with more reason, fear that too wooden an exercise of regulatory powers by the responsible agencies may deprive them of the use of a valuable intermediate even when effective precautions may be taken. A more serious difficulty, shown up by the muddled division of responsibility in the United States between the Environmental Protection Agency (responsible for the administration of the Toxic Substances Control Act) and the Occupational Safety and Health Administration is that of making sensible arrangements for the protection of the public at large, and of people occupationally exposed to larger concentrations of the same materials.

In Britain, as earlier in the United States, a further complication has arisen — the interest of the trades unions in the design of the regulations and in their administration. This development is entirely to be welcomed. Even the most diehard opponents of organized labour would agree that unions are better occupied in the protection of the health of their members than in some of the other things they do. Industry as a whole, and the community at large, can only benefit from a proper understanding by its workpeople of the occupational risks they are asked to run — and from their informed consent that risks of some magnitude, ideally small but probably not identical with zero, should be run. The time that might now be spent in Britain by the Health and Safety Executive on reconciling the interests of the trades unions and the chemical manufacturers would be well worthwhile.

Unfortunately, good sense is in danger of being overtaken by events. The Health and Safety Executive, applauded ever since its creation as a novel and autonomous entity within the British constitution, is much in need of the safety valve of which its special place deprives it — an expression of a range of prejudiced

opinion in the House of Commons. (Is the House of Lords too busy to oblige?) The second draft of its regulations on the notification of new substances is indeed needlessly onerous, too cut and dried, and the executive should take the time to produce a third, whatever they say in Brussels. The trouble, now, is that the executive has to watch out not only for the European Commission but for Mr Clive Jenkins, the secretary of the trade union called the Association of Scientific, Technical and Managerial Staffs.

Mr Jenkins's union has in the past few years taken a close and often constructive interest in problems of occupational health and safety. More than a year ago, it produced an arresting (if controversial) document about the occupational hazards of new chemicals. Ten days ago, Mr Jenkins issued a lengthy commentary on the second draft of the Health and Safety Executive's proposed notification regulations and made some cogent points. Chemical companies are indeed too defensive of their superficial financial interests, and too unwilling to let independent outsiders enquire into their affairs. The costs of complying with regulations have been systematically (that is, consistently) exaggerated. And so on. He erred only in the most rudimentary way — by supposing that there is some state of grace in which occupational risks are absolutely avoidable. His problem, and the executive's, is rather how to strike a balance between the possibly carcinogenic risk of a novel chemical and the possibility that somebody, or some group, will be out of work. Who will persuade whom of that?

Damming estuaries

The hope that there might be some other way — any other way - of generating electricity than those now used is likely to be widely paraded in the weeks ahead. The United Nations conference on alternative energy sources due to open in Nairobi next week will be an obvious forum (see also page xxx). And nobody should deride the innocent declarations of faith in windmills, water-wheels or wood (now called biomass) that there will be. There are unfortunately still too many circumstances, in still too many developing countries, in which any way of generating a modicum of mechanical power from some indigenous source is much needed. But those who assemble in Nairobi should also take to heart the message hidden in last week's report of Sir Hermann Bondi's study of the feasibility of an electricity-generating barrage across the Severn estuary, between England and Wales (see Nature 30 July, p.401). The conclusion of this thorough piece of work is that if British taxpayers want the kinds of benefits that a barrage across the Severn estuary would provide, they would be best advised to build nuclear power stations and not a dam.

The argument is simple but instructive. A dam across an estuary can be used to generate electricity in several obvious ways — by letting the incoming tide drive turbines in a dam, by letting water impounded at high tide run back through turbines on the ebb tide or (as in the scheme operated by Electricité de France on the estuary of the Rance since 1966) arranging that water in an estuary will drive turbines at both the ebb and flood tides. The amounts of energy that can in principle be won from such schemes are huge—the Bondi committee estimated that even the most modest of all possible dams across the Severn could yield 13 TWh of electricity, something like six per cent of present electricity demand in the United Kingdom, roughly the amount of electricity that would be

generated by the use of 7 million tons of coal a year, year in and year out. The snag is that not a single kilowatt-hour of this electricity would accrue until the whole dam across the Severn estuary had been built — a process likely to take 15 years and to cost £5,660 million without allowing for the cost of paying interest on this substantial sum of money while construction is under way. But is not any scheme that produces energy from a renewable resource, such as the slopping one way and another of water in a tidal estuary, inherently worthwhile? That is what the enthusiasts for the Severn barrage have been saying almost since the beginning of the electricity supply industry itself. Unfortunately, a careful reading of the Bondi committee's first report shows the argument to be false.

As in other industrially developed countries, the British demand for electricity is a reasonably well-defined function of the time of day and the season of the year. Inevitably, the daily pattern of demand is linked with the movement of the Earth about the Sun and not with the movement of the Moon about the Earth, with the result that maxima of demand coincide only by accident with tidal maxima. Similarly, seasonal variations bear no constant relationship to the occurrence of spring or neap tides, which are related to the relative alignment as seen from the Earth of the Sun and the Moon. None of this is surprising. The consequence is, however, that a dam across the Severn would be capable of generating electricity at times only randomly related to those at which electricity demand is greatest. Accordingly a Severn barrage (or any other tidal scheme for generating electricity) will not substantially reduce the need for building other kinds of generating plant to satisfy the maximum demand on the system. (This blunt statement is a little over-gloomy, for electricity utilities would in principle be able to plan routine maintenance of conventional plant on a lunar calendar and thus obviate the need for some capital investment.) Such benefits as there are from tidal schemes, however, consist only in the avoidance of fuel costs at conventional power stations, some of which may be shut down when barrage schemes are ready to generate electricity. Overall, the crucial question is whether, over the lifetime of a barrage, the initial capital cost will be justified by the conventional fuel (coal, oil and uranium) that would be saved.

The Bondi committee, which includes several enthusiasts for big dams, puts a brave face on this daunting prospect. On the basis of a calculation of the present value of the conventional fuel that a Severn barrage would save, it concludes that the most modest version of such a scheme would just about pay for itself. There will be much technical interest in the way in which this calculation has been carried out — and curiosity will be satisfied only when the full report of the study is published two months or so from now. Already, however, it is clear than even this calculation is over-optimistic. The committee has assumed in calculating costs and benefits that the annual rate of interest on capital is either 5 per cent or 7 per cent — much less than the cost of the money the British government is having to borrow to finance less ambitious projects than the Severn barrage. But that is only half the story. The Bondi committee, with its hand firmly on its heart, makes the honest declaration that the economic benefits of investing in nuclear power stations would be roughly twice those of investing in the Severn barrage.

None of this implies that schemes for the exploitation of renewable energy should not be examined. The Bondi study is a model of how such exercises should be carried out. But enthusiasts for the use of renewable energy resources should acknowledge that conclusions such as those that have now put paid to dreams of a barrage across the Severn are likely to afflict many other projects for generating electricity in unconventional ways. After all, the properties of a stream of electrons in an electrical conductor are independent of the source of the electrical potential that drives them. By making uneconomic judgements about the virtues of different sources, it is possible to waste vast amounts of wealth that might be used in other ways. This is not to say that the United Nations conference at Nairobi should promptly adjourn, and amalgamate with the meeting of the major electricity utilities at the next World Power Conference, for there

is much that might be done by a sober appraisal of small power sources for use in places where there is hardly any power of any kind available at present. But, in the long run, the hard economics that have ruled out the Severn barrage in Britain are likely also to apply elsewhere.

Cricket and patriotism

The British, much depressed for several years by problems in Ulster and of economic misjudgement and mismanagement, have been so curiously uplifted in the past few weeks by a royal wedding and their success in beating the Australians twice at cricket that the question must be faced whether the psychological benefits of these public spectacles can be more widely spread. For if there is the slightest chance that peoples elsewhere might be given the courage to face intractable problems — or perhaps even the excuse to forget about them for a time - by a suitable patriotic display, should not governments other than the British be ready with plans for suitable ceremonies? Precedents set by the Roman emperors, however politically prudent 2,000 years ago, would no longer be acceptable, while the chances are small that governments will value royal weddings so highly that they would embark on the constitutional upheavals necessary to introduce monarchies of their own. On the face of things, only the game of cricket is potentially adaptable to other circumstances than the British, and even there the prospects are not bright.

The difficulty with cricket is that it is not widely understood. There is, however, no reason to accept the complaint of those not subjected to an English education that the game is inherently incapable of being understood. The way in which cricket has spread through much of the British Commonwealth gives the lie to this canard; the fact that West Indian cricketers are probably now more skilled than any others is a sign that cricket could become an international vehicle for national contentment, even chauvinism. To that end, the most urgent need is for a deeper understanding of what cricket is about. It may even be necessary to simplify the rules.

The fundamental principles of cricket are by no means unique. As in baseball, one person throws a ball at another, who is equipped for his defence with a piece of wood called a bat. Superficially, cricket is more objective than baseball in that a batsman's defence is held to have been unsuccessful if the ball strikes one of three pieces of wood planted on the ground behind him, and not merely on the say-so of an umpire who has to judge whether the ball passes at the right height over an object laid in the ground. What puzzles potential devotees of cricket is that the ballthrowing act is routinely performed in each of two opposing directions and that there appears to be no limit to the number of people who can be invited to throw the ball. It is also puzzling to many that ball-throwers (called "bowlers") may legitimately bounce their projectiles on the ground in front of the hapless batsmen, thus introducing an element of disconcerting randomness into their trajectories.

Fortunately, these faults are easily remediable. One-ended cricket played on an adhesive surface (so that cricket balls could not be bounced) would plainly make it possible for others than the Old Commonwealth to understand and thus to enjoy a game to which they are at present denied access. The time taken by cricket matches could be reduced if batsmen were given less substantial pieces of wood. For obvious economic reasons, it would be necessary to reduce the duration of international matches from five days to five hours, which to a first approximation would require that the presenting surface of the standard bat should be reduced by approximately five-sixths. The disadvantage that it would then be difficult for batsmen to accumulate large individual scores - one of the prerequisites of national cricket heroes — could be offset by multiplying by six the present rewards for batsmanship. No doubt there would be many in places where cricket is understood who would resist these innovations. But should tradition be allowed to stand in the way of a wider distribution of the benefits that cricket might bring?

Stanford sells gene-splicing licences

Boyer, Cohen patent may yet be challenged

Washington

In a move expected to bring in annual royalty payments of over a million dollars within the next four or five years, Stanford University announced this week the terms under which it is prepared to let commercial companies use the genesplicing techniques developed in the early 1970s by Dr Stanley Cohen of the university's medical school and Dr Herbert Boyer of the University of California in San Francisco.

The techniques have since become the basic tools for many widely used genetic engineering processes. University researchers who wish to use them may continue to do so free of charge, but companies marketing products based on their use will be required to pay a royalty fee varying from one per cent of net product sales below \$5 million to 0.5 per cent on annual sales of over \$10 million.

Before reaching the commercial stage, any company wishing to use the techniques in its research can purchase a non-exclusive licence from Stanford for an initial fee of \$10,000, and an annual sum of the same amount. An incentive offered to those who act quickly is that any company which signs up before the end of 1982 can credit five times the licence fee against future royalties, up to a maximum of 50 per cent of the royalties earned in any one year.

Stanford will deduct 15 per cent from fees and royalties earned to servicing the licensing arrangements. After that the remaining sum will be divided equally between Stanford and the University of California. Stanford's share will be further divided, with a third going to the university's medical school and a third to its departments of medicine and genetics. Dr Cohen, who holds joint appointments in the two departments and who would under the university's normal rules be entitled to the remaining third, has assigned his share back to the university, to be divided between biomedical research and support for postdoctoral fellowships. Dr Boyer has also waived any rights to personal royalties.

Despite being one of the leading research universities in the United States, with an annual research budget of over \$100 million, Stanford has so far lacked any major money-spinning patents comparable, for example, with the widely-used dental fluoride developed at the University of Indiana. University officials are hoping that the income from the patent on the Cohen/Boyer techniques, filed in

1974 and granted last December, will change this, although president Donald Kennedy says he does not anticipate that royalty income from this source "will significantly alter our present financial projections".

The patent granted by the Patent Office is broad, covering the series of steps used by Drs Cohen and Boyer to replicate and express exogenous genes in microorganisms. It describes the techniques used to cleave a plasmid or virus DNA, to insert a new gene to provide a biologically functional replicon, to place the latter in a microorganism cell, and finally to isolate the transformants in order to produce cells in which the DNA molecules in the modified plasmid can be replicated and expressed.

The breadth of the patent — and the ubiquity of the techniques' current usage — means the university is expecting a large number of licensing requests. In a statement issued on Monday it estimated that more than 200 companies in the United States may already be involved, and that half may sign up for the licences in the near future.

At the same time, this breadth could lay the patent open to challenge from other scientists who claim to have made significant contributions to the results which were patented by the universities solely in the names of Cohen and Boyer. Murmurs of possible legal challenges have surfaced in the past, although the relatively low licence fee — as well as the fact that the royalties will go to the universities and not to the individual scientists — may well dampen any conflict.

Stanford is requiring that any company obtaining a licence must agree to use the techniques in compliance with the guidelines for physical and biological containment of experiments drawn up by the National Institutes of Health.

The university has also welcomed a recent ruling by the International Trade Commission forbidding the unlicensed import of goods made abroad with techniques patented in the United States. Previously it had been feared that companies might try to avoid the licensing fee and royalty payments by setting up production facilities in other countries.

David Dickson

Chevènement wins control of science

France's civil science is now almost totally in the hands of one man, Jeane-Pierre Chevenment, the left-wing minister of state for research and technology (see *Nature* 2 July, p.3).

Chevènement has been battling for weeks with other ministers — notably two successive ministers of industry — for the power he believes he needs to direct France's scientific future. Now he has won.

A decree published last week gives him control of the budget for all government-funded civil research, development and technology. The budgets of all public bodies concerned with such matters will henceforth be ascribed to his ministry, giving him around FF20,000 million (£2,000 million) to play with each year.

Nevertheless, the formula which gives Chevenement these powers is complicated, and is likely to be read very closely by all concerned.

Chevènement will, however, have total authority over the Centre National de la Recherche Scientifique (CNRS), which plays the leading role in supporting basic science in France. CNRS has 1,200 laboratories (including a third of all university laboratories) most of them managed jointly with the universities or other institutions but 250 of them completely independent. CNRS is a dominating influence in basic research in France, with the possible exception of mathematics; it has a staff of 25,000, of whom 8,000 are researchers.

Previously CNRS was under the authority of the ministry of universities,

and some now think that the separation from that ministry may complicate the management of joint laboratories: Chevenement is primarily interested in science as the driving force of industrial development, which may not match the views of some university departments. But he has also described science as an essential element of "culture" (music to the ears of President François Mitterrand) and wishes further to improve the international standing of French science.

Other bodies over which Chevenement will have total authority are the Délégation Générale à la Recherche Scientifique et Technique, which will act as his secretariat; the Agence Nationale pour la Valorisation de la Recherche (aimed at turning government-sponsored research into industrial innovations, through venture-capital grants); and two ministerial services, the Mission Interministérielle pour le Développement de l'Information Scientifique et Technique, and the Délégation à l'Innovation et à la Technologie.

Over other organizations, such as the Commissariat à l'Energie Atomique and the Centre National d'Etudes Spatiales, Chevènement will have control through the indirect (but powerful) lever of the budget; and also, if the relevant paragraph in the decree is so interpreted, through appointments.

This paragraph gives Chevenement the task of undertaking "all reform" of public bodies concerned with research, including "all measures have an impact on the

politics of scientific employment" — which he must countersign.

Chevenement will also "be associated with" France's efforts in international scientific cooperation, in cooperation with the foreign minister M. Claude Cheysson.

The decree outlining Chevenement's powers has required negotiation at the highest possible level, and has been signed by President Mitterrand, Prime Minister Pierre Mauroy, the foreign minister and ministers of industry and education.

The national colloquium on science and technology will now take place on 13-16 January 1982, and Chevenement is laving great emphasis on its role in defining a new politics of science in France, and the major "loi-programme" for science which he is to put before parliament next year. The colloquim is to have six principal sessions: on the cultural contribution of science and scientific responsibility; on the internal division of money for science (scientific and technical options, big science and so on); the role of science in helping France to climb out of the recession and create employment; the management of scientists (contracts of employment and so on); the role of other bodies influencing science (big industry, for example); and the political and structural means adopted by Chevènement himself. Robert Walgate

No rapprochement

The usually covertly political nature of education in France came out into the open last week with the sacking of 14 of the 28 regional education officials.

These officials administer education from primary to university level within the "Académies", territories which encompass several of the French departments; ever since General de Gaulle revised their powers in 1967, they have become increasingly political figures. Madame Saunier Seïté, Giscard d'Estaing's minister of universities, was previously head of an Académie; and others became junior ministers or ministerial advisers.

In effect, this was inevitable under de Gaulle's ordinance, which loosened their hold on power and so made them more reliant on the goodwill of the government. Now fourteen unlucky incumbents have been found to be tarred too heavily with the brush of the previous administration, and must go.

This will make room for a "profound reform", the minister of education, M. Alain Savary, said last week in a press statement. The new administrators must not make politics, he said; they must obey the politics of the government. Their predecessors, by contrast, had been active against the new government both before and after the election. Some had stood as candidates for opposition parties. The deposed director of the Paris Académie, for example, had been Saunier Seïté's chief adviser.

Robert Walgate

Alternative energy conference

Realism the theme

Washington

At least five heads of state, including Canadian Prime Minister Pierre Trudeau and Mrs Indira Gandhi of India, will be among the participants at the United Nations Conference on New and Renewable Sources of Energy (UNERG) which starts in Nairobi next Monday. Their presence, together with energy and other ministers of industrialized and developing nations, is held by the conference organizers to indicate a measure of success in getting across their view of the political importance of alternatives to oil, coal, gas and nuclear energy.

But political weight alone will not guarantee a successful conference. UNERG is likely to set a very different tone from the large UN conferences of the 1970s, when global issues such as environmental pollution and the "human habitat" were confronted in a spirit of optimistic idealism.

From the beginning, UNERG has been organized with a more pragmatic outlook. It was, for example, agreed by a narrow vote of the UN General Assembly that nuclear power would not be included on the "new energy technologies" agenda, a move supported by many industrialized nations which feared the result would be too difficult to handle, but opposed by developing nations on the grounds that its exclusion - together with any explicit reference to conventional energy sources - would inevitably skew any results. But perhaps the main stimulus for pragmatism is the general feeling that grandiose schemes for new international bodies (such as the UN Environmental Programme, set up after the Stockholm conference in 1972), or even for a commitment to significant increases in development aid funds, are unlikely to gain support in an international mood of austerity.

In such a context, the tangible achievements of the Nairobi meeting will inevitably be limited. But Secretary General Enrique Iglesias, seconded to direct conference preparations from his permanent position as head of the Economic Commission for Latin America, remains confident that it can still have a real impact by bestowing legitimacy on energy sources frequently omitted from development planning.

One of the most practical parts of the conference has already been completed — a series of technical reports on fourteen different types of new and renewable energy sources, from wind energy to draught animals. The quality of the reports is mixed; but some, such as that produced by an international panel on wind energy, have met with wide approval. And the preparation of national contributions for the conference is said to have catalysed thinking about alternative forms of energy

production and energy planning in general — particularly in some of the developing countries — that had previously been virtually non-existent.

How these will evolve into practical initiatives remains to be seen. Several industrialized countries are said to be keen to support the setting up of research and training institutes for the various energy sources, wood-fuel being the most frequently quoted example. And the Society for International Development is promoting the idea of an international network of energy research institutes along the lines of the agricultural research network run by the World Bank.

The political debate will inevitably focus on the two factors which tend to dominate all international meetings of this nature: money (development aid contributions, primarily from the industrialized nations), and power (the organization of the UN bureaucracy).

Initially the developed and developing nations will be less far apart than at the UN Conference on Science and Technology for Development (UNCSTD) in Vienna in August 1979, where the Group of 77 arrived at the bargaining table with a proposal for a new fund to support Third World research with a budget of \$2,000 to \$4,000 million. This time the developing countries have accepted that such proposals are unrealistic. Any extra support for development of new energy technologies is therefore likely to come from changes in existing arrangements.

The conference organizers originally hoped that the meeting would coincide with the creation of a new "energy affiliate" by the World Bank, a proposal put forward by the bank's then President Robert McNamara last year as a device for raising capital for energy production schemes. So far, though, this has been vetoed by the Reagan Administration on the grounds that investment should, where possible, be left to the private sector.

With the World Bank's initiative stalled — and general agreement that the Nairobi conference should not try to set up a new energy fund — one of the most likely outcomes is an agreement that energy projects should receive a set proportion of the funds raised through a new "financing system" for science and technology, which has been in the planning stages since the Vienna meeting two years ago.

If approved in principle by the General Assembly later this year, the "financing system" would probably take over responsibility for projects at present financed through a two-year interim fund, also established at Vienna, operated by the UN Development Programme. The interim fund already supports several energy projects, such as research into the use of wind power in Mauritius and the introduction of more efficient wood-stoves into the Sahel region of Africa.

To a large extent, however, the most significant meetings will not take place in

Nairobi at all, but will be those surrounding the summit meeting planned for Mexico in October under the auspices of the Brandt Commission. This meeting, which will be attended by President Reagan, is expected to set the tone for negotiations between developed and developing nations for the first half of the 1980s; as a result, it will provide the setting within which any results from Nairobi will inevitably be judged.

David Dickson

To the gulags

The last two members of the Moscow "Working Commission to Investigate the Use of Psychiatry for Political Purposes", Irina Grivnina and Feliks Serebrov, were last month tried and convicted on charges of anti-Soviet activity. Ms Grivnina was sentenced to five years' internal exile, Mr Serebrov to four years in a labour camp plus five years internal exile.

The "Working Commission" was established in 1977 as part of the general human rights monitoring movement in the Soviet Union, and its members made determined efforts to visit "patients" confined in psychiatric hospitals because of their political beliefs. The commission produced a samizdat (information bulletin) giving details of its findings, and where possible provided colleagues abroad, on a confidential basis, with case notes of the patients investigated. In most cases these notes showed that by non-Soviet standards there were no grounds for compulsory hospitalization.

For these activities commission members have either been forced to emigrate (like Dr Volshanivich) or arrested and charged with disseminating anti-Soviet slander (Article 190/1), or with anti-Soviet propaganda and agitation (Article 70/1). According to Soviet judicial theory, as reiterated recently by Evgenii Smolentsev, Deputy Chairman of the USSR Supreme Court, "in the practice of Soviet courts, there are not and there cannot be convictions for religious and political beliefs" and hence any claim that there are prisoners of conscience in the Soviet Union is anti-Soviet propaganda.

According to TASS, Mr Serebrov pleaded guilty under Article 70/1 and admitted knowing that the documents he helped to prepare would be distributed in the West. TASS further recorded that he had "repented" of these actions, but some Moscow sources deny this "repentance", saying that in such a case a far lighter sentence would be expected.

The British Medical Association has tabled a motion for the September meeting of the World Medical Association in Lisbon, condemning both the use of psychiatric methods for political repression and the suppression of the "Moscow Working Commission".

Vera Rich

UK engineering council

New lambs for old

A new body to safeguard the quality of British engineers is to be set up by Royal Charter, the government announced last week. The Engineering Council, as the body will be known, is the culmination of eighteen months of heated debate since the Committee of Inquiry into the Engineering Profession, chaired by Sir Monty Finniston, recommended that there should be a new organization to oversee the education and registration of engineers.

The Engineering Council's first parttime, unpaid chairman is Sir Kenneth Corfield, chairman of Standard Telephones and Cables Limited. The Department of Industry, which is putting up £1 million to get the council started, will appoint 15-24 board members — for which it has already received 300 suggestions — and a permanent secretariat in the autumn. After a three-year transition period, the council will elect its own members and will be expected to be selffinancing mainly from fees charged for registration, its chief business.



Corfield; engineering's chartered chief

The council is a disappointment to many. Some consider it a poor substitute for the statutory Engineering Authority that Sir Monty's committee had asked for. One issue in the debate has been the relationship between a new body and engineering institutions, especially the Council of Engineering Institutions, which have traditionally chartered engineers and promoted their separate interests through their own Royal Charters. Sir Monty and his supporters fear that the new Royal Charter gives the institutions too much influence. Certainly the new council will not have funds for the improvement of engineering education or even the support of students, as Finniston had asked.

The charter lays down two main roles for the council, each of which it is empowered to delegate in part to the engineering institutions: to determine standards and criteria for the education, training and experience of engineers and to keep a register of those meeting the criteria. Engineers will be eligible to apply for three categories of registration; as professional engineers, technician engineers or engineering technicians. Registration for each category will be in three stages, the first after completing an approved course, the second after training and the third after work experience. Provision is also made for those entering the profession through unorthodox routes. And all those now registered through the Engineers' Registration Board of the Council of Engineering Institutions will automatically be registered with the new council at stage three.

The role of the Engineering Council as registering authority calls into question the future of the Council of Engineering Institutions (CEI). That body, however, expects to carry on with business as usual for perhaps two years until the new council is operating fully. Any change in its status will then mean revoking its own Royal Charter, a move which cannot be taken without a two-thirds majority among its members. The battle for responsibilities could continue much against the wishes of Sir Monty's committee and others who hoped to break the CEI's grip.

Judy Redfearn

University Grants Committee

Biting the bullet

The University Grants Committee considered earlier this year whether it should resign rather than administer the British government's 8.5 per cent cut in support for the universities, according to Dr Edward Parkes, the committee's chairman, in evidence to the House of Commons Select Committee on Education. But, in the end, the committee decided to soldier on, not wishing to be a "fair-weather committee" and believing itself to be the only group with a sufficiently detailed knowledge of the British university system.

Some Members of Parliament were clearly disappointed that the committee had not given them the tangible weapon against the government that a mass resignation would have provided. The select committee was also chagrined that Dr Parkes declined to hand over copies of his correspondence with the Secretary of State for Education, Mr Mark Carlisle, in which — according to his evidence — he had warned the British government of too rapid a contraction of the university system.

Although Dr Parkes's evidence, like that of a delegation from the Committee of Vice-Chancellors and Principals, was taken in private, a transcript of the proceedings was made public last week after the witnesses had confessed themselves puzzled that the hearing had been held in private. Perhaps the select committee had been hoping that its witnesses would have been more open, even gossipy.

Much of Dr Parkes's evidence concerned the criteria his committee had used for allocating funds to the various universities. The relatively favourable treatment for science is apparently accounted for by the "swing back to science" evident in recent applications from would-be students. Within biology, Dr Parkes said that the committee had taken the opportunity to encourage courses and departments with economic potential. Departments of social science. whose student numbers are to be cut collectively by 12 per cent in the next few years, will suffer chiefly at the "soft end" partly on the grounds that student demand is falling and partly because staff-student ratios in some of the departments concerned are too small for good research to be feasible. The grants committee had not, however, been influenced to any substantial extent by "manpower considerations".

Nor, according to Dr Parkes, had regional considerations played a part in his committee's planning except in special circumstances — the argument, for example, that the Universities of Glasgow and Strathclyde should between them be able to provide a full range of courses in higher education to satisfy the needs of students from south-west Scotland, where regional loyalties are strong.

The qualifications of students entering universities had counted in the grants committee's calculations, but Dr Parkes said last week that the committee had tried to strike a balance between institutions with high-quality entering students and those apparently able to provide less highly qualified students with good degrees and useful adult careers.

On the contentious issue of the research records of various departments, Dr Parkes rejected the criticism that too much attention had been given to the success with which departments were able to recruit grant support from the publicly funded research councils. Instead, he argued that industrially supported research usually provided university departments with a measure of overhead support, with the result that the grants committee could legitimately confine itself to the public provision of research support.

The joker in Dr Parkes's pack appears to be the quality of teaching in individual departments and universities, which was also one of his committee's criteria. Insisting that judgements of this kind must necessarily be to some extent subjective, and that little could be done to develop objective criteria based on graduation results, he will not have stilled the charge of prejudice made in the past few weeks by several universities.

Dr Parkes agreed, however, with the vice-chancellors that his committee's ignorance of the other sector of British higher education, represented principally by the 26 polytechnics, is a serious obstacle to sensible planning.

US university research

Industry to provide

Washington

Increased incentives to private companies which support basic research in US universities were contained in a tax package, backed by President Reagan, which was passed by Congress on Monday this week.

The tax incentives were considerably broader than the Administration had initially proposed and unlike that part of the bill dealing with cuts in personal taxation, credits for spending on research received wide bi-partisan support.

Initially the Administration, in its tax package put to Congress in March, had concentrated on shortening the period in which research and development equipment could be written off. Both House and Senate have now passed bills under which such equipment can be fully depreciated over three years, two years less than is allowed for other capital equipment.

The protracted debate over these proposals gave individual congressmen a chance to add their own suggestions for reducing the tax burden on the private sector. One of these is the idea, originally raised by Representative Charles Vanik, that companies should receive substantial credit for money used to support basic research in universities.

When the tax bill was passed to the House Ways and Means Committee, groups such as the Association of American Universities and the American Council on Education argued that such an addition would help offset reduced federal support for university research.

Dr Donald Kennedy, president of Stanford University, told the oversight subcommittee of the House Science and Technology Committee in June that the additional tax credits, which had been put forward in a separate bill sponsored by Congressman James Shannon, would "significantly invigorate" the relationship between industry and universities "without some of the hazards that I see in the present helter-skelter pattern of affiliation".

In the same vein Dr Paul Gray, president of Massachusetts Institute of Technology, told the Ways and Means Committee that tax credits would create an "urgently needed" increase in the flow of corporate funds for university research. At present about \$210 million — or 3.5 per cent — of university research funds is provided by the private sector, the bulk of the rest coming from federal government.

Responding to such arguments, the committee not only included a tax credit for any increased research expenditures, but confirmed that existing tax provisions affecting both gifts direct to academic institutions and to third-party tax-exempt organizations, such as foundations, would persist.

However Dr Kennedy and Dr Gray were

Australasian fellowships

The council of the Royal Society has set up a new research fellowship designed to further the United Kingdom's scientific collaboration with Australia and New Zealand. Main purpose of the fellowship is to improve access to major "facilities" such as unique features of the geological, oceanographic or biological environment, astronomical centres and nuclear physics and biotechnology laboratories.

The scheme is aimed at British and Australasian postdoctoral scientists wishing to undertake research or to learn new techniques. The fellowships last from three to twelve months, and are the result of discussions between the Royal Society, its Australian and New Zealand equivalents, the UK Science and Engineering Research Council and Australasian government departments. The Australasians have yet to find the funds for the scheme, so the Royal Society, encouraged by £15,000 from BP, has forked out £50,000 for the first year of the enterprise. Interested scientists and engineers, or their potential hosts, are invited to apply. Philip Campbell

unable to convince Mr Donald Regan, Secretary of the Treasury, that the extra credits for support of university research should be included in the bill.

The bill was eventually defeated after a bitter fight over the timing of cuts in personal taxes on the floor of the House. But the Republican-sponsored measure which replaced it, with the support of several conservative Democrats, adopted virtually identical language in its section on tax credits for research expenditures within the private sector.

There were some differences. The House bill, for example, includes corporate expenditure for university research in the base from which the additional expenditures, which would be eligible for the tax credit, are calculated. This will substantially reduce the size of the credits over those supported by the committee. In addition, the House bill specifically excludes tax credits for the support of research in the social sciences and the humanities.

The result is that there will be a small credit for basic research as well as a provision that research equipment given to a university, provided it is less than two years old, can be counted by a company as a charitable contribution.

The major question now is whether all the proposals made in the House bill will be agreed by the Senate. At present, however, the Senate version does not include the tax break for basic support in universities. Representatives from the two legislative bodies met this week to iron out their differences; in the absence of significant opposition the university tax credits survived into the final bill.

David Dickson

Brazilian research funding

Sliding backwards

Rio de Janeiro

There is uncertainty and alarm among Brazilian scientists following a series of forums held during the past few months at the request of the Brazilian Association for the Advancement of Science and involving the heads of the various funding agencies. Still no official government policy has been announced, and scientists are very much in the dark about the future of Brazil's science funding.

With inflation at 110 per cent this year and confronted with shrinking research budgets and graduate scholarships, research scientists, particularly those at universities, are concerned by suggestions made by Gerson Ferreira Filho, new director of the Financing Agency for Projects and Studies (FINEP), that his agency would abandon its policy of financing all institutions and only approve funding for specific projects. Support for institutional funding, he said, would be transferred to the Ministry of Education.

The Minister of Education, General Rubem Ludwig, in his turn, has hinted at the possibility of changing the status of 19 federal universities to that of "foundations", capable of "more financial flexibility". This would force the universities to balance their budgets by charging high tuition fees, making Brazil's education system even more discriminatory than it is now. Even with 65 per cent of the Ministry of Education's budget going to higher education, academics point out that the amount is ridiculously small. Education's share of the national budget has fallen from eleven per cent 10 years ago to less than five per cent today. And the Secretary for Higher Education, although admitting at one of the forums that "we've reached the last line of defence at the bottom of the financial pit", has made no proposals for improving the situation in the immediate future.

Another area of concern is the size of the support provided by FINEP, which amounts to \$100 million per year for all fields of science. In principle other funds are available from FINEP for industrial development projects, but these are in the form of loans to private industry and government agencies, rather than academic institutions. Funds are allocated without external review, and this lack of participation of the scientific community was strongly criticized recently in a document signed by fourteen scientific associations.

The problems of inflation have hit particularly hard at those receiving grants and scholarships from the National Centre for Research (CNPq). The recent introduction of fixed ceilings for scholarships was strongly denounced at the forums, and CNPq, with a meagre \$10 million budget, has announced a policy of

increasing the number of scholarships on offer by reducing the size of each one. CNPq's deputy director has agreed to increase the value of grants already awarded to keep them in line with inflation. Fixed ceilings, however, would apply for all new grants and there are rumours that CNPq's own budget will not be adjusted for inflation next year.

When, at one of the forums Senhor Ferreira claimed that this year's budget was 38 per cent higher than in 1980, he was interrupted by one of Brazil's leading economists, Professor Maria da Conceicao Tavares, who demanded "honesty in words", saving that because of inflation a 38 per cent absolute increase implied an effective cut of 60 per cent. Suggestions from administrators that scientists and professional organizations should lobby the government for more funds were quickly rejected by the scientists present, who pointed out that lobbying requires an effective Congress. In Brazil, however, Congress does not even legislate over budgetary matters - as these have been the sole responsibility of the Presidency since 1967.

Maurice Bazin

Soviet plate tectonics

Open approval

Moscow radio has denied Soviet prejudice against the "new global tectonics". On the contrary, according to Moscow radio's world service, there is a "healthy climate" of "perfectly normal scientific controversy" about continental drift and plate tectonics. The radio's science correspondent, Boris Belitskii, said that Western speculations that there are political overtones to the controversy are "quite absurd".

As evidence, Belitskii cited two tributes which appeared in Soviet learned journals last year on the centenary of the birth of Alfred Wegener. One article, said Belitskii, was written by a strong opponent of Wegener's views, Dr Evgenii Milanovskii, while the other was written by an enthusiastic supporter, Dr Portnov, thereby making nonsense of Western "mischiefmaking insinuations".

Nevertheless, Belitskii acknowledged that many Soviet geologists do not support plate tectonics, considering that the theory cannot account for many aspects of continental structure and evolution.

Whether this leads to bias, or a stifling of opinion, is not clear. Some who know the Soviet Union say that discussion among geologists is now quite free. At the same time, geophysical articles in the general and semi-popular media tended, until recently, not to support plate tectonics. One of the first approving popular articles was a progress report on the Baikal-Amur Mainline railway which observed in passing that, owing to continental drift, the line, when completed, would be some 50-70 cm longer than originally planned. Vera Rich

Chinese university development

Help from afar

Washington

The World Bank and its soft-loan subsidiary, the International Development Association, have agreed to lend \$200 million to the People's Republic of China to support the country's efforts in meeting its present shortage of trained scientists and engineers. The loan is the first to have been made to the republic since the country assumed China's representation at the World Bank from Taiwan last May and is the biggest loan ever made specifically for building up a nation's scientific and technological base.

Half of the money will be provided by the World Bank at its standard interest rate of 9.6 per cent a year over 20 years, and the other half comes interest-free over 50 years from the International Development Association. The loans will go directly to supporting China's University Development Project, which includes among its aims an increase in the enrolment of science and engineering students at 26 leading universities from 92,000 to 125,000.

In addition, the money will be used to introduce graduate degree programme, to improve the general quality of teaching and research, and to strengthen the management of universities and the Ministry of Education.

According to World Bank officials, the government of the People's Republic of China will contribute an extra \$95 million to the University Development Project as part of a general effort to increase undergraduate enrolment by 7 per cent a year up to 2.2 million in 1990. Graduate programmes will raise their enrolments from virtually zero to 200,000 by 1990.

Of the total amount of \$295 million for which the project has at present budgeted, about \$160 million will be used to buy instructional and research equipment. Most of this will be obtained through international competitive bidding, a standard requirement of World Bank loans. Preference will be given to local manufacturers, but World Bank officials admit that "the amount of equipment contracts to be awarded to local suppliers through international competitive bidding is expected to be small".

China's determination to proceed with its plans for science and technology education contrast with its decision substantially to reduce previous commitments to the purchase of capital research equipment. Plans for obtaining both a particle accelerator — which was to have been built to designs produced by the US Department of Energy — and a telecommunications satellite which was to have been provided through the National Aeronautics and Space Administration, have both been shelved because of the lack of available capital.

David Dickson

CORRESPONDENCE

Creation "copout"

SIR — In a critique of American Creationism (Nature 2 July, p.95), Darnbrough, Goddard and Stevely state "no plausible theoretical model exists which provides a mechanism for the spontaneous generation of nucleic acids as informational macromolecules specifying polypeptides which themselves mediate the replication and expression of that information". This statement is much like the definition of the same scientific chicken-egg problem by J. Monod (1971) and K. Popper (1974).

One can argue plausibility indefinitely because the assessment of that quality is highly subjective. However, there has appeared. through experimental demonstration, a laboratory model of a cell-like structure with many of the properties required. This structure is composed of lysine-rich and acidic thermal polyamino acids that complex with each other to form cell-like structures. Both in solution and in suspensions of particles, these (selfordered) polyamino acids catalyse simultaneously the formation of pentides and polynucleotides from ATP and free amino acids (see ref.2). This model so far leaves unanswered many of the questions about the origin of the genetic code, but it demonstrates that the question is not as scientifically imponderable as Popper and Darnbrough et al. have suggested. It does provide answers to some questions, such as how the two kinds of macromolecular synthesis could first have been closely coordinated. The sequence theoretically derived from those experiments is: amino acid sets → protocells → nucleic acid + protein

I would not criticize Darnbrough et al. for not being aware of advances as recent as these (further details in the press). However, to attack an area of science because it has not yet reached a given stage, and then to argue a need for resorting to supernatural explanations because specific scientific answers are not reported, consolidated or agreed upon, is what is referred to in an American idiom as a "copout".

SIDNEY W. FOX

University of Miami, Coral Gables, Florida, USA

- 1. Fox, S. Nature 205, 328-340 (1965).
- Fox, S. & Nakashima Bio Systems 12, 155-156 (1980).

Attack on Tamuz

Sir — The editorial entitled "Making Israel Atone for Tamuz" (Nature 18 June, p.523) has shocked and dismayed many of your faithful readers. It appears that Nature, a journal that has hitherto enjoyed an unparalleled reputation in the scientific community for publishing innovative and careful scientific observations, is now embarking on a new course as a "yellow sheet" of political comment. One can only fear that subjective, biased, political diatribe as represented by your editorial will sully and finally displace the elegant work that has heretofore been the mark of publications in Nature.

I must admit that the editorial was cleverly written. Under the guise of a great concern for the reputation of the Non-Proliferation Treaty the editorial proceeds to mount a political attack on Israel and depicts Iraq, a signatory of the treaty, as an innocent, wholesome and wronged party, even deserving financial redress through "international legal processes" for the destruction of its means for producing awesome weapons.

Scientists are wont to deal with observations and to derive probability statements for predicting future phenomena based on past observations. Let us apply some logic to the editorial at hand. Unfortunately, although Iraq is a signatory of the Non-Proliferation Treaty, past actions have shown that in the case of Iraq "the sword is mightier than the pen". In the course of history the signatures of dictatorial governments have proved worthless. Iraq, by its own declaration, has maintained a continued state of war with Israel and has recently branched out in its military adventurism by an unprovoked attack against its neighbour, Iran. Iraq has been at the forefront of frenzied calls for the total destruction of Israel and has given both financial and military support to the terrorists who have revelled in the wanton murder of innocent civilians, especially women and children. Parenthetically, I did not note editorials in Nature either denouncing the bombing attack by Iran, albeit unsuccessful, against the nuclear reactor in Iraq, or decrying the slaughter of innocent women and children in Israel by terrorist attackers.

The editorial does not at all address the question as to why Iraq was stockpiling uranium suitable for the manufacture of atomic weapons. Much faith is placed in international commissions and the cursory inspections of the Iraqi reactor. It is ludicrous to believe that a government bent on production of nuclear weapons could not hide such facilities. Furthermore, what good is the knowledge that nuclear weapons are being produced once all the production capabilities, including the raw materials, are in place? Would a contrite editorial in Nature bring back to life the many thousands of casualties that would result from even one atom bomb dropped on Israel? Or might the response to such an unthinkable event be equivalent to the world response witnessed during the Nazi outrages? Even the United States government was quite concerned by Iraqi intentions as evidenced by the testimony of Mr Roger Richter before the US Senate Foreign Relations Committee.

As a native-born American, and a former officer in one of the US uniformed services, I am offended by the ugly term "Zionist vote". Fortunately, the majority of American voters speak to reality, and feel an affinity for Israel as the bastion of an open and democratic society in an area where tyrants and dictators prevail.

Unfortunately, the threat of nuclear retaliation is perhaps the only deterrent that Israel has against total annihilation by its truculent neighbours. Indeed, it appears that not only does Israel require this deterrent against its threatening neighbours, but perhaps also against such editorials as that in *Nature*.

Finally, aside from the question as to

whether one does or does not justify the Israeli destruction of the Iraqi nuclear reactor, I believe that one can assert that editorials of political diatribe do not belong in a premier scientific journal such as *Nature*.

S.Z. HIRSCHMAN

Mount Sinai School of Medicine of the City University of New York, New York, USA

Certain points raised in this letter have been discussed in a subsequent editorial (Nature 16 July, p. 185) — Editor, Nature.

Researchers insecure

Sir - "Poaching" of ideas and staff between competing institutions is not a new phenomenon (A.J.S. Davies, Nature 2 July, p.96), though I doubt if it has (yet) reached serious proportions. A more serious problem, exacerbated in recent years by economic constraints, is that research teams usually include several young postgraduate and postdoctoral workers financed by short-term grants of 1-3 years' duration. The present system by which these are made available does not permit, let alone encourage, such scientists to remain in the teams where they begin to develop their expertise. There are two powerful reasons in particular for this. One is the present dire shortage of permanent posts in the academic sphere. The second is that it now appears to be the general policy that a young scientist should not be the recipient of more than 2 three-year grants from such bodies as the Medical Research Council. Consequently, after 6 years, such people may be obliged to find other employment anyway.

In most cases, the prime motivating force for a young scientist is not so much the chance to earn a large salary as reasonable security in order to develop his or her own skills and ideas over the long term. To be in a constant state of anxiety for the future, no matter what one achieves, seriously undermines one's ability to do this. Sadly, neither the universities nor the major funding bodies (the government and research councils) in Britain seem to have grasped this simple concept.

A certain degree of flexibility for movement between institutions is highly desirable, but many young scientists would welcome a contract that bound not only them but also their employer in the long term. Present conditions in Britain promote academic paralysis by destroying flexibility — those with secure jobs stick to them — while denying encouragement and opportunity to the young, and with that the long-term success and wellbeing of research teams.

MICHAEL W. RUSSELL

University of Alabama, Birmingham, Alabama, USA

Erratum

In a letter published in *Nature* 14 May, p. 104, the name of one of the members of the (US) Xeroderma Pigmentosum National Registry was omitted. The full list of members is: Alan D. Andrews, James L. German III, Kenneth H. Kraemer and W. Clark Lambert.

NEWS AND VIEWS

Towards a total human protein map

from Brian F.C. Clark

Now that such powerful techniques are available for the sequencing of DNA and the study of genome organization, some scientists are turning to what might be the next large-scale challenge — the separation, identification and measurement of the gene products.

At a recent workshop*, the possibility of making a complete catalogue of animal cell proteins using two-dimensional gel electrophoresis was discussed. In man there are at least 30,000 different proteins (although maybe only 10,000 in a given cell type) and the technique has already been used to separate and catalogue more than 1,200 of them (R. Bravo et al. Cell Biol. int. Rep. 5;93, 1981).

At the workshop it was confidently stated that it will be possible to detect 2,000 to 3,000 proteins with present technology. Further progress can be made by fractionating cellular components before carrying out two-dimensional separation. This will bring many proteins present in small amounts to the level at which they can be detected.

There are three main problems in trying to produce a catalogue of proteins for human or any other animal cells: how to make methods of protein identification reliable and reproducible; how to store the immense amount of data produced in a usable form; and how to name and catalogue the separated proteins.

The question of reproducibility is clearly the most serious problem and needs to be solved first. At present gel chemistry is not under complete control and, among the many parameters discussed, it was generally agreed that the first essential is the standardization of the commercial production of ampholytes for the first separation by isoelectric focusing. Attempts have been made to overcome standardization problems by R. Bravo (University of Aarhus), L. Anderson (Argonne National Laboratory) and J. Garrels (Cold Spring Harbor Laboratory) and a representative of a commercial concern expressed confidence that the requirements of the research scientists could be met in the future. Indeed, an

arrangement was to be made to provide specified laboratories with samples of ampholytes until all appropriate criteria were met. Since future automatic measurements and clinical applications depend on the reproducibility of the technique, this arrangement was of great significance for the workshop.

In the two-dimensional separations it is now usual to take a total cellular protein extract which can be radioactively labelled, denature it, and apply it to two different first dimensions. Isoelectric focusing is used to separate neutral and acidic proteins and non-equilibrium pH gradient electrophoresis for the basic proteins. After the first two dimensions have been run, each separated sample is applied to an identical polyacrylamide gel containing SDS to provide the second dimension of electrophoretic separation.

J. Garrels' laboratory routinely standardizes total protein extracts by using a number of different concentrations of polyacrylamide and several pH ranges. In a simpler, two-dimensional standardization (in cases for which it is justifiable to lose some basic protein resolution) his group runs only a broad-range (pH 3.5 – 10) isoelectric focusing electrophoresis for the first dimension

After separation the protein locations on the gel have to be visualized. Discussion was rather brief but there was a general consensus that fluorography provided a better and more accurate method than autoradiography. With unlabelled proteins the recently used silver stains are much more sensitive than Coomassie blue but produce variable results with gels more than one millimetre thick.

The problems of data handling are obviously immense - even at the present time more than 1,300 proteins may be recorded in different cell types and under different conditions, and L. Anderson reported that his laboratory has already run 40,000 separations. There was, however, much optimism about current progress. Already it seems that automatic measurements of protein locations, together with computer storage and retrieval, will be possible in the laboratories of N. and L. Anderson, J. Garrels, K. Lonberg-Holm (Dupont), L.E. Lipkin and P.F. Lemkin (NIH) and E.P. Geiduschek (University of California, San Diego)

within the next year. Since these laboratories have access to medium-sized computer facilities, it was reassuring to small laboratories that A. Freiburghaus (University of Cambridge) was also optimistic of finishing his plans for general automatic measurement of two-dimensional gels using more standard university facilities.

There was general agreement on the best approach to the third problem, that of the naming and cataloguing of the proteins. It was felt that, at present, any system relying on accurate positional information was doomed to failure and that it is far too early to try to set up a master nomenclature. Estimates of when this might be possible varied from three to five years. With this problem in mind, the Aarhus group described the advantages of labelling small numbers of cells with ¹⁴C -labelled amino acids so that raw data may also be preserved for long periods.

Different nomenclatures will probably be necessary for different cell types. It was agreed that the current different nomenclature systems used in different laboratories should continue but with the intention of switching to a master plan when the separations can be suitably reproduced. Meanwhile, it was agreed that laboratories involved in cataloguing cell proteins should exchange samples and marker proteins so as to be able to crossrefer to separations in different laboratories. If the cross-referencing were done, then it should be possible at future workshops to assemble the available information (tissue specificity, modifications, hormonal responses) for the 100 or so most prominent proteins and to publish a compendium of such data with representative maps. The problem of protein identity was also discussed and a general appeal was made to anyone working with purified identified proteins to allow them to be located on the standardized two-dimensional gel separations.

The workshop forecast that within a short time several data sets of catalogued proteins will be established in the USA and Europe. Possible locations would be the Argonne National Laboratory, Cold

Brian F.C. Clark is Professor in the Division of Biostructural Chemistry, Department of Chemistry, Aarhus University.

[•] The EMBO workshop on 'The standardization of a numbering system of animal cell proteins separated by twodimensional gel electrophoresis' was held at Aarhus University, Institute of Chemistry, June 30 and July 1 1981, and was organized by J.E. Celis of the Department of Structural Biochemistry, Aarhus University.

Spring Harbor Laboratory and the European Molecular Biology Laboratory.

It was agreed that it was desirable to support a number of future research projects to obtain as complete a catalogue of proteins as possible for a small number of centrally important cell types. Probably the laboratories of L. Anderson, J. Garrels and J.E. Celis would concentrate at first on HeLa, human fibroblast, human lymphocyte and mouse fibroblast cell types. The choice of the last cell type arose from the availability of genetic data for the mouse which, in future, will allow changes in the protein map to be related to changes in genotype. In this respect J. Klose (University of Berlin) described promising results from experiments in which he identified a small number of protein changes in mouse cells treated with the mutagen methylnitrosourea.

The great potential of the method in clinical diagnosis was described by E. Jellum (University of Oslo) and L. Anderson. Jellum described the 'Janus collection', an impressive stock of human blood samples from 63,000 patients set up in Oslo in 1973. Since the patients have had samples taken yearly, when they develop a

new disease it is possible to search through their protein record for several years before the disease was detected. Clearly a catalogue of human gene products could make it possible to detect metabolic irregularities and diseases, such as cancer, which affect gene expression.

In terms of current costs, J.E. Celis's group has estimated that one two-dimensional gel analysis can be made for thirty dollars, using ¹⁴C-amino acid labelling of cells. For the development of a computerized program for handling large numbers of reproducible analyses and the establishment of a human protein catalogue, the Argonne National Laboratory has estimated that a sum of the order of ten million dollars is required. The general impression given by the research workers involved was that a grant of a million dollars a year would be feasible from US granting agencies.

The meeting ended on an optimistic note with the belief that present technical problems can be solved, and plans for cooperation and for communication of unpublished data among the laboratories which are developing the technique can be implemented.

hormone.

Mouse mammary tumour virus (MMTV) is a RNA virus that may be transmitted as a virion, producing mammary carcinomas in female mice and productive infection in rodent tissue culture cells. Like other RNA tumour viruses, it replicates by synthesizing a viral DNA copy. MMTVlike DNA is also present as an endogenous provirus in the genome of mice. In mammary tumour cells and in virusinfected cells, glucocorticoid hormones rapidly and dramatically enhance the rate of synthesis of MMTV RNA. Two recent papers from the Swiss Institute for Experimental Cancer Research describe the cloning of MMTV DNA, the introduction of that DNA into mouse tissue culture cells and the regulation of MMTV RNA expression in the cells thus transformed. In one case, the full genome of MMTV was cloned from the closed circular DNA of infected rat hepatoma cells (Buetti and Diggelman Cell 23: 335. 1981). In a second approach, the MMTV provirus and flanking host cell sequences were cloned from a mouse liver DNA library (Hynes, Kennedy, Rahmsdorf and Groner Proc. natn. Acad. Sci. U.S.A. 78: 2038, 1981). In each case the cloned DNA was introduced into thymidine kinasedeficient mouse L-cell fibroblasts, cells that have glucocorticoid receptors. The now-standard technique of co-transfection with DNA containing the herpes thymidine kinase-proficient cells was used to introduce the MMTV DNA. In this way, L-cell clones, each carrying multiple copies of the MMTV DNA, were obtained. Treatment of the transformed cells with the glucocorticoid dexamethasone resulted in a 5-10-fold increase in the level of poly-(A)-containing MMTV RNA. Expression of the cloned DNA was thus shown to respond to hormone in a manner similar to that found in cultured mouse mammary tumour cells or virus-infected rodent cells.

Hormonal regulation of another cloned gene is reported by Kurtz of the Cold Spring Harbor Laboratory (Nature 291; 629, 1981). The rat protein α_{2U} globulin is synthesized in liver and excreted in urine. It is regulated in vivo by multiple hormones. including glucocorticoids, which enhance, and oestrogens, which suppress, its synthesis. The protein is encoded by a multi-gene family. Two of these genes were cloned and the cloned DNA introduced into L cells using the herpes thymidine kinase co-transfection technique. L-cell clones transformed with one of these genes showed dexamethasone-induced enhancement of α_{211} globulin mRNA levels in 8 of 12 cases, and transformation with the other gene conferred a positive response in 4 of 9 clones. Again, individual L-cell clones each carried multiple a_{211} globulin gene copies.

The question of how glucocorticoids regulate genes is but a fragment of the problem of differential gene expression, of how genes are turned off and on by differ-

Hormonal regulation of cloned genes

from Philip Coffino

How do steroid hormones work? Glucocorticoids and the sex-related steroids, oestrogens, progesterones and testosterones, have profound effects on development and differentiation. A consensus exists on the early and late steps of their action. The hormones enter a cell and bind to a specific cytoplasmic receptor. The steroid-receptor complex undergoes a structural change that results in its entry into the nucleus. In the nucleus a series of events ensue that lead to an increased rate of transcription from specific genes, with a consequent increase in the steady-state level of mRNAs generated from these transcripts. (Other mechanisms, such as changes in mRNA stability, may also play a part in some cases.) The nature of the events involved in transcriptional regulation by the steroid-receptor complex in the nucleus is obscure.

This problem is a special case of a more general one: how is gene expression controlled to produce cellular differentiation

Philip Coffino is in the Departments of Medicine and of Microbiology and Immunology, University of California, San Francisco, and is currently on sabbatical at the Institut de Chimie Biologique, Strasbourg.

and modulated by hormones and other physiological effectors in differentiated cells? Cloning of eukaryotic genes in recent years has yielded considerable information on gene structure, but relatively little on regulation. Cloned genes, for example globin and ovalbumin, have been placed in cells and shown to function there. However, in their new environment, these genes did not respond to the regulatory signals that work in vivo. Recently, a series of papers has demonstrated that hormonedependent control of expression can take place in cloned genes put back into cells. This work and natural extensions of it promise to help elucidate the molecular mechanisms of steroid hormone action.

An attractive model, derived from our understanding of how gene expression is managed in bacteria, postulates that the steroid hormone-receptor complex binds with specificity and high affinity to a region of DNA which lies near the gene whose transcription it regulates. The model predicts, as has now been found possible in two distinct systems, that isolated DNA containing the regulable gene, introduced into a hormone-responsive cell, should show regulation dependent on the

entiation and by physiological changes. The studies described here show that, placed together, a cell and a cloned gene can modulate the level of that gene's RNA product in response to a glucocorticoid. The nature of the contribution of each is not resolved. Does the hormone-receptor complex interact directly with sequences in or near the cloned gene, in flanking host cell sequences or at more distant sites? Do all copies of the gene function, or only some, and if the latter, what distinguishes the responsive copies? Is the regulation shown here transcriptional or does it lie at the level of RNA stability or processing? Why do the cloned genes work at all in L cells? In the case of MMTV the gene is already there in the form of a provirus, but is not expressed, while the normal functional environment of the α_{211} globulin gene is a liver cell, not a fibroblast. Can these differences in function be accounted for in terms of chromatin structure, DNA methylation, the nature of flanking sequences or in some other way?

It is probable that we will soon have answers to those and related questions. The MMTV α_{211} and other hormone-responsive genes, natural or constructed, will be sequenced, cut, modified and asked to perform in cells, and the transformed cells' DNA and chromatin will be picked at with nucleases and restriction enzymes. Experiments of this kind will define the elements needed to make the system work and this understanding will guide efforts to design a fully defined hormone-regulated in vitro transcription system constituted of purified components. A crucial early step of this ambitious programme has been achieved and the rest will find no lack of willing hands.

Eruption mechanics on the Earth, Moon and Mars

from G. Wadge

THE discovery of volcanic eruptions on Io by the Voyager 1 spacecraft1 was exciting visual proof that volcanism, in this case based on sulphur, is a major process on the surface of at least one planetary body other than Earth. There is abundant, though less spectacular, visual evidence of past volcanism on Mars and the Moon; most of this volcanicity occurred in the early history of these planets. Volcanologists are faced with the problem of how to interpret this evidence in terms of eruptions which ended billions of years ago. One of the most productive methods has been to compare the known magma properties and conduit systems of volcanoes on Earth with the equivalent putative values for the other planet, deduce what volcanic landforms these eruption mechanics should produce and see whether these agree with the observations. Recently Wilson and Head2 reviewed the mechanics of erupting basaltic magma on the Moon using this approach. Their conclusions, perhaps rather surprisingly, generally emphasize the similarities between terrestrial and lunar flood basalt eruptions.

On both bodies magma rises to less than 2 km below the surface before gases begin to exsolve, mainly H₂O and CO₂ on Earth, and CO on the Moon. Wilson and Head assume that CO is the main lunar volatile phase and is soluble in silicate magmas —

but there is little direct evidence. The rates of rise of magma before this vesiculation are limited by the degree of cooling and the attendant increase in viscosity, and by the yield strength of the magma. Wilson and Head find that a very small range of conduit widths (0.1-4 m) is adequate to explain the whole spectrum of terrestrial eruptions, from the magma that barely reaches the surface to gigantic eruptions exemplified by the Columbia River flood basalts. The most striking feature of lunar volcanism is the great length and volume of many of the recognizable mare flows. This study clearly shows that the feeding fissures of these huge, high-effusion rate eruptions need to have been only slightly wider than those on Earth, perhaps 10-25 m. We see little evidence of these source fissures on the Moon and should not expect to, for they are too narrow.

Lunar basaltic magmas came from deeper sources (~100 km) than most terrestrial basalts and were also relatively denser compared with the overlying crust. Buoyancy is presumed to be the main force responsible for magma rise on the Moon as it is on the Earth. Thus lunar magma pressures should have been lower and the effusion rates less than terrestrial values. This conclusion is contrary to the observed size of the lava flows. A hydrostatic solution to this problem was proposed by Solomon³ involving regional loading outside the mare basins. Wilson and Head now show that magma density contrasts were of the same order as those calculated for Earth (a few hundred kg per m³). The large magnitude of the lunar eruptions was due to the local extensional tectonic environment around the mare which allowed particularly wide fissures to develop. Lunar effusion rates were probably not exceptionally large compared with those for terrestrial flood basalts. What is remarkable is that some of the calculated eruption volumes (10³-10⁴ km³) represent a significant fraction of the total volume of mare volcanism (~10² km³).

CO concentrations in lunar basalts were probably about an order of magnitude less (a few hundred p.p.m.) than average volatile contents of terrestrial basalts. As magma rose to the surface, this relative deficiency of volatiles would be compensated by an increased release of energy per unit mass due to the decompression to zero atmospheric pressure at the surface. The resultant disruption of magma into fragments would allow wide dispersion but the lack of an atmosphere would preclude the complex differential sorting processes typical of terrestrial pyroclastic eruptions. Instead, spectacular fountains of magma clots must have blanketed large areas around lunar vents, and in many cases these clots, uncooled by atmospheric interaction, would have recoalesced on hitting the ground to form lava flows.

Unlike the Moon, Mars has a thin but appreciable atmosphere and a gravity field intermediate between those of the Moon and Earth. One would expect martian volcanism to be a hybrid of the characteristics displayed by its planetary neighbours. Preliminary calculations, again by Wilson and Head4, indicate that it should resemble Earth volcanism more than that of the Moon. They predict vigorous, convecting eruption columns, growth of gas bubbles in magma to about four times the equivalent size on Earth, and the common occurrence of high-pressure, explosive vulcanic eruptions caused by magma interaction with sub-surface ice. Of course, we know less about the tectonic setting and much less about magma compositions on Mars than we do for the Moon. If these factors are not too different from the values assumed by Wilson and Head, then martian eruption mechanics should make the development of ignimbrites, by the collapse of large emption columns, even more common than on Earth. Can we see these ignimbrites? The answer given by Greeley and Spudis in their review⁵ of the Mariner and Viking data is that despite several claims for their identification, unequivocal proof is lacking. Failure to identify ignimbrites on Mars could necessitate revision of the eruption models of Wilson and Head although it is more

G. Wadge is at the Lunar and Planetary Institute, Houston.

Masursky, Schaber, Soderblom & Strom Nature 280, 725 (1979).

^{2.} Wilson & Head J. geophys. Res. 86, 2971 (1981)

Solomon Proc. 6th Lunar Sci. Conf. 1021 (1975).
 Wilson & Head Abstr 12th Lunar Sci. Conf. (1981).

Greeley & Spudis Rev. geophys. Space Phys. 19, 13 (1981).

probable that we need to look longer and more closely.

The calculations of Wilson and Head assume that the pressure gradients driving erupting magma are equal to the lithostatic gradients — a reasonable assumption for generalized modelling. It is clear that this does not hold for all eruptions on Earth. Repeated fracturing and deformation of

the surface during the eruption of central volcanoes is proof of this. Mars has huge central volcanoes which, like their terrestrial counterparts, exhibit a variety of evolutionary forms and undoubtedly have complex internal structures. In future we will need to incorporate elements of these massive surface structures into models of martian eruption mechanics.

Sedimentary sulphides

from Philip Trudinger and Preston Cloud

THE recognition that present reserves of minerals may be nearing exhaustion has increased interest in the help that models of ore genesis might give in the search for deep-seated deposits of commercial-grade minerals. That some strata-bound base metal sulphide ores may have formed syngenetically, that is, in the early stages of sedimentation, by deposition in sedimentary pile rather than by hydrothermal replacement was first proposed in the early 1950s. Since then, syngenetic models have been proposed for many deposits and often imply lowtemperature deposition with the involvement of biological activity in mineralization. A timely review of evidence for and against biological and other syngenetic processes in sulphide ore genesis was provided at a symposium* held in Australia earlier this year.

The characteristics of strata-bound sulphide ores were described by L. Gustafson (Australian National University, Canberra). The strata-bound sulphide and sediment-hosted lead-zinc and copper ores are features of the oxygenous Earth, and are no older than 2 Gyr. The deposits are in tectonically active intracratonic settings, commonly associated with volcanism, with indications of aridity or hypersalinity. The local settings are ones in which groundwater can move to shallow sites of sulphide deposition. The deposits are characteristically associated with redbeds, either beneath or interbedded with them. This is particularly true of copper ores, most of which are found in reduced sediments, often at a redox interface; for example, between red and green sediments. Zoning patterns are usually related to 'plumbing systems', both stratigraphically and areally. The timing of metal fixation

relative to deposition and diagenesis, and the temperature of mineralization, are of crucial importance but are, as yet, poorly understood. Sulphur isotope values reflect processes at the sites of deposition rather than the source of the sulphur. Finally, unlike volcanogenic deposits which range across the copper-zinc axis on a lead-zinc-copper ternary diagram, the strata-bound copper and lead-zinc deposits mostly cluster in the copper corner or line up along the lead-zinc edge respectively.

Gustafson emphasized the problems arising from variable usage of the terms syngenesis, diagenesis (processes taking place after sedimentation) and epigenesis (processes taking place after sediments are compacted). Different scientists draw their boundaries at different places. He concluded that most of the deposits in question are diagenetic, with metals derived from within the sedimentary sequence but outside the areas of ore deposition. Local reduction of sulphate is probably responsible for deposition of most copper and some lead-zinc, but more than one source of sulphur and mechanism of fixation exists. Variations are, however, observed towards both later epigenetic, and earlier syngenetic, processes.

Several papers dealt with specific ancient strata-bound sulphides - especially the Mount Isa ores of north-west Queensland. the McArthur deposits of Northern Australia, and the Mt Gunson copper deposits of the Stuart Shelf, South Australia. Methods of investigation included isotope tracers, mass-transfer modelling, fluid inclusion palaeothermometry and palaeosalinity, detailed stratigraphy and petrology and biogeochemistry. A consensus of opinion favoured a penecontemporaneous origin for the lead-zinc-silver ores at Mount Isa and McArthur, compared with an epigenetic hydrothermal origin for the associated copper ores at Mount Isa. The ore metals and most of the sulphide were introduced by exhalative processes although there is evidence that some of the sulphide in pyrite was of biogenic origin. Local karst-associated mineralization also occurs in the McArthur region, which further displays redox-related mineralization in the Woologorang Formation, some 3,000 m below the well known HYC deposit.

Further evidence against 'biogenic' lead-zinc mineralization is the presence of saddle dolomite in carbonate host rocks of many lead-zinc occurrences. The mineral indicates relatively high temperatures (up to 150°C) and is perhaps formed along with sulphides during inorganic sulphate reduction.

At Mt Gunson, copper-iron sulphide minerals hosted by laminated mudstones and dolostones are of two generations. The sulphur itself was introduced at the same time as ancient coastal sediments and then reacted with sedimentary iron to form pyrite. This was replaced by copper introduced along faults and other channelways.

C.C. von der Borch (Flinders University, South Australia) outlined how studies on modern depositional systems can lead to a better understanding of sedimentary controls on ore deposition. Noteworthy are deposits in oceanic rift zones (for example, the Red Sea and East Pacific Rise), which provide information on the sources of metal-enriched hydrothermal fluids, and contemporaneous deposition of metal sulphides, in settings analogous to some major ancient deposits. By contrast, modern shallow marine, subaerial and lacustrine sediments, while often resembling ancient counterparts that host stratiform and strata-bound ores, rarely contain metal sulphide concentrations significantly above background.

The main factor controlling metal sulphide accumulation in reducing sediments remote from hydrothermal influences appears to be the supply of reactable metals rather than of sulphide. In many present-day shallow marine sediments, and in some deep-sea sediments, bacterial sulphate reduction is of sufficient intensity to generate sulphide concentrations one to two orders of magnitude higher than those actually observed. In the Holocene paralic carbonate complexes in northeastern Spencer Gulf, South Australia, low levels of metals limit mineralization even though the hydrological regime of this environment resembles those inherent in a number of models of ore genesis.

Potential environments for large-scale biogenic sulphide deposition are those in which metal-enriched groundwaters mix with organic-rich, sulphidic sediments—perhaps exemplified by the Denali chalcopyrite—pyrite deposit in Alaska. The possibility of deposits containing sulphide of both hydrothermal and biogenic origin

^{*}A symposium on Sulphide Mineralization in Sediments: Current Status of Syngenetic Theory was held at the Australian Academy of Science, Canberra, from March 2 to 4, 1981. It was sponsored by the Baas Becking Geobiological Laboratory and formed part of the programme of IGCP Project 157 on Early Organic Evolution and Mineral and Energy Resources. It was attended by over 100 scientists from the mining and petroleum industries, universities and government research laboratories. Abstracts and several full papers will be published in December 1981 in the Bureau of Mineral Resources Journal of Australian Geology and Geophysics 6, No. 4.

Philip Trudinger is in the Baas Becking Geobiological Laboratory, Canberra, and Preston Cloud is Emeritus Professor at the University of California, Santa Barbara.

is suggested by isotopic studies on the McArthur deposit and on the modern sediments of the rift-related Guaymas Basin, Gulf of California.

Although it is now well established that bacterial sulphate reduction is a significant and rapid biochemical process in modern anoxic sediments, caution was advised in extrapolating local rates of sulphate reduction to regional models of mineralization. Rates are affected by many factors, including abundance of organic matter, rate of flow of interstitial water, bioturbation and, for intertidal sediments, the amount of time the sediment is wetted.

Sulphur isotope data are frequently used in inferring the origins of mineral deposits and in interpreting the diagenesis of sulphur. The interpretation of such data is not, however, without difficulties. Patterns of δ^{34} S frequencies in sulphides of modern sediments can be related to the supply of sulphate (open versus closed systems) but not to geological settings. In the reducing sediments of the Bali Trough, 32S in pyrite increased with depth, a trend similar to those reported for cores from the McArthur and Kupferscheifer deposits but which at present defies explanation. However, broad trends in the isotopic compositions of ancient minerals with time are perhaps more amenable to interpretation and suggest that sulphate became the major sulphur component in the hydrosphere, and bacterial sulphate reducers widespread, at about 2 Gyr BP. Anomalous δ^{34} S distributions in 2.7-2.8 Gyr old sulphides of the Michipicoten and Woman River deposits could have resulted from reduction of an oxidized species (sulphite?) other than sulphate.

Further papers dealt with the production and diagenesis of organic matter, the driving force for all biogeochemical processes, and the significance of organic carbon in ancient deposits. Benthic oxygen-evolving prokaryotes were seen as major organic carbon producers in marginal aquatic environments of arid and semiarid climates. Both field and laboratory studies indicated, however, that the bulk of the organic matter is rapidly remineralized, particularly by sulphate-reducing bacteria.

In relatively young sediments, specific organic molecules of diagenetic origin provide clues to sources of organic matter (for example, marine versus non-marine), depositional environment (oxic compared with anoxic) and maturity of organic matter. With time, carbonaceous material undergoes a complex series of reactions leading to loss of volatiles and formation of different products - kerogen, fossil fuels or graphite. Even graphite may be oxidized to CO₂ by water at above 200°C, and can be removed from the system without trace. Thus, while reduced carbon (or graphite) occurs in sediments of all ages, including the 3.8 Gyr old metasediments at Isua, south-west Greenland, its significance can prove difficult to determine. Nevertheless,

considerable progress has been made towards identifying major biogeochemical evolutionary events in Proterozoic and older history through the use of carbon isotopic data on kerogens, together with fossil and other geochemical evidence. These data are now all consistent with the view that oxygenic photosynthesis had evolved by the late Archaean and that, thereafter, first the hydrosphere and then the atmosphere were progressively oxygenated.

The symposium highlighted advances made over the past decade in our knowledge of mineral deposits and modern

depositional systems. It stressed the need for continuing multidisciplinary research on the evolutionary cycles of elements involved in the making of either sedimentary ore deposits or fossil fuels. These cycles are, or may be, affected by biological processes at some stage in their history. Whether that is called syngenetic or diagenetic seems to be more a matter of discipline and definition than of timing. To the extent that we detected consensus, it was to the effect that major sulphide deposits seem to include both post-depositional sedimentary and later hydrothermal components.

Synthesis and function of metallothioneins

from J. Kägi, T.L. Coombs, J. Overnell and M. Webb

As cadmium is a ubiquitous component of the Earth's minerals, its transfer to animals and man through food chains cannot be avoided. Since the metallic ion is cumulative, long-lived mammalian species, even in uncontaminated environments, can accumulate appreciable body burdens of Cd2+ during their lifetimes. Most of this Cd2+ is stored in the liver and kidneys as a soluble lowmolecular-weight metalloprotein which contains not only appreciable amounts of Cd2+ and Zn2+, but also an extremely high number of cysteine residues. The metalloprotein, named 'metallothionein', is a metallo-derivative of the sulphur-rich apoprotein, thionein. Thionein is an inducible protein and synthesis of usually short-lived metallothioneins, in which Zn2+ and Cu+ are the major bound cations, occurs when the intakes or body burdens of these 'essential metals' are

An explosive increase in interest in these metalloproteins followed observations in the 1960s that they accumulate in the liver and kidneys of rabbits in response to administration of cadmium salts. As there was evidence from Japan of a relationship between cadmium pollution and the Itai Itai disease, the hypothesis was put forward that the inducible synthesis of metallothionein formed a defence mechanism. Evidence soon accumulated that besides participating in cadmium sequestration, metallothioneins play a fundamental part in the homeostasis of certain essential cations, principally Zn2+ and Cu2+, a function undoubtedly dependent upon their primary, secondary and tertiary structures. Analysis of recent developments in the chemistry of metallothioneins, with its implications in biochemistry, nutrition, toxicology and medicine, formed the main objective of a recent meeting*.

Sequencing and physico-chemical

Sequencing and physico-chemical studies (J.H.R. Kägi, University of Zurich) show how the primary and tertiary structures have been conserved during many evolutionary stages. From UV, circular dichroism and magnetic circular dichroism spectroscopic studies on experimentally prepared cobalt(II)- and nickel(II)-metallothioneins, as well as the natural zinc- and cadmium-proteins, M. Vasak (University of Zurich) concluded that the cysteine residues are bound by their thiolate groups to these metals in a tetrahedral configuration. Electron paramagnetic resonance and magnetic susceptibility studies of the cobalt derivative suggest that the metal sites are joined in clusters with some of the cysteinyl residues serving as bridging ligands between the metals, as exemplified by an adamantane-type structure.

Mammalian copper-metallothionein and the potential biological significance of its inducibility in some tissues by copper administration were discussed by I. Bremner (Rowett Institute, Aberdeen). The binding of copper to metallothionein differs from that of other metals and the metal to sulphur stoichiometry is much lower. The copper of anaerobically prepared metallothioneins is bound in the cuprous state in the form of Cu(I)-thiolate complexes. The binding mode is consistent with unusual luminescence properties observed in copper-metallothionein from Neurospora crassa (K. Lerch, University of Turiob)

That metallothioneins transfer metal to

J. Kägi is in the University of Zurich, T.L. Coombs and J. Overnell in the Institute of Marine Biochemistry, Aberdeen and M. Webb in the MRC Toxicology Unit, Carshalton.

^{*}A post-FEBS 2-day international meeting on metallothionein torganized by J. Overnell and T.L. Coombs) was held on April 4 and 5 at the University of Aberdeen, Scotland.

apo-metalloproteins was supported by K. Lerch and by U. Weser and H. Hartmann (University of Tübingen). They showed reconstitution of different copperdependent apoproteins with Neurospora and yeast copper-metallothionein respectively. However, no single function can be ascribed to metallothionein. M. Webb and K. Cain (MRC Tox cology Unit, Carshalton) demonstrated that in rats the protein can act as a mobile zinc and copper reserve in maintaining homeostasis or as a control agent for zinc and copper uptake in fetal and neonatal life stages. The detoxication role for controlling cadmium and mercury may well be a carry-over of this fetal mechanism into adult life.

Introducing the topic of metallothionein biosynthesis, R. Palmiter and D. Durham (University of Seattle) described experiments carried out on mouse metallothionein gene structure. Recombinant cDNA and genomic clones corresponding to mouse metallothionein-1 have been isolated and characterized. The DNA sequence indicates that this gene spans 1,100 base pairs with two intervening sequences (see Palmiter Nature 292; 267, 1981). The gene is regulated at the transcriptional level by heavy metals and less efficiently by glucocorticoid hormones both in vivo and in isolated cell lines.

Several cell lines that were selected for resistance to cadmium were shown to have amplified their metallothionein genes, allowing enhanced synthesis of this metalbinding protein. In an elegant study using DNA recombinant techniques, the regulatory portion of the mouse metallothionein gene was fused to the structural gene of thymidine kinase, rendering this enzyme inducible by Cd²⁺. Furthermore, evidence was presented that the inducibility of the mouse metallothionein gene is controlled by DNA methylation.

The application of high-pressure liquid chromatography to the assessment of homogeneity, structural investigations and the isolation of fragments from enzymatic and chemical cleavage of metallothioneins was described by K.J Wilson (University of Other communications emphasized the importance of these metalloproteins in the homeostatic control of specific metals not only in mammalian species but also in yeasts, marine arthopods, crustaceans, eels and sea-birds. From the significant developments that were reported in all fields it seems that the elucidation of the mechanisms of synthesis, degradation, gene regulation and of the structural arrangements at the metal-binding sites of the metallothioneins is 'just around the corner'.

Treating urea cycle defects

from Vicente Rubio and Santiago Grisolía

THERE is much interest in the treatment of inborn errors of the urea cycle. As pointed out by Smith¹ in her excellent review, the prospects for patients with deficiencies of this cycle are far better now than in the recent past. Therefore, we regret her omission of N-acetylglutamate synthetase deficiency and of N-carbamoyl glutamate as a therapeutic agent for this condition.

N-acetylglutamate, synthesized by N-acetylglutamate synthetase, is the physiological activator of mitochondrial carbamoyl phosphate synthetase. Deficiency of N-acetylglutamate synthetase should induce hyperammonaemia. The deficiency cannot be treated by administration of N-acetylglutamate because this compound is deacylated by cytosolic deacylases and it does not permeate the mitochondrial membrane. However, carbamoyl phosphate synthetase is also activated by analogues of N-acetylglutamate, such as N-carbamoyl glutamate2, which is resistant to deacylases and which, when injected into rats, can be found in the mitochondrial matrix of liver cells3.

Therefore, it is not surprising that

Vicente Rubio and Santiago Grisolía are at the Instituto de Investigaciones Citologicas de la Caja de Ahorros de Valencia, Valencia, Spain. Bachmann et al.⁴, who recently described the first case of hyperammonaemia due to N-acetylglutamate synthetase deficiency, found that N-carbamoyl glutamate is indeed extremely effective in the treatment of this condition.

As indicated by Smith, increases in the levels of N-acetylglutamate leading to greater activation of carbamoyl phosphate synthetase may improve control of ammonia levels in ornithine carbamoyl transferase, arginosuccinate synthetase and arginosuccinate lyase deficiencies and in partial defects of carbamoyl phosphate synthetase. Thus, although N-carbamoyl glutamate should be regarded as the specific treatment for N-acetylglutamate synthetase deficiency, its efficacy in these conditions should also be tested. N-carbamoyl glutamate may also be useful for patients with propionic methylmalonic acidaemias, for which decreased levels of N-acetylglutamate are postulated as a cause for the hyperammonaemia associated with these syndromes.



100 years ago

THE COMET OF 25 JUNE

The appearance of a large comet has afforded an opportunity of adding to our knowledge of these bodies by applying to it a new means of research. Owing to the recent progress in photography it was to be hoped that photographs of the comet and even of its spectrum might be obtained and peculiarities invisible to the eye detected.

It was obvious that if the comet could be photographed by less than an hour's exposure there would be a chance of obtaining a photograph of the spectrum of the coma, especially as it was probable that its ultra-violet region consisted of but few lines. In examining my photographs of the spectrum of the voltaic arc, a strong band or group of lines was found above H, and on the hypothesis that the incandescent vapour of a carbon compound exists in comets, this band might be photographed in their spectrum.

Accordingly at the first attempt a photograph of the nucleus and part of the envelopes was obtained in seventeen minutes, on the night of June 24, through breaks in the clouds. On succeeding occasions, when an exposure of 162 minutes was given, the tail impressed itself to an extent of nearly ten degrees in length.

I next tried by interposing a direct-vision prism between the sensitive plate and the object-glass to secure a photograph which would show the continuous spectrum of the nucleus and the banded spectrum of the coma. After an exposure of eighty-three minutes a strong picture of the spectrum of the nucleus, coma, and part of the tail was obtained, but the banded spectrum was overpowered by the continuous spectrum.

I then applied the two-prism spectroscope used for stellar spectrum photography, anticipating that, although the diminution of light would be serious after passing through the slit, two prisms, and two object-glasses, yet the advantage of being able to have a juxtaposed comparison-spectrum would make the attempt desirable, and, moreover, the continuous spectrum being more weakened than the banded by the increased dispersion, the latter would become more distinct. Three photographs of the comet's spectrum have been taken with this arrangement with exposures of 180 minutes, 196 minutes, and 228 minutes, and with a comparison spectrum on each. The continuous spectrum of the nucleus was plainly seen while the photography was in progress. For the present it suffices to say that the most striking feature is a heavy band above H which is divisible into lines, and in addition two faint bands, one between G and h. and another between h and H. I was very careful to stop the exposures before dawn, fearing that the spectrum of daylight might become superposed on the cometary spectrum.

It would seem that these photographs strengthen the hypothesis of the presence of carbon in comets, but a series of comparisons will be necessary, and it is not improbable that a part of the spectrum may be due to other elements.

HENRY DRAPER

271, Madison Avenue, New York From Nature 24, 4 August, 308, 1881

^{1.} Smith, 1. Nature, News and Views 291, 378 (1981).

Grisolia, S. & Cohen, P.P. J. biol. Chem. 198, 561 (1952).

^{3.} Rubio, V. & Grisolia, S. Enzyme (in the press).

Bachmann, C. et al. New Engl. J. Med. 304, 543 (1980).

REVIEW ARTICLE

A one-receptor view of T-cell behaviour

Polly Matzinger

Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK

The discovery of T cells and their behaviour has forced a re-evaluation of the immunological relationship between self and not-self. T cells seem to respond against foreign antigens only when the latter are in some form of association with self molecules encoded by the major histocompatibility complex. This has raised the question of whether T-cell recognition may depend on two separate receptors. I present here the case for a model of T-cell behaviour based on a single receptor.

"I shall always regard the differentiation between self and not-self as crucial to all immunological theory."—F. M. Burnet.

Consider an immune response to a virus infection. B cells respond by producing antibodies which bind to virus particles and neutralize them. T cells, however, do not deal directly with the virus itself but instead interact with other cells. For example, T helper cells collaborate with B cells in the production of antibody while T killer cells prevent viral replication by lysing virus-infected cells.

These differences in T- and B-cell function necessitate different modes of antigen recognition. Whereas the receptors on B cells (antibodies) should be able to bind free virus particles, the receptors on T cells should not. A T cell which, like antibody, chases down the bloodstream after virus particles is redundant, and it is not surprising that T cells do not bind viruses or other antigens alone. Instead they recognize two entities: the foreign antigen in question (which makes them antigen specific) and a special cell-surface marker (which targets them onto cells).

The cell-surface markers that serve as T-cell guidance molecules¹ constitute a set of highly polymorphic membrane glycoproteins encoded by the major histocompatibility complex (MHC). Some (K and D) are found on almost all cells of the body and are specifically recognized by T killer cells, while others (the I antigens) are found mainly on cells of the immune system and guide T helper cells.

The recognition of MHC products by T cells is extremely precise. They distinguish between the products of self and foreign MHC alleles and their response to foreign molecules has long been the major barrier to successful tissue transplantation. They also recognize allelic differences when using their own MHC molecules as guidance proteins. For example, a virus-infected animal of MHC type 'A' contains activated T cells capable of recognizing and destroying only virus-infected cells which express the A allele. This is known as 'MHC restriction' of T-cell recognition.

A key question raised by MHC restriction concerns the nature of the T-cell receptor. Do T cells bear two separate receptors each of which recognizes one of the two components (antigen and MHC) or do they bear one receptor which recognizes a complex of the two molecules? The two-receptor viewpoint, known as dual recognition, has the advantage of requiring no special assumptions about the two antigens. They can simply be molecules floating freely in cell membranes. However, it has the disadvantage of requiring many assumptions about the genetics, structure, development and affinity of T-cell receptors. Nevertheless most models of T-cell behaviour have been constructed on the basis of dual recognition ²⁻¹⁰.

It is time to reconsider the alternative known as the 'altered self' or 'interaction antigen' model¹¹⁻¹⁴. Based on a single T-cell receptor, it depends on one long-standing but difficult assumption about interactions between molecules in membranes. My

argument here is that this assumption still serves surprisingly well to explain the most important aspects of T-cell behaviour.

The assumptions

The assumption underlying the interaction antigen model is that T cells recognize neither antigen nor MHC molecules alone but rather molecular complexes composed of the two. This idea can be divided into two parts: (1) molecules in membranes can form associations (some transient, some more stable) which exhibit new antigenic determinants absent from the uncomplexed molecules; and (2) T cells are triggered to mature or differentiate only when they bind to MHC molecules on the surface of a specialized antigen-presenting cell.

Assumption (1)

Assumptions may be justified either because they make sense a priori or because of their ability to explain new data. I accept the idea of an interaction antigen purely on the basis of economy and predictive ability. First proposed in 1974, it has continued to explain many aspects of T-cell behaviour. A possible view of how such molecular interactions might occur was developed by Cohen and Eisen in 1977. They pointed out that, because cell membranes are essentially planar, the effective concentration of membrane molecules is exceedingly high. This high concentration generates a large number of molecular interactions, some of which will involve MHC molecules 15.

A molecule floating freely in a membrane conforms to the ionic, hydrophobic and electrostatic environment. The same molecule, when complexed (however briefly) with another, will find itself in an altered environment and may thus take on a different conformation. In complexes of MHC and antigen, three kinds of new determinant may be formed: (1) changes in the conformation of the antigen, (2) changes in the conformation of the MHC protein, and (3) new surfaces involving both antigen and MHC.

If such complexes exist, why are they not often detected in co-capping studies? Most such complexes, based on high concentrations rather than innately high affinity, will not be very stable and may be detectable only in certain conditions. Consider three interacting molecules: an MHC protein, an antigen and antibody directed against the antigen. Three equations govern their interactions:

MHC-antigen-antibody complex (3)

Depending on the antigenic determinant to which the antibody binds, the reaction depicted by equation (3) may predominate or not occur at all. The presence of an antibody directed against a determinant not available on the MHC-antigen complex will reduce the amount of free antigen and drive all three equations to the left, diminishing the number of MHC-antigen complexes. An antibody with greater affinity for the complexed antigen than for the free form will stabilize the complex, driving equation (2) to the right and co-capping the MHC and the antigen ¹⁶⁻¹⁹. These considerations should apply to T-cell receptors as well as to antibodies. In addition, the T-cell receptor, being membrane bound, may have a greater opportunity to achieve multipoint binding and thus further stabilize any molecular interaction to which it binds.

In principle, any molecule in the cell membrane should be able to interact with any other, yet the evidence is that T cells recognize only complexes that contain MHC molecules. This brings us to assumption (2).

Assumption (2)

Because recognition of MHC molecules is so important to T cells, it has been tempting to speculate that such recognition is genetically predetermined, that the germ-line genes code for a set of T-cell receptors directed specifically against MHC molecules. However attractive this assumption may seem, it cannot stand alone, and those who use it must explain (1) how T cells see non-MHC antigens, (2) how the gene pool manages to contain receptors able to bind new mutants in MHC molecules, and (3) how T cells choose from the gene pool those receptors which recognize the particular MHC allele(s) expressed by the body in which they live^{3-6,8,20}.

Because of these problems and, more importantly, because such a genetic bias is unnecessary, the interaction antigen model contains no such assumptions about the T-cell receptor gene pool and begins with a random repertoire (which may, in principle, contain receptors capable of binding any molecule alone or in combination with any other). How then are T cells restricted to recognition of antigen in the context of MHC molecules?

An early interaction antigen model¹¹ introduced the antigenpresenting cell as the determining factor in MHC restriction, proposing that T cells are selectively activated only when they bind to MHC molecules on the antigen-presenting cell surface (a concept generally accepted by all models of T-cell behaviour).

The general idea is that antigen-presenting cells participate actively in T-cell induction in two ways. First, they present antigens as well as MHC molecules to virgin T cells. Second, when a T cell binds to one of these MHC molecules, the antigen-presenting cell is induced to produce a differentiation signal. It is this differentiation signal which activates the T cell.

T cells should be able to bind in several ways to MHC molecules on the surface of antigen-presenting cells. Some will bind specifically to the MHC itself while others will bind to interaction determinants found on molecular complexes containing MHC. Like the antibodies discussed earlier, these latter may stabilize the complex, and the T cell will thus be treated like one which has bound free MHC. In general, any T cell which binds strongly to an MHC complex, no matter which part of the complex it binds, will be signalled to differentiate. Any T cell which binds any other complex or free molecule, failing to bind MHC, will not trigger the presenting cell and will not be activated. Thus, beginning with a random repertoire, one finishes with a functional set of T cells that are MHC restricted.

T-cell ontogeny and induction

Although all interaction antigen models are based on the same two assumptions, they differ in their development of specific points¹¹⁻¹⁴. In what follows I have tried to draw on those ideas which lead to the simplest overall view.

No assumptions were made about the germ-line genes coding for T-cell receptors. A simple idea would be that B-cell variable-region genes also code for T-cell receptors, but we can as easily start with a T-cell precursor population carrying any random set of specificities.

This repertoire of specificities must be modulated early in development to remove or inactivate T cells directed against self²¹⁻²³. Self tolerance is learned anew within each individual

and must involve at least two types of T cell: those specific for the self-MHC molecules alone and those specific for associations of other self molecules with self-MHC. It is unclear whether cells specific for isolated non-MHC molecules must be tolerized. Every potentially inducible cell must be tolerizable²⁴, but, as T cells specific for isolated non-MHC molecules fail to bind MHC, they are not inducible and should never be activated in the normal animal. However, were they to be activated by a cross-reactive complex of foreign antigen plus MHC, they could become autoreactive. One reason for supposing that such cells may not be tolerized is that it would require tolerance to have a different specificity from induction. Perhaps they form the basis of autoimmune disease. If so, one could predict that the destructive phase of some autoimmune diseases might not be MHC restricted.

Whether tolerance induction occurs in the thymus or in the periphery²⁵, and whether it occurs by mutation²⁰, deletion^{22,23} or suppression²⁶, parsimony suggests that the self antigens are presented by the same sort of antigen-presenting cell as those which present foreign antigens. The supposition then is that T-cell precursors are made tolerant when they bind to self-MHC molecules (or any complex containing self-MHC) on the surface of an antigen-presenting cell. This has the added attraction of allowing tolerance to occur to tissue antigens not normally intrinsic to cells of the immune system itself. Perhaps, as with B cells^{27,28}, an antigen recognition event may lead to tolerance in a young T-cell precursor and activation in a mature T cell.

Given a random repertoire depleted by self tolerance, several sorts of T cell will be available to bind to a foreign antigen X. Some will bind to X alone on the surface of the antigenpresenting cell but, failing to bind to MHC molecules, will not be activated. Others will bind in one of several ways to MHC-X complexes and will be activated. These T cells will retain their specificity and exercise their functions only when they encounter X in the same form as that with which they were activated, namely on the surface of a cell carrying the same MHC alleles.

MHC restriction

When Zinkernagel and Doherty first proposed 'altered self' recognition, the one thing known about MHC restriction was that T cells interacted only with syngeneic cells. The rules of T-cell behaviour are now better defined. How has the interaction antigen model held up?

Their original finding was that a mouse of MHC type A, immunized against an antigen X, contains activated T cells specific for targets carrying X+A and not targets carrying $X+B^{10,29-35}$. This simply reflects conservation of specificity—activated T cells respond best to the antigenic complexes with which they were primed. Because there are similarities between different MHC alleles as well as differences, X+A should have some determinants in common with X+B and a certain level of cross-reaction should exist. When T-cell responses are titrated far enough, such cross-reactions are usually seen $^{36-39}$.

It has since been shown that MHC-restricted T cells do not bind free MHC or free antigen. Competition experiments show that neither free MHC nor free antigen will block T-cell activity^{32,40-43}. Furthermore, to be bound by T cells, MHC and antigen must not only be cell bound, they must be together in the same cell membrane^{29,44,45}. This is, of course, predicted if T cells recognize only interaction antigens, whereas dual recognition models must explain it a posteriori.

A further problem for at least some dual recognition models is posed by T-cell reactions against cells bearing foreign MHC. These models explain MHC restriction by supposing that T cells cannot function unless both of their receptors are engaged, one receptor being specific for antigen and the other for self MHC. However if a target carries only foreign MHC alleles, the anti-self receptor should be useless and the T cell helpless. To deal with this, some dual recognition models suggest that alloaggression is a 'special' case in which T cells need bind only one of their receptors. In contrast, the interaction model does not invoke such 'special cases'—T cells need only one receptor

whether recognizing a foreign-MHC antigen or some modification of a self-MHC product.

For similar reasons, it is easier to accommodate 'aberrant recognition' in terms of the interaction antigen model. Since the original experiments on MHC restriction, it has become clear that T cells can recognize antigens presented with MHC molecules not found on their own tissues 1. These experiments are of course complicated by responses against the foreign MHC itself: an individual of MHC type A, immunized with antigen-presenting cells carrying MHC B and antigen X, makes such a strong response against B that it is virtually impossible to determine whether a response against antigen X also occurs. This complication can, however, be overcome by the removal of T cells reactive against B. In many experiments (but not all 12-54), removal of such T cells reactive to B has revealed a population which can recognize X + B. In short, an animal that is itself MHC type A carries T cells capable of recognizing X + B.

This is predicted by the interaction antigen models. Because the specificity of the functional T-cell population is selected only at the time of antigen encounter, there is nothing to exclude the recognition of X + B. An antigen-presenting cell carrying B can activate T cells as efficiently as a presenting cell carrying A. Some dual recognition models can also incorporate this finding^{2.8}, but most ignore it.

It is not surprising that the interaction antigen idea deals well with MHC restriction as it was originally created for precisely that purpose. How does it fare with other aspects of T-cell behaviour?

Immune response genes

Immune response (Ir) genes code for MHC proteins and determine whether an animal can respond to a particular foreign antigen. For example, a mouse of MHC type A may respond to a particular antigen X, whereas a mouse of MHC type B does not55-58. The two assumptions underlying the interaction antigen view of MHC restriction allow at least three ways of explaining the Ir phenomena. (1) Some MHC alleles are able to form complexes with X while others, because of their structure, may create inappropriate environments for complexes to form. If no complex forms, no T cell can be activated (reviewed in ref. 59). (2) For any complex of X with MHC, there may be some determinants for which there is no T-cell receptor gene 12,14,50 (3) The induction of self tolerance creates gaps in the functional T-cell repertoire. The adult animal, having lost the ability to respond against its own tissues, cannot respond against antigens which cross-react with those tissues 12,14

The first two explanations are straightforward. The third, while more complicated, can explain both MHC-linked and some non-MHC-linked immune response genes. It rests on the idea that a mouse of MHC type A is tolerant of many self components complexed with A. If an antigen X mimics self + A, the A-type mouse would be unable to respond to it. This does not mean that X itself must be identical to a 'self' component: it is the complex of X+A that is relevant. If X+A mimics any self + A complex, then an A mouse will not respond to X. However, an MHC B-type mouse may respond to X, implying that X+B carries some determinants not found on self + B.

How do these ideas stand up to our current understanding of Ir genes? An early model attributing nonresponsiveness to self tolerance predicted that responsiveness would be recessive on the dominant. However, the discovery of MHC restriction allows a re-examination of this idea. If strain A is a nonresponder to X and strain B a responder, then explanation (3) above states that X + A mimics self + A while X + B carries determinants which do not cross-react with self + B. The F_1 progeny of an $A \times B$ mating will be tolerant of both self + A and self + B and will be unresponsive to X + A, but they will see the non-cross-reactive determinants on X + B and thus responsiveness will be dominant.

The crucial point is that, to T cells, X+A and X+B are entirely different antigens. Tolerance to one has no implications

for tolerance to the other and thus T cells of both responder and nonresponder genotypes should 12,49,59 and do $^{6,61-64}$ respond to X if it is presented with a responder-type MHC allele.

Some immune responses are governed by two MHC genes (α and β) which exhibit a peculiar complementation pattern in that some alleles complement while others do not 65-69. (For example, if A, B and C are nonresponders, the $(A \times B)F_1$ may respond while the $(A \times C)$ and $(B \times C)$ do not.) If α and β code for the two chains of the Ia guidance molecules 70,71, then the simplest explanation is that a particular pair of α and β chains forms an Ia molecule which can associate in a recognizable way with X, while another pair cannot. The $(A \times B)F_1$ between two nonresponder strains will express the ineffective pair of Ia molecules of its parents $(\alpha^a \beta^a$ and $\alpha^b \beta^b$) and also two new types $(\alpha^a \beta^b$ and $\alpha^b \beta^a$). It is unpredictable whether a particular combination of α and β chains will form appropriate Ia molecules. However, some of the recombinant molecules may carry determinants which allow a response to X.

For some time all known MHC-linked Ir genes were located in the I region, provoking speculation that this might be the location of the T-cell receptor. However, Cunningham and Lafferty reasoned from the interaction antigen viewpoint that the known Ir genes mapped to the I region because the responses analysed had always been those of T helper cells, which use the I-region molecules as guidance molecules. They predicted that Ir genes for T killer responses would map to the K and D regions 11 —a finding 73 now accepted as a matter of course.

Some T-cell responses are controlled by genes outside the MHC as well as by MHC-linked Ir genes⁷⁴⁻⁷⁸. Whereas most models ignore these non-MHC immune response genes, the tolerance model is predicated on their existence and predicts their genetic behaviour. If a mouse of MHC type A is unresponsive to X because X + A cross-reacts with self + A, then the lack of response is controlled by two genes: one coding for MHC 'A', the other for the 'self' cell-surface molecule. Why then do most Ir genes map only to the MHC? I presume that this is due to the lack of polymorphism in the genes coding for other cell components. If the non-MHC surface molecule (such as a hormone receptor) has no alleles, then every strain carrying MHC 'A' will be tolerant of the same self + A complex and response to X will seem to be exclusively controlled by the MHC genes. However, if alleles of the self molecule do exist, then some strains may be tolerant of an allelic form of self + A which does not mimic X+A. In this way two mice may differ in their responses to X even if both carry the same MHC product. The critical prediction from the tolerance model is that, unlike the MHC-linked Ir genes, the non-MHC immune response genes should lead to dominant unresponsiveness. The progeny of a nonresponder (self¹+A) by responder (self²+A) mating will express and be tolerant of both self1 and self2 along with A. They should therefore be unresponsive to X.

Ir gene-controlled suppression: some mice appear to be unresponsive to an antigen because of suppression rather than deletion⁷⁹⁻⁸¹ and it has been suggested that this is inconsistent with cross-reactive self tolerance¹². However, I disagree. As self tolerance may be mediated or at least maintained by suppression^{82,83}, it is but a short step to suggest that nonresponsiveness due to cross-reactive self tolerance may also involve suppression.

Alloaggression

MHC molecules are themselves uniquely strong antigens^{84–88}. Two decades ago it was suggested that this strength reflected a high frequency of reactive lymphoctyes⁸⁶ and it is now known that as many as 10% of T cells can be specific for any foreign MHC molecule^{87,89–93}. Why is there a such high frequency of T cells specific for molecules which the individual meets only in artificial circumstances?

Although many explanations have been proposed^{2-6,8,11,20,94-99}, the interaction antigen model offers a new one¹³ which follows directly from the assumptions created for MHC restriction without any additions or special cases. The

argument is that if T cells from an MHC type-A mouse confront B-type antigen-presenting cells, they will recognize not only B but also all the complexes that B makes with the other molecules in the membrane. Thus the T cells are not responding only against the B antigens but against a very large number of antigenic complexes, and the frequency of reactive cells will be correspondingly high.

To analyse why this should occur let us return to self tolerance. A mouse is tolerant of its own MHC alleles and of all complexes they form with other self cell-surface molecules. It has no need and no opportunity to become tolerant of MHC type B either alone or complexed with other molecules. So, although mouse A is tolerant of its own components as seen with A, it will respond to these same components (as well as any new ones) associated with B. The result of immunizing with cells carrying foreign MHC molecules should thus be the massive and highly heterogeneous 100 response that is seen.

Does this interpretation account for other features of alloreactive responses? A single mutation at the MHC can elicit strong immune responses—this requires an explanation. It is known that in responses against conventional antigens, a single mutation at the MHC can profoundly change the T-cell response to the antigen associated with it 101. For example, T cells directed against X+A may not recognize X+A^M (the mutant). By analogy, T cells tolerant of self+A may not be tolerant of self+A^M. The mutant MHC will interact with normal surface molecules to generate a set of interaction antigens different from those formed by the parental MHC type. The mutant cells will thus present a large number of antigenic determinants to be recognized by parental lymphocytes.

If the majority of alloreactive T cells do not recognize MHC products except as interaction antigens with other cell-surface molecules, one would expect that purified MHC molecules would not interfere with alloreactions at any concentration. Although a few alloreactive cells should be directed against the foreign MHC molecules themselves, the effect of blocking them would be too small to be detected reliably. It has, indeed, been shown that purified MHC molecules do not block alloreactions^{41,42}.

Muddying the waters

So far I have shown how two assumptions deal with the principal features of T-cell behaviour. There is, however, one set of experimental results which has been used as evidence against the interaction antigen viewpoint. These are the experiments showing thymic 'bending'.

Homozygous MHC type-A mice which are irradiated and then reconstituted with $(A \times B)F_1$ bone marrow contain a peripheral set of F₁ T cells and also F₁ antigen-presenting cells that can present antigens in association with both A and B MHC molecules. The result of immunizing such a chimaera against antigen X should be the activation of two sets of T cells, one specific for X+A and the other for X+B. However, the experiments show that (A × B) T cells which have matured in an A animal respond preferentially to antigens associated with A, whereas maturation in a B animal confers a bias towards X+ $B^{6,62,102-105}$. Further testing showed that the organ responsible for this bias was the thymus $^{105-108}$. One interpretation has been that T-cell precursors are positively selected in the thymus 3-9,20 In an A thymus, T-cell precursors which bind to A would differentiate into functional T cells whereas those specific for B would not mature appropriately. Thus, even if X were later presented with both A and B, the response would be directed only against X + A.

Naturally such an interpretation is difficult for an interaction antigen model. There is no obvious way to select T cells for A+X or B+X in the absence of X. However, I disagree with the positive selection interpretation on two grounds. First, it depends on a special set of assumptions which are not required to explain other aspects of T-cell behaviour. Second, the 'aberrant recognition' experiments (see above) show that normal T

cells can recognize antigens presented with non-self MHC alleles. In fact, in one comparative study, T cells from $(A \times B) \rightarrow A$ chimaeras did not respond against virus + B, while normal A-type T cells, from which cells reactive to B had been removed, made good responses to virus + B^{51} . Both sets of T cells had matured in an A thymus, yet one was able to recognize X + B whereas the other was not.

So what is going on in the chimaeras? Obviously the thymus is having some effect on the T-cell repertoire long before encounter with antigen, but need it be a positive selection? Could it be the reverse?

Unlike the normal A mouse, an $(A \times B) \rightarrow A$ chimaera must be tolerant of B, yet, early on, its thymus contains only A-type antigen-presenting cells¹⁰⁹. How then does tolerance to B occur? There are broadly two ways in which tolerance may be induced. Natural self tolerance may occur either by deletion of self-reactive cells and/or by the induction of suppressor cells that function to suppress anti-self responses. A possible clue to the mechanism of tolerance in the radiation chimaeras comes from another type of $(A \times B) \rightarrow A$ chimaera: the classic neonatally tolerant mouse. Strain A mice which have been injected at birth with (A × B) cells are chimaeras 110,111 tolerant of B 112 and often exhibit immune responses 'bent' towards A113-115 Tolerance to B in these chimaeras can involve suppressor cells 116-119. Are the radiation chimaeras similarly suppressed 120? If this is the case, the suppression may extend to B+X (see refs 121, 122) while tolerance to A, occurring by the normal thymic route, allows a response to A + X.

Until these issues are resolved it does not seem appropriate to encumber the interaction antigen model with a new set of assumptions merely to explain a thymic positive selection which may not exist. The model predicts that the functional T-cell repertoire is the result of selection at the time of antigen encounter and I leave it at that.

Antigen encounters can be separated roughly into two sets: encounter with self antigens, which affects the repertoire by removing those specificities directed against self, and encounter with foreign antigen which increases the proportional representation of T cells reactive to the complex of that foreign antigen with MHC. If, unexpectedly, the thymic bias does turn out to reflect a positive selection for self MHC, then it will be time to act in accordance with current immunological practice—to add another assumption.

Recapitulation

The two basic assumptions are: (1) molecules in membranes interact to form new antigenic determinants, and (2) T cells are triggered when they bind to MHC molecules on an antigenpresenting cell (either for tolerance or reactivity).

T-cell development: (1) Pre-T cells enter the thymus and express receptors from a pool which may be as random as that of B cells (perhaps the same pool). (2) T cells capable of binding to MHC molecules on antigen-presenting cells in the thymus are inactivated, including T cells which recognize self non-MHC molecules in association with self MHC. All other T cells mature and peripheralize. (3) At the time of antigen encounter, T cells which bind to antigen in association with MHC molecules on the presenting cell are activated.

Ir genes: Three types of defect control immune responses: (1) the antigen cannot associate with the MHC molecule; (2) the T-cell receptor gene pool lacks a receptor for a particular interaction determinant; and (3) the complex of antigen + MHC mimics self.

Alloaggression: The high frequency of alloreactive cells exists because normal non-MHC membrane molecules associate with the MHC on foreign antigen-presenting cells to create a very large and heterogeneous set of antigens that in turn is recognized by a large and heterogeneous set of T cells.

Conjectures

A model is only as good as its predictions. The following predictions are not made by dual recognition models and are

Fourth, a mouse of MHC type A should be able to respond to

both self and non-self antigens complexed with MHC type B

If any of the following are true, then the model presented here is

wrong and cannot be saved with additional assumptions or any

special pleading. (1) If T cells which are MHC restricted at one stage (such as effector function) are found to be unrestricted at

an earlier stage (such as activation or tolerance). (2) If a somatic

cell hybrid of two T cells of different restriction specificities has

the mixed specificity, that is, if one parent sees X+A and the other sees Y + B and the hybrid sees in addition X + B and Y + A.

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van Leeuwen, A., Goulmy, E. & van Rood, J. J. J. exp. Med. 150, 1075 (1979).
 Bevan, M. J. Proc. natn. Acad. Sci. U.S.A. 74, 2094 (1977).
 Lemonnier, F., Burakoff, S. J., Germain, R. N. & Benacertaf, B. Proc. natn. Acad. Sci.

127. Finberg, R., Burakoff, S. J., Cantor, H. & Benacerraf, B. Proc. nam. Acad. Sci. U.S.A. 75,

129. Kappler, J. W., Skidmore, B., White, J. & Marrack, P. J. exp. Med. 153, 1198 (1981).

U.S.A. 74, 1229 (1977)

Von Boehmer, H. et al. Eur. J. Immun. 9, 592 (1979)

5145 (1978).

supported by NIH Fellowship AI06052.

(this follows from the explanation for alloaggression).

Refutations

therefore distinguishing characteristics of the single receptor/interaction antigen viewpoint.

First, it follows from assumption (1) that MHC-restricted antibodies should exist. If interaction antigens exist and can be recognized by T cells, they should be seen by B-cell receptors as well. MHC-restricted antibodies should be a small fraction of the total antibody made against any antigen and will often be missed; however, possible candidates do exist^{123,124}. It is the dual specificity of T cells which led to the suggestion that they express two receptors. If single antibodies can be MHC restricted, there should be no need to postulate two receptors for T cells.

Second, some T cells specific for MHC A associated with X should bind B+Y or even Y alone. There is no reason to expect that a new antigenic determinant will be unique to one particular complex. It has already been shown that some T cells specific for self MHC+X cross-react on allogeneic MHC, that is, A+X=B¹²⁵⁻¹²⁸. A type of cross-reaction which would not be predicted

by dual recognition models is A + X = B + Y (or A + X = Y). Third, some non-MHC-linked Ir genes⁷⁴⁻⁷⁸ should show dominant unresponsiveness (see the explanation of tolerance).

Davis, S., Sheater, G. M., Mozes, E. & Seia, M. J. Immun. 113.
 Immun. Rev. 38 (1978).
 Benacerraf, B. & Germain, R. Immun. Rev. 38, 70 (1978).
 McDevitt, H. O. & Benacerraf, B. Adv. Immun. 11, 31 (1969).
 Chesebro, B. W. et al. Eur. J. Immun. 2, 243 (1972).

Cheselot, B. W. & Marrack, P. J. exp. Med. 148, 1510 (1978). Longo, D. L. & Schwartz, R. H. J. exp. Med. 151, 1452 (1980). Hodes, R. J., Hathcock, K. S. & Singer, A. J. Immun. 123, 2823 (1979). Dorf, M. E. & Benacerraf, B. Proc. natn. Acad. Sci. U.S.A. 72, 3671 (1975).

Note added in proof: Very recently refutation (2) was tested 129. Not a single clone was found that had mixed specificity. Günther, R. & Rüde, E. J. Immun. 115, 1387 (1975) 1. Mitchison, N. A. in Strategies of Immune Regulation (ed. Sercarz, E.) (Academic, New Günther, R. & Rude, E. J. Immun. 115, 1387 (1975).

Melchers, I. & Rajewsky, K. Eur. J. Immun. 5, 753 (1975).

Dorf, M. E., Twigg, M. B. & Benacerraf, B. Eur. J. Immun. 6, 552 (1976).

Dorf, M. E. & Stimpfling, J. H. J. exp. Med. 146, 571 (1977).

Cook, R. G., Vitetta, E. S., Uhr, J. W. & Capra, J. D. & exp. Med. 149, 981 (1979).

Silver, J. J. Immun. 123, 1423 (1979). York, 1980). Janeway, C. A. Jr, Wigzell, H. & Binz, H. Scand. J. Immun. 5, 993 (1976). Langman, R. E. in Rev. Physiol. Biochem. Pharmac. 81, 1 (1978). Cohn, M. & Epstein, R. E. Cell. Immun. 39, 125 (1978). Blanden, R. V. & Ada, G. L. Scand. J. Immun. 7, 181 (1978). Von Boehmer, H., Haas, W. & Jerne, N. K. Proc. natn. Acad. Sci. U.S.A. 75, 2439 (1978). Fathman, C. G. & Hengartner, H. Nature 272, 617 (1988). Simpson, E. & Gordon, R. D. Immun. Rev. 35, 59 (1977). Droege, W. Immunobiology 156, 2 (1979). Williamson, A. R. Nature 283, 527 (1980) McDevitt, H. O. & Chinitz, A. Science 163, 1207 (1969).
 Gasser, D. L. J. Immun. 105, 908 (1970). Seshi, B. Curr. Sci. 48, 919 (1979). Zinkernagel, R. M. & Doherty, P. C. Nature 251, 547 (1974) Jasser, D. L. J. Immun. 105, 908 (1970).
 Lilly, F., Jacoby, J. S. & Coley, R. C. in Immunogenetics of the H-2 System (eds Lengerové, & Vojitskova, M.) 197-199 (Karger, Basel, 1971).
 Gasser, D. L. & Silvers W. K. Adv. Immun. 18, 1 (1974).
 Keck, K. & Momayezi, M. J. Immun. 121, 1612 (1978).
 Kapp, J. A., Pierce, C. W., Schlossman, S. & Benacerraf, B. J. exp. Med. 140, 648 (1974). Cunningham, A. J. & Lafferty, K. J. Scand. J. Immun. 6, 1 (1977). Schwartz, R. H. Scand. J. Immun. 7, 3 (1978). Matzinger, P. & Bevan, M. J. Cell. Immun. 29, 1 (1977).
 Matzinger, P. thesis, Univ. California, San Diego (1980). Cohen, R. J. & Eisen, H. N. Cell Immun. 32, 1 (1977).
 Schrader, J. W., Cunningham, B. A. & Edelman, G. M. Proc. natn. Acad. Sci. U.S.A. 72, Debré, P., Waltenbaugh, C., Dorf, M. E. & Benacerraf, B. J. exp. Med. 144, 272 (1976).
 Benacerraf, B. & Dorf, M. Cold Spring Harb. Symp. quant. Biol. 41 465 (1976). 5066 (1975). Transplantn Rev. 26 (1975). Bourguignon, L. Y., Hyman, R., Trowbridge, I. & Singer, S. J. Proc. natn. Acad. Sci. U.S.A. 75, 2406 (1978).
 Zarling, D. A., Keshet, I., Watson, A. & Bach, F. H. Scand. J. Immun. 8, 497 (1978). Immun. Rev. 46 (1979). Immun. Rev. 46 (1979).
 Gorer, P. A. J. Path. Bact. 47, 231 (1938).
 Cunce, S., Smith, P., Barth, R. & Snell, G. D. Ann. Surg. 144, 198 (1956).
 Simonsen, M. Prog. Allergy 6, 349 (1962).
 Dutton, R. W. J. exp. Med. 123, 665 (1966). Gomard, E. et al. Eur. J. Immun. 8, 228 (1978).
 Jerne, N. K. Eur. J. Immun. 1, 1 (1971). Owen, R. D. Science 102, 400 (1945).
Billingham, R. E., Brent, L. & Medawar, P. B. Phil. Trans. R. Soc. 239, 357 (1956). Dutton, K. W. J. exp. Med. 123, 605 (1906).
 Cantrell, J. L. & Hildemann, W. H. Transplantation 14, 761 (1972).
 Nisbet, N. W., Simonsen, M. & Zaleski, M. J. exp. Med. 129, 459 (1969).
 Ford, W. L., Simmonds, S. J. & Atkins, R. C. J. exp. Med. 141, 681 (1975).
 Bevan, M. J., Langman, R. E. & Cohn, M. Eur. J. Immun. 6, 150 (1976).
 Lindahl, K. F. & Wilson, D. B. J. exp. Med. 145, 508 (1977).
 Swain, S. L., Panfili, P. R., Dutton, R. W. & Lefkovits, I. J. Immun. 123, 1062 (1979). 23. Burnet, F. M. The Clonal Selection Theory of Acquired Immunity (Cambridge University Press, 1959). Press, 1959).
Bretscher, P. & Kohn, M. Science 169, 1042 (1970).
Doherty, P. C. & Bennink, J. R. Scand. J. Immun. 12, 271 (1980).
Gershon, R. K. & Kondo, K. Immunology 21, 903 (1971).
Nossal, G. J. V. & Pike, B. L. J. exp. Med. 141, 904 (1975).
Lederberg, J. Science 129, 1649 (1959).
Bevan, M. J. J. exp. Med. 142, 1349 (1975). 94. Burnet, F. M. Nature 226, 123 (1970).
95. Thomas, L. in Cellular and Humoral Aspects of the Hypersensitive States (ed. Lawrance, S.) 529 (Cassell, London, 1959). Simonsen, M. Cold Spring Harb. Symp. quant. Biol. 32, \$17 (1967) Kindred, B. & Shreffler, D. C. J. Immun. 109, 940 (1972). Katz, D. H., Hamaoka, T. & Benacerraf, B. J. exp. Med. 137, 1405 (1973). Warner, N. L. Cold Spring Harb. Symp. quant. Biol. 32, 523 (1967) (Discussion). Burakoff, S. J. et al. J. exp. Med. 148, 1414 (1978). Shearer, G. M. Eur. J. Immun. 4, 527 (1974).
Gordon, R. D., Mathieson, B. J., Samelson, L. E., Boyse, E. A. & Simpson, E. J. exp. Med. Cunningham, A. J. Cell. Immun. 19, 368 (1975). Sherman, L. A. J. exp. Med. 151, 1386 (1980). 144, 810 (1976).
34. Miller, J. F. A. P., Vadas, M. A., Whitelaw, A. & Gamble, J. Proc. natn. Acad. Sci. U.S.A. Klein, J. Adv. Immun. 26, 55 (1978)
 Bevan, M. J. Nature 269, 417 (1977) 73, 2486 (1976). 35. Transplantn Rev. 29. (1976). Sprent, J. J. exp. Med. 147, 1838 (1978). Zinkernagel, R. M. et al. J. exp. Med. 147, 882 (1978). 13. Iransplantin Rev. 29 (1976).
 14. Peavey, D. L. & Pierce, C. W. J. Immun. 115, 1515 (1975).
 15. Lindahl, K. F. & Wilson, D. B. J. exp. Med. 145, 508 (1977).
 16. Teh, H. S., Phillips, R. A. & Miller, R. G. J. Immun. 120, 425 (1978).
 17. Teh, H. S., Phillips, R. A. & Miller, R. G. J. Immun. 121, 1711 (1978). Zinkernagel, R. M. et al. J. exp. Med. 147, 892 (1976).
 Waldmann, H., Pope, H., Bettles, C. & Davies, A. J. S. Nature 277, 137 (1979).
 Zinkernagel, R. M. et al. J. exp. Med. 147, 897 (1978).
 Fink, P. J. & Bevan, M. J. J. exp. Med. 148, 766 (1978).
 Miller, J. F. A. P., Gamble, J., Mottram, P. & Smith, F. E. Scand. J. Immun. 9, 29 (1979). Plata, F. & Levy, J. P. Nature 249, 271 (1974). Plata, F. & Levy, J. P. Nature 249, 271 (1974).
 Todd, R. F. III, Stulting, R. D. & Amos, D. B. Cell. Immun. 18, 304 (1975).
 Stulting, R. D., Todd, R. F. III & Amos, D. B. Cell. Immun. 20, 54 (1975).
 Basten, A., Miller, J. F. A. P. & Abraham, R. J. exp. Med. 141, 547 (1975).
 Swierkosz, J. E., Rock, K., Marrack, P. & Kappler, J. W. J. exp. Med. 147, 554 (1977).
 Watt, T. S. & Gooding, L. R. Nature 283, 74 (1980).
 Doherty, P. C. & Bennink, J. R. J. exp. Med. 149, 150 (1979).
 Heber-Katz, E. & Wilson, D. B. J. exp. Med. 142, 928 (1975).
 Pierce, C. W., Kapp, J. A. & Benacerraft, B. J. exp. Med. 144, 371 (1976).
 Thomas, D. W. & Shevach, E. M. Proc. natn. Acad. Sci. U.S.A. 74, 2104 (1977).
 Wilson, D. B. I. indahl, K. F. Wilson, D. H. & Sprent, I. Levn. Med. 146, 361 (1977). Longo, D. L. & Schwartz, R. H. Nature 287, 44 (1980).
 Lubaroff, D. M. & Silvers, W. K. J. Immun. 111, 65 (1973) Nakić, B., Mikuška, J., Kaštelan, A., Springer, O. & Silobrčić, V. Immunology 18, 119 (1970). Brent, L., Brooks, C. G., Medawar, P. B. & Simpson, E. Br. med. Bull. 32, 101 (1976). Kindred, B. Cell. Immun. 20, 241 (1975). Zinkernagel, R. M., Callahan, G. N., Streilein, J. W. & Klein, J. Nature 266, 837 (1977). Waldmann, H., Pope, H., Brent, L. & Bighouse, K. Nature 274, 166 (1978). Roser, B. J. & Dorsch, S. Nature 258, 233 (1975).
 Gorczynski, R. M. & MacRae, S. J. Immun. 122, 747 (1979) Wilson, D. B., Lindahl, K. F., Wilson, D. H. & Sprent, J. J. exp. Med. 146, 361 (1977). Doherty, P. C. & Bennink, J. R. J. exp. Med. 150, 1187 (1979). Gorczynski, R. M. & MacRae, S. J. Immun. 122, 747 (1979).
 Holfaft, V., Chutta, J. & Hášek, M. Nature 274, 895 (1978).
 Reiger, M. & Hilgert, I. J. Immunogenetics 4, 61 (1977).
 Smith, F. I. & Miller, J. F. A. P. J. exp. Med. 151, 246 (1980).
 Yowell, R. L., Araneo, B. A., Miller, A. & Sercarz, E. E. Nature 279, 70 (1979).
 Schwartz, M. et al. Proc. natn. Acad. Sci. U.S.A. 73, 2862 (1976).
 Ivanyi, P., Melief, C. J. M., van Mourik, P., Vlug, A. & de Greeve, P. Nature 282, 843 (1976). Sprent, J. & von Boehmer, H. J. exp. Med. 144, 617 (1976). Shearer, G. M. & Schmitt-Verhulst, A. Adv. Immun. 25, 55 (1977). Sprent, J. Immun. Rev. 42, 108 (1978).

Benacerraf, B. & McDevitt, H. O. Science 175, 273 (1972).

Lilly, F., Graham, H. & Coley, R. Transplantn Proc. 5, 193 (1973).

Davis, S., Shearer, G. M., Mozes, E. & Sela, M. J. Immun. 115, 1530 (1975).

ARTICLES

Effect of ions on the light-sensitive current in retinal rods

K.-W. Yau, P. A. McNaughton & A. L. Hodgkin

Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG, UK, and Department of Physiology and Biophysics,
The University of Texas Medical Branch, Galveston, Texas 77550, USA

The effect of ions on the light-sensitive current of retinal rods was studied by sucking the inner segment into a tightly fitting capillary with the outer segment projecting into a flowing solution. This new method showed that the light-sensitive pathway, in which Na^+ is the normal carrier of current, has an ionic selectivity different from that of other known sodium channels. External calcium has a striking effect on the current, which increased about 20-fold when all calcium was removed. Reducing the sodium concentration gradient greatly prolonged the response to a flash of light, as would be expected if internal calcium blocks sodium channels and if light releases calcium which is subsequently extruded by a sodium-calcium exchange mechanism.

THE experiments described here were carried out with a new method which gives quantitative information about the effects of ions on the current generated by an isolated photoreceptor. The method is an extension of that developed by Baylor, Lamb and $Yau^{1.2}$ and in its usual form depends on sucking the inner segment of an isolated rod into a recording capillary while leaving the outer segment projecting into a flowing solution whose composition can be changed rapidly. This arrangement (Fig. 1a) allows one to examine the effects of substances on the light-sensitive pathways in the outer segment; similar experiments on the inner segment may be carried out by inverting the cell as in Fig. 1b. Light responses obtained with the two arrangements were of similar shape and size and of the same polarity with respect to the rod.

A piece of retina from a dark-adapted toad (Bufo marinus) was chopped into small pieces in Ringer's solution under IR light, yielding a preparation that consists of many detached outer segments, which did not give light responses, and a few fairly complete cells, that is, outer segments still attached to inner segments containing a nucleus but without synaptic endings. When the inner segment of such a cell is sucked into a closely fitting pipette containing Ringer's solution it gives currents of the usual form and magnitude for several hours.

In addition to changing the light-sensitive current, test solutions may set up a junctional current that arises from the small difference between the junctional potentials at the two boundaries of the test solution. Unlike the light-sensitive current, the junctional current did not change sign when the rod was inverted, nor was it much altered by replacing a complete rod by an inert outer segment. When the results were corrected for this junctional current, it was found that changing external ions altered the dark current but usually had little effect on the current in the presence of a bright light, which remained close to zero.

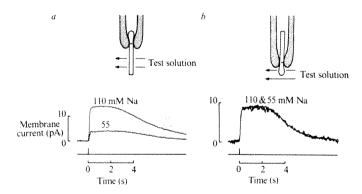
The records in Fig. 1 show that halving the sodium concentration round the outer segment reduced the light response to one-third but had no effect on the response when applied to the inner segment. This illustrates the difference between the two parts of the cell and shows that there was no appreciable leakage of ions through the tight seal at the mouth of the capillary.

Ionic selectivity of the light-sensitive channel

As expected from previous work³⁻⁹, replacement of sodium by choline in the solution bathing the outer segment abolished the light response in a rapid and reversible manner. Figure 2 shows the relationship between the maximum response and external sodium concentration with 1 mM Ca in the external solution. A similar curve is obtained with 0.1 mM Ca in the external solu-

tion, except that the currents are scaled up by a factor of 3-5 (see below).

We looked for an inverted response in the complete absence of external sodium but could detect no response of either polarity in sodium-free choline solutions containing the normal amount of external calcium (1 mM). This failure to see an inverted response may be due to (1) the recording limit of $\sim 0.1 \, \mathrm{pA}$, (2) the probable hyperpolarization when the dark current is cut off in low external sodium, (3) the possible closure of sodium channels resulting from the rise in internal calcium which might follow removal of external sodium¹⁰. The responses seen in sodium-free solutions containing 0 Ca–EGTA are discussed below.



a, Above, recording arrangement with the rod inner segment drawn into the Ringer-filled pipette and the outer segment exposed to test solutions. Membrane current was recorded with a current-to-voltage transducer connected to the pipette. Below, averaged responses to identical bright flashes in the presence of either 110 mM Na or 55 mM Na + 55 mM choline In plotting the traces the baseline has been shifted so that the dark levels superimpose. b, Experimental conditions similar to a, except that the rod outer segment was inside the pipette and the inner segment exposed to test solutions. Outward membrane current across the outer segment is taken as positive (upwards) in both cases; responses are low-pass filtered at 30 Hz (6-pole); temperature 19 °C. Time separation between recordings in a and b (which were obtained on the same rod) was ~2 h, during which the maximum response of the cell had declined somewhat. Light monitor trace below the responses indicated timing of 20-ms flash ($\lambda=500$ nm). The flashes delivered 37 photons μm^{-2} (unpolarized), corresponding to 740 photoisomerizations (Rh*) per flash for an outer segment with an effective collecting area of about $20~\mu\text{m}^2$. The Ringer's solution in all experiments contained (mM): NaCl, 110; KCl, 2.5; MgCl₂, 1.6; CaCl₂, 1.0; HEPES (neutralized with tetramethylammonium hydroxide to pH 7.6), 10; glucose 3. Test solutions were similar except for the specific substitutions stated. Calcium buffers were made with EGTA, HEDTA and Mg-EDTA to cover a range of 10^{-5} – 10^{-8} M Ca²⁺ using the stability constants in ref. 29. The seal resistance with the rod in position was 12 M Ω in a and 8 M Ω in b. In other experiments seal resistances varied between 5 and 15 MO.

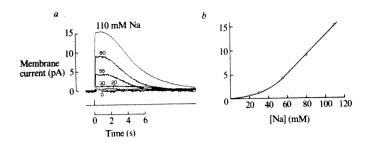


Fig. 2 Dependence of light-sensitive current on external sodium. a, Responses of an isolated rod to bright flashes (delivering 1,534 Rh* per flash) with different sodium concentrations bathing the outer segment. Temperature 19 °C. Choline was used as substitute in the low-sodium solutions. The 110 mM Na solution (Ringer) was applied between different low-sodium solutions and the response in a represents the grand average of 34 responses all within the range 14-16 pA. The other responses are averages over 3-10 flash trials. Note the prolongation of the plateau at external sodium concentrations < 55 mM. b, Plot of the response peaks in a versus external sodium concentration. 1 mM Ca²⁺ present throughout.

Lithium was able to substitute for sodium in carrying the inward dark current, but was less effective. Complete replacement of sodium with lithium reduced the current from 15.4 to 0.6 pA in a representative experiment. With a solution in which all but 20 mM of sodium had been replaced by choline the current was 0.56 pA. As 20 mM Na is almost as effective as 110 mM Li we conclude that the permeability ratio P_{Li}/P_{Na} is about 1:5 in these conditions. A similar ratio was also obtained with sodium present throughout. In the presence of 55 mM Na, replacing 55 mM choline with lithium caused an increase in current from 4.0 to 6.3 pA. Earlier in the same experiment an increment in sodium concentration from 55 to 80 mM caused an increase in current from 4.2 to 9.0 pA. Assuming that the current increase was proportional to the sodium increment in this concentration range (see Fig. 2), we calculate that 13 mM Na is equivalent to 55 mM Li, and therefore that the permeability ratio is 1:4.2. The permeability ratio was also not significantly affected by reducing the external calcium to as low as 1 µM even though in low calcium the dark current is greatly increased.

The other alkali cations behaved quite differently. With zero external sodium, application of 110 mM potassium, rubidium or caesium produced no detectable current in the presence of 1 mM Ca, but at lower concentrations small currents were observed. In 100 μ M Ca, tentative values of the permeability ratios were $P_K/P_{Rb}/P_{Cs}/P_{Na}=0.02:0.015:0.01:1.0$. In the presence of external sodium, however, potassium and to a lesser extent rubidium, but not caesium, actually reduced the existing dark current. In one experiment, increasing potassium from 2.5 to 20 mM, with sodium present as the main cation in both cases,

reduced the dark current to 36%. This effect cannot be attributed to potassium leaking into the pipette and depolarizing the inner segment because when the rod was inverted in the pipette and the same solutions applied to the inner segment, the current was reduced only to 75%. It therefore seems that external potassium and rubidium ions have some kind of blocking action on the inward sodium currents in the rod outer segment.

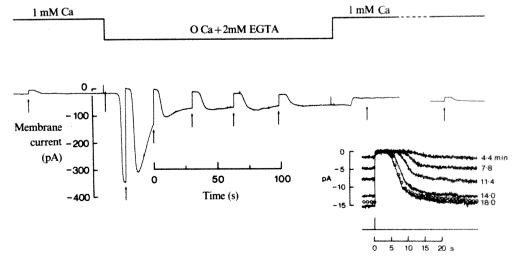
These results show that the sodium conductance in rods differs in its ionic selectivity from other known sodium conductances. In nerve, for example, lithium substitutes equally for sodium¹¹⁻¹³ and external potassium has no direct blocking effect¹⁴. It remains for future work to show whether the light-sensitive conductance is a pore or a carrier system.

Effects of external calcium on dark current

In agreement with Yoshikami and Hagins¹⁵, altering the external calcium concentration caused large changes in dark current and light response. In Fig. 3, 0 Ca plus 2 mM EGTA increased the dark current and light response from 15 to 340 pA. This large dark current, which depended on external sodium, was not maintained probably because the cell gained sodium and lost potassium. On restoring Ringer's solution, the light response often disappeared completely for a few minutes and when it returned it was smaller and much longer than normal. However, a full sized response was usually restored within 10-20 min or sooner if the exposure to 0 Ca was shorter. Details of the changes in response during recovery are given in the inset of Fig. 3. Evidence that the lengthening of response and reduction in photocurrent after a period in low calcium were caused by entry of sodium is provided by the facts that they occurred only if sodium was present during the period in low calcium, and they disappeared if the rise in dark current was prevented by exposure to a bright light during the application of 0 Ca.

Raising external calcium from 1 to 5 mM decreased the light-sensitive current to about one-quarter and lowering it from 1 to 0.1 mM increased the current by a factor of 3-6. In one rather complete experiment, replacing 1 mM Ca with 10 μM Ca increased the current from 20 to 170 pA whereas 0 Ca-EGTA gave 320 pA, both measurements being repeated twice. The initial effect of altering calcium concentration was to scale the quantal response in proportion to the dark current. In the above experiment, tests with weak flashes showed that the peak current produced by absorption of a single quantum was 0.1 pA in 5 mM, 0.35 pA in 1 mM and 2.5 pA in 10 µM Ca. The approximate proportionality between the quantal response and dark current indicates that the fraction of channels blocked by a single photon remains roughly constant in spite of the large increase which calcium reduction must produce either in the total number of sodium channels open in the dark or in the conductance of individual channels.

Fig. 3 Low-gain record showing the effect of removing external calcium on the photocurrent in a toad rod. Inward current is shown downwards from the level in bright flashes. Flashes each giving 5.2×104 Rh* were delivered at times indicated by arrows. Mg was present throughout at 1.6 mM, K at 2.5 mM and Na at 110 mM. Inset, details of the same experiment showing recovery of dark current and light response (unaveraged) in Ringer's fluid. Numbers give the time after restoring Ringer; open circles show the response before applying 0 Ca. Inward current shown downward from the level after bright flash, which remained constant during the recovery period.



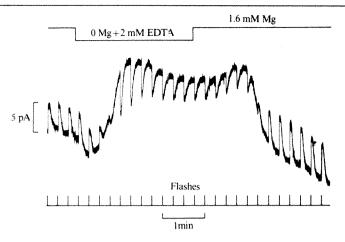


Fig. 4 Inversion of rod light response in 0 Mg and 0 Ca solution. Outward membrane current across the outer segment is upward. Solutions contained 110 mM choline chloride, 2.5 mM KCl, 0 Ca, 2 mM EGTA and 1.6 mM MgCl₂ or 0 Mg with 2 mM EDTA. The bright flashes, which elicited saturating responses, were estimated to give 6,500 Rh* in the outer segment. The solution change in this experiment was slower than in other experiments, with a time lag between change in tap position and solution change around the outer segment of about 0.5 min.

Experiments with a rapid flow system in which external calcium was changed from 1 to 5 mM and back showed that the restoration of dark current lagged behind the solution change at the pipette by ~ 1 s and that the restoration of dark current after reducing calcium occurred much faster than the return of current after a bright flash. The following values were obtained in two experiments with a particularly fast flow: time of solution change measured between 10 and 90% limits = 0.6 s; time for dark current to increase between the same limits after lowering $[Ca]_0$ from 5 to 1 mM = 1.6 s; time for dark current to increase between same limits after bright flash = 10 s. Another relevant comparison is that the maximum rate of increase of current on reducing calcium from 5 to 1 mM was 9 pA s⁻¹, whereas the corresponding value after a bright flash was 1.6 pA s⁻¹. In these experiments the speed of the solution change was obtained from the change in junctional current measured in the presence of a bright light.

The speed with which external calcium ions affect the sodium conductance is explained simply if they act directly on the outside of the membrane, but an internal action cannot be ruled out. If calcium ions act internally, as has sometimes been proposed¹⁶, one must elaborate the calcium transmitter hypothesis to accommodate the observation that the recovery of dark current after a bright flash is so much slower than the recovery when external calcium ions are reduced. One modification is to assume that calcium release continues after the light flash is over. Another is to suppose that calcium shuts channels, but that a more complicated process is involved in opening them again.

Light responses in the absence of external sodium

Using voltage recording, E. R. Griff, L. H. Pinto, B. L. Bastian and G. L. Fain (personal communication) showed that in solutions containing calcium buffered to 10⁻⁸ M with EGTA, replacement of sodium by choline did not abolish the response but gave a residual response of normal polarity. We have observed the same phenomenon when recording current but only if the choline-EGTA solution contained magnesium. With no magnesium we obtained an inverted response, that is, an outward dark current that was suppressed by light (Fig. 4). These magnesium-sensitive currents appeared only if the calcium concentration was less than ~1 µM. Their appearance might be explained if the effects of calcium removal on cation conductance are brought about by unmasking negatively charged sites which attract sodium and magnesium ions into or near the light-sensitive channel. Thus, if calcium removal created a negative potential well large enough to increase sodium permeability by 20, it should on a simple basis increase magnesium permeability by 400. The effect of magnesium removal in inverting the EGTA response has recently been confirmed in similar conditions with intracellular voltage recording (V. Torre, E. Pasino, M. Capovilla and L. Cervetto, personal communication).

Effects of alterations of external and internal sodium

Alterations in external sodium have a striking effect on the time course of the light response. Reducing the sodium concentration from 110 to 20 mM prolonged the response to strong and weak flashes (Fig. 5). It also made the cell more sensitive in the sense that low sodium increased the fraction of the maximum current suppressed by each absorbed photon. The correlation between time to peak and amplitude illustrated by the scaled responses in Fig. 5c is reminiscent of the desensitizing effects of background

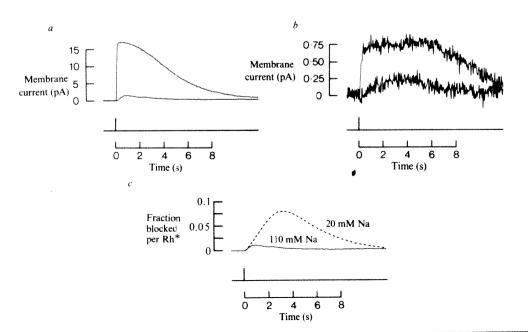


Fig. 5 Change in sensitivity and response time course in the presence of low external sodium, a. Averaged responses to bright and dim flashes in Ringer's solution (110 mM Na) before and after applying low sodium. Bright flash gave 1,660 Rh* and dim flash 8.1 Rh*. b, Averaged responses to bright and dim flashes in 20 mM Na. Bright flash gave 1,660 Rh* and dim flash 4.14 Rh*. Note the substantial prolongation of the responses. 1 mM Ca present throughout. c, Comparison of dim flash responses in 110 mM and 20 mM [Na]₀. Responses have been scaled to show the fraction of dark current blocked by one photoisomerization (Rh*). Response in low [Na]o (dotted curve) smoothed by eye.

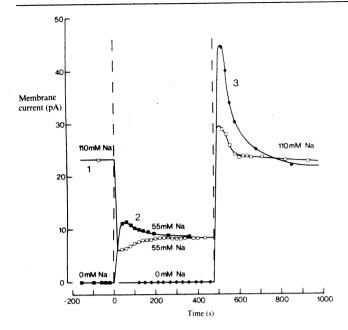


Fig. 6 Time course of change in response amplitude to bright flashes (1,660 Rh* per flash) with partial or complete replacement of external sodium by choline. 1 mM Ca present throughout. Note overshoot when sodium is increased and undershoot when it is decreased. Curve 1 ([]): [Na]₀ was 110 mM up to time 0, then 55 mM for 480 s and finally 110 mM again. Curve 2 (■): [Na]₀ was 0 from -480 s to time 0 and then 55 mM for 480 s. Curve 3 (♠); [Na]₀ was 0 from time 0 to 480 s and then 110 mM. The three curves were obtained consecutively on the same rod.

lights¹⁷, with an increase in sodium gradient being roughly equivalent to a rise in background light intensity.

The effects of external sodium on response duration can be explained if a flash of light releases calcium ions which are then extruded to the external solution by sodium-calcium exchange 18-22 A similar argument can account for the greatly prolonged responses seen in solutions in which external sodium is replaced by lithium.

Conditions which should increase internal sodium concentration, such as treatment with strophanthidin or previous exposure to low calcium (Fig. 3), also slowed the response to light flashes. This might be explained if internal sodium inhibited calcium transport across either disk or surface membrane.

The transient component in the response to changes in external sodium and calcium

A striking feature of the light-sensitive current is that after a change in external sodium or calcium the current does not approach its steady value monotonically but with an overshoot or undershoot of the kind shown in Fig. 6. An overshoot is seen when the dark current is raised by increasing external sodium or reducing external calcium and an undershoot when the dark current is decreased by ionic changes of the opposite kind. Figure 6 illustrates the effect of changing [Na]₀ at 1 mM Ca. The phenomenon is suggestive of a regulatory system in which sodium conductance varies inversely with internal sodium concentration. Such an effect might depend either on a direct inhibitory action of internal sodium or an indirect one, possibly mediated by the changes in internal calcium concentration that are likely to follow changes in internal sodium concentration²

For small perturbations from the normal condition in Ringer's solution, the half time with which the dark current returns is about 1 min, but shorter values and steeper-than-exponential return are seen for large overshoots (as in Fig. 6), and much longer half times and an S-shaped return after conditions likely to give a large increase in internal sodium, for example, after several minutes in 110 mM Na+0 Ca.

Possible effect of reducing internal calcium on sensitivity to light

Exposure of >10 min to solutions such as 20 mM Na, 0 Ca, 0 Mg, 2 mM EGTA, sometimes reduces the sensitivity of the rod by several orders of magnitude, perhaps because the disks in the rod have lost most of their calcium24. In these conditions the rod exhibits the phenomenon of 'superlinearity' in which doubling the intensity of a weak flash may increase the response by a factor of ≥10. This behaviour might be explained by assuming that (1) several calcium ions are required to block each sodium channel, (2) with normal calcium one photon releases sufficient calcium ions to block many channels in a small region, and (3) after treatment with 0 Ca-EGTA the disks contain so little calcium that several photons must be absorbed simultaneously in a small region to release enough calcium to block any sodium channels at all.

Conclusions

These experiments with isolated rods support the widely held view that sodium ions normally carry the light-sensitive current into the outer segment of vertebrate photoreceptors. They also show that the current which flows in normal conditions is only a small fraction of that observed in low calcium. When taken with the rapid effect of external calcium on sodium conductance the result may imply that the sodium pathway is closed for about 95% of the time by combination with external calcium ions, and that light blocks the pathway completely by closing the inside for 100% of the time. Such a mechanism might allow a finer grading of response and less dark noise than if there were fewer pathways open for most of the time.

The present experiments do not prove that calcium is the internal blocking agent released by light, as first suggested by Hagins²⁵, nor do they provide any information about the role of cyclic GMP, another potential transmitter26-28. They do, however, provide some indirect evidence for the calcium hypothesis, particularly when taken in conjunction with recent work on the light-activated extrusion of calcium 18,19. Thus, the strong influence of the sodium concentration gradient on the time course of the light response fits rather well into a scheme in which some of the calcium ions released by light are transported to the outside by exchange with external sodium. The powerful effects of calcium ions in suppressing sodium current when applied externally also makes it easier to assume that they may have a somewhat similar effect on the inside of the membrane.

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- Yau, K.-W., Lamb, T. D. & Baylor, D. A. Nature 269, 78-80 (1977)
- Baylor, D. A., Lamb, T. D. & Yau, K.-W. J. Physiol., Lond. 288, 589-611 (1979). Arden, G. B. & Ernst, W. Nature 223, 528-531 (1969).
- Sillman, A. J., Ito, H. & Tomita, T. Vision Res. 9, 1443-1451 (1969). Yoshikami, S. & Hagins, W. A. Abstr. 14th a. Meet. biophys. Soc. WPM-13 (1970).
- Korenbrot, J. I. & Cone, R. A. J. gen. Physiol. 60, 20-45 (1972). Cervetto, L. Nature 241, 401-403 (1973).
- Capovilla, M., Cervetto, L., Pasino, E. & Torre, V. J. Physiol., Lond. 317, 223-242 (1981).
 Brown, J. E. & Pinto, L. H. J. Physiol., Lond. 236, 575-591 (1974).
- Fain, G. L. & Lisman, J. E. Prog. Biophys. molec. Biol. 37, 91-147 (1981).
 Hodgkin, A. L. & Katz, B. J. Physiol., Lond. 108, 37-77 (1949).
- Chandler, W. K. & Meves, H. J. Physiol., Lond. 180, 788-820 (1965). Hille, B. J. gen. Physiol. 59, 637-658 (1972).
- Hille, B. J. gen. Physiol. 59, 637-658 (1972).
 Frankenhaeuser, B. J. Physiol., Lond. 160, 40-45 (1962).
 Yoshikami, S. & Hagins, W. A. in Blochemistry and Physiology of Visual Pigments (ed. Langer, H.) 245-255 (Springer, New York, 1973).
 Hagins, W. A. & Yoshikami, S. Ann. N. Y. Acad. Sc. 307, 545-561 (1978).
 Baylor, D. A., Mathews, G. & Yau, K.-W. J. Physiol., Lond. 309, 591-621 (1980).
 Gold, G. H. & Korenbrot, J. I. Proc. natn. Acad. Sci. U.S.A. 77, 5557-5561 (1980).
 Yoshikami, S., George, J. S. & Hagins, W. A. Nature 286, 395-398 (1980).
 Blaustein, M. P. & Hodgkin, A. L. J. Physiol., Lond. 200, 497-527 (1969).
 Daemen, F. J. M., Schnetkamp, P. P. M., Hendriks. Th. & Bonting, S. L. in Vertebrate Photoreception (eds Barlow, H. B. & Fatt, P.) 29-43 (Academic, London, 1977).
 Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, R. A. J. Physiol., Lond. 200.

- 23. Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, R. A. J. Physiol., Lond. 200, 431-458 (1969)

- Lipton, S. A., Ostroy, S. E. & Dowling, J. E. J. gen. Physiol. 76, 747-770 (1977).
 Hagins, W. A. A. Rev. Biophys. Bioenging 1, 131-158 (1972).
 Hubbell, W. L. & Bownds, M. D. A. Rev. Neurosci. 2, 17-34 (1979).
 Lipton, S. A., Rasmussen, H. & Dowling, J. E. J. gen. Physiol. 70, 771-791 (1977).
- Miller, W. H. & Nicol, G. D. Nature 280, 64-66 (1979).
- 29. Martell, A. E. & Smith, R. M. Critical Stability Constants Vol. 1 (Plenum, New York, 1974)

The p21 src genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes

Ronald W. Ellis*, Deborah DeFeo*, Thomas Y. Shih*, Matthew A. Gonda*, Howard A. Young*, Nobuo Tsuchida*, Douglas R. Lowy* & Edward M. Scolnick*

* Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Maryland 20205, USA
† Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland 21701, USA
‡ Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104, USA
§ Dermatology Branch, National Cancer Institute, Bethesda, Maryland 20205, USA

The Harvey and Kirsten strains of murine sarcoma virus encode enzymatically and serologically related p21 src proteins which are required for virally mediated cellular transformation. The genes in each virus encoding p21 show such extensive divergence from each other that cloned probes from these genes detect distinct sets of cellular genes in the DNA from several vertebrate species. These data suggest that cellular p21 sarc genes constitute a divergent family of vertebrate genes that can regulate the growth of cells.

THE Harvey and Kirsten strains of murine sarcoma virus (Ha-MuSV and Ki-MuSV) are replication-defective retroviruses that were isolated from independent mouse tumours induced by murine leukaemia viruses which had been passaged through rats 1,2. Ha-MuSV and Ki-MuSV are capable of both transforming fibroblasts in cell culture and inducing sarcomas and erythroleukaemias in susceptible mice3. The only known gene product of the viruses is a 21,000-molecular weight (MW) protein (p21) which is required for cellular transformation⁴ and is present at relatively high levels in virally transformed cells; a related but distinguishable p21 is constitutively expressed at low levels in normal cells of many vertebrate species⁵. Thus, p21 is a member of the class of normal cellular proteins whose coding sequences have been incorporated into the RNA genomes of various transforming retroviruses, such as Rous sarcoma virus⁶, Abelson murine leukaemia virus⁷ and feline sarcoma virus⁸. The phosphoproteins derived from these normal cellular proteins are required for transformation by their respective viruses and are ATP-using protein kinases⁹⁻¹¹. Viral p21 differs from the other kinases in that it specifically binds guanine-containing nucleotides and can transfer the γ-phosphate of GTP to a threonine residue on p21 (ref. 12).

The nucleic acid sequences of Ha- and Ki-MuSV are derived from both murine leukaemia virus and rat cellular DNA 13,14. In the 5.5-kilobase (kb) RNA genome of Ha-MuSV, which has been analysed in much greater detail than that of Ki-MuSV, the rat sequences (4.5 kb) have been divided into two components: (1) 1.0 kb in the 5' half of the genome representing the viral transforming (src) and p21-encoding gene¹⁵; (2) 3.5 kb derived largely, if not exclusively, from replication-defective, non-transforming, retrovirus-like sequences which are highly reiterated in rat DNA and which express themselves in some rat cells as '30S' RNA 16,17. Recently, using a molecularly cloned probe from the Ha-MuSV p21 src sequences, we have cloned two related cellular genes (sarc) from the rat¹⁸. These p21 sarc genes are distinct from each other in that one is colinear with the viral src sequences while the other has three introns in its coding sequence; nevertheless, with appropriate in vitro molecular reconstructions, both genes can induce cellular transformation mediated by high-level expression of strongly related, yet distinguishable, p21 sarc proteins18

The gross structure of Ki-MuSV is very similar to that of Ha-MuSV. The murine leukaemia virus components of these two viruses are highly related and their rat cellular components show extensive homology^{13,14}. However, RNA heteroduplex analysis has revealed segments of rat sequences in the 5' half of

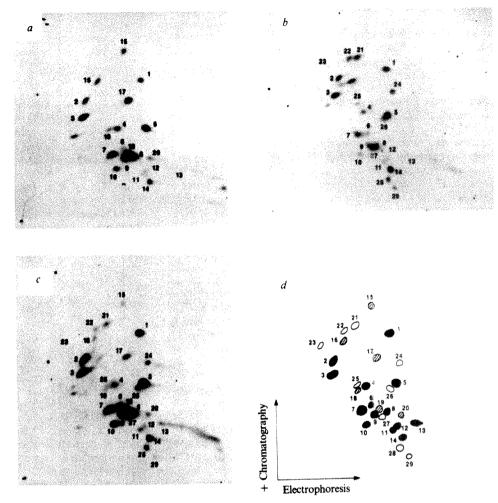
the two genomes which show little homology ¹⁴. Comparison of these heteroduplexing data with our recently published data suggests that this segment of poor homology in Ha-MuSV corresponds to the 1.0-kb src sequence which encodes p21. These results were quite striking as the p21s encoded by Ha-MuSV and Ki-MuSV had shown only minor serological and electrophoretic differences¹⁹, and we had not anticipated an apparently larger divergence in their nucleic acid coding sequences.

We have now analysed in greater detail the relationship between the p21s of Ha-MuSV and Ki-MuSV, and we have performed a more detailed structural comparison of the src genes of Ha-MuSV and Ki-MuSV using molecular clones of each virus. We have found that there is only a short segment [0.3 kilobase pairs (kbp)] of incomplete homology between their non-30S (that is src) components. Furthermore, using cloned fragments of each src sequence as radiolabelled probes for Southern blots of cellular DNA from a variety of species, we have shown that cellular sarc genes corresponding to Ha-src and Ki-src are detected as conserved genes in the DNA of several

	Table 1 Infecti	Infectivity of viral DNA clones		
DNA clones	Treat	ment	Foci per µg o	
H-1	Intact	in pBR322	6.0×10^{3}	
p-14	Self-lig		0	
HB-11	Self-lig		(7)	
KBE-2	Self-lig	,	(5)	
p-14+HB-11	Ligatio	,	9.0×10^{2}	
p-14+KBE-2	Ligatio		3.1×10^{2}	

The infectivity of the DNA preparations was tested through transfection onto NIH 3T3 cells using the calcium precipitation procedure 22,34. Clone H-1 DNA was used as a positive control. All other DNA clones were purified free of their pBR322 vectors by restriction endonuclease digestion (using enzymes from Bethesda Research Laboratories and New England Biolabs according to the suppliers' specification), agarose gel electrophoresis and electroelution³⁵. Purified inserts were ligated using T4 DNA ligase (New England Biolabs). DNA preparations (0.05-0.40 µg per dish) were transfected using calf thymus DNA as carrier (25 µg ml⁻¹), and foci of transformed cells were quantified from duplicate dishes. Representative foci were removed from the dishes using a cloning cylinder, so that the viral aetiology of the cellular transformations could be tested by p21 immunoprecipitation (see Fig. 5 legend for experimental details). The foci in parentheses are discussed in the text. Although these data are not related directly to the location of the viral src genes, we observed that HB-11 and KBE-2 DNA reproducibly induced low numbers of foci in these assays. However, when the DNA from such foci was analysed by Southern blotting, no new bands were detected homologous to the HB-11 or KBE-2 DNAs, and none of these foci expressed enhanced levels of p21. We do not understand the origin of these 'background' foci.

Fig. 1 Tryptic peptide analysis of p21 from cells transformed by Ha-MuSV or Ki-MuSV. MDCK dog cells transformed by Ha-MuSV, and C127 mouse cells transformed by Ki-MuSV, each in 60-mm dishes, were labelled for 22 h at 37 °C with 2 ml of lysine/arginine-free Dulminimal becco-Vogt modified essential medium supplemented with 2% dialysed fetal calf serum and 125 µCi each of ¹⁴C-lysine and ¹⁴C-arginine (Amersham, 350 mCi mmol⁻¹). Cells were lysed with 2 ml buffer containing 50 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 100 mM NaCl and 1% Triton X-100. After centrifugation for 30 min at 150,000 g, the cleared lysates were immunoprecipitated by antisera containing antibodies to Ha-MuSV p21 from tumour-bearing rats as described previously 4,19. The antigen-antibody complexes were precipitated by Staphylococcus aureus containing protein A. The precipitated proteins were analysed by discontinuous polyacrylamide gel electrophoresis in 0.1% SDS and were visualized by fluorography. The protein bands from the polyacrylamide gels, fixed diphenyloxazole, were excised for tryptic peptide analysis. Gel slices were swelled and digested in 1 ml of 0.1 M NH4HCO3 containing 20 g of DCC-treated trypsin (Sigma, Type X1) at 37 °C for 16 h. 20 µg of fresh trypsin were added and incubation continued for another 8 h. The eluted tryptic peptides were lyophilized twice and finger-



printed by electrophoresis for 70 min at 400 V in 30% formic acid on cellulose thin-layer plates (Schleicher and Schuell) followed by ascending chromatography in buffer containing *n*-butanol, acetic acid, pyridine and water (15:3:10:12 v/v). Peptide spots were visualized by fluorography, a, Ha-MuSV p21; b, Ki-MuSV p21; c, Ha-MuSV p21 mixed with equal c.p.m. of Ki-MuSV p21; d, interpretative sketching of c, where Ha-MuSV-specific, Ki-MuSV-specific, and common tryptic peptides are indicated by hatched, open and solid circles respectively.

vertebrate species. However, the individually conserved Hasarc and Ki-sarc gene(s) in each species are markedly divergent from one another, being present on different restriction fragments of DNA from that species.

Tryptic peptide analysis of Ha-MuSV and Ki-MuSV p21s

Our laboratory has demonstrated the close relationship between the Ha-MuSV and Ki-MuSV p21s by the criteria of serological cross-reactivity, electrophoretic mobility and guanine nucleotide-binding properties. To assess further, by an independent parameter, the relatedness between these src proteins and, indirectly, their genetic sequences, we performed two-dimensional tryptic peptide analysis on the p21s of Ha- and Ki-MuSV. To visualize every tryptic peptide except the most C-terminal one, virally transformed cells were labelled with 14C-lysine plus ¹⁴C-arginine. As shown in Fig. 1, 29 peptides have been resolved of which 6 are unique to Ha-MuSV p21, 9 are unique to Ki-MuSV p21, and 14 are shared by the two. Therefore, approximately two-thirds of Ki-MuSV and Ha-MuSV p21 peptides are shared, and this close, yet divergent, structural relationship has formed the basis for a detailed comparison of the genes encoding each viral p21.

Biological relationship between Ha- and Ki-MuSV molecular clones

Before studying the Ki-MuSV src sequences in detail, we sought biological evidence for the location of the Ki-MuSV src sequences relative to the well characterized Ha-MuSV src

region. The molecular cloning of the genomes of Ha-MuSV and Ki-MuSV has been reported. These clones, referred to as clone H-1 and clone 4 respectively 20,21 (Fig. 2), were derived from unintegrated closed circular DNA molecules isolated shortly after cell infection by the viruses. Consequently, the cloned sequences are circularly permuted with respect to their linear viral genomes. As our initial step towards analysing the Ki-MuSV src sequences, we investigated whether these transforming sequences indeed were in a position analogous to their location in Ha-MuSV, as the earlier data had suggested. An appropriate direct biological assay would be the testing of the transforming activity of subgenomic Ki-MuSV fragments in the calcium phosphate precipitation-mediated DNA transfection assay22. An initial problem in this determination was the inability of viral src sequences alone to induce efficiently formation of foci of transformed cells. The viral long terminal repeat (LTR) sequences, highlighted as a solid box on clone H-1 in Fig. 2, prove useful in overcoming this problem. The LTR, presumably containing an efficient promoter of viral RNA transcription, is an amalgam of sequences from both the 5' and 3' termini of the RNA genome, which are juxtaposed by transfer of nascent DNA between templates during reverse transcription²³. Ligation of LTR DNA to both the transforming sequences of Ha-MuSV²⁴ and Moloney-MuSV²⁵, as well as the cellular sarc sequences related to these two viruses 18,26, has resulted in enhanced transforming activity mediated by these DNAs. Accordingly, a large subgenomic Ha-MuSV fragment containing the LTR was cloned in pBR322 and designated clone p-14 (Fig. 2). The derivation of Ha-MuSV clone HB-11, which contains the entire viral src sequence, has been described18.

Ligation of these two clones at their common BamHI terminus resulted in efficient induction of transformed cell foci (Table 1). as expected. Therefore, a subgenomic Ki-MuSV fragment, residing in clone 4 in an analogous position to that of the HB-11 src clone in clone H-1, was cloned in pBR322 and designated clone KBE-2 (Fig. 2). Ligation of Ha-MuSV clone p-14 to Ki-MuSV clone KBE-2 at the BamHI terminus resulted in efficient focus formation (Table 1), confirming that clone KBE-2 did contain the Ki-MuSV src sequences. This result is consistent with published data which implicate the 5' half of the Ki-MuSV genome as required for transformation²¹. As expected, cells from foci induced by HB-11 or KBE-2 DNA ligated to the LTR fragment contained markedly elevated levels of p21 (data not shown). These results indicate that the viral p21 src genes of Ha-MuSV and Ki-MuSV are located in the same region of each respective viral genome.

Fine structural comparison of Ki- and Ha-MuSV src genes

Having confirmed the general location of the src sequences within the Ki-MuSV genome, we then compared these sequences with their Ha-MuSV counterparts. Clones KBE-2 and HB-11 were hybridized for heteroduplex analysis (Fig. 3, d-f). The 1.1-kbp segment '11+8+12' (Fig. 3f) in clone HB-11 represents precisely the published location and size of the Ha-MuSV src sequences relative to the 5'-terminal BamHI site in the clone ^{15,27}. Similarly, segments '6' and '10' (Fig. 3f) in clone HB-11 coincide with the reported location of rat 30S sequences. Heteroduplexing of clone KBE-2 to the rat 30S clone shows that segments '1' + '3' (Fig. 3c) correspond to the rat 30S sequences and segment '2' represents sequences specific to Ki-MuSV. Segments '7–9' in Ki-MuSV DNA (Fig. 3f) correspond to the Ki-specific segment '2' in Fig. 3c, and segment '8'

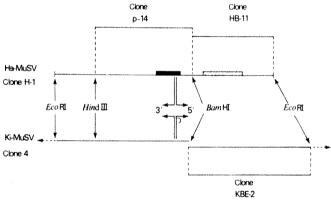


Fig. 2 Structural relationships among the viral clones. Ha-MuSV and Ki-MuSV RNA genomes had been molecularly cloned from preintegration circular DNA forms. Advantage was taken of the fact that retroviral RNA is reverse-transcribed after cellular infection into closed circular double-stranded DNA. Based on the uniqueness of particular restriction endonuclease sites, unintegrated viral DNA was purified from the Hirt supernatant of infected cells, cleaved with EcoRI (Ha-MuSV) or BamHI (Ki-MuSV), and cloned at these respective restriction sites in pBR322. (Ha-MuSV DNA originally had been cloned in λ gtWES · λ B³⁶ but subsequently was recloned in pBR322.) Consequently, Ha-MuSV clone H-1 and Ki-MuSV clone 4 are circularly permuted with respect to their RNA genomes, such that the termini of the genomes are juxtaposed in the DNA clones. The 5' and 3' halves of the viral genomes are demarcated as indicated on the DNA maps. The subgenomic fragments p-14, HB-11 and KBE-2 were derived from their respective parental clones by double digestion with the indicated restriction endonucleases, preparative gel electrophoresis and molecular cloning at the appropriate sites in pBR322. Clone p-14 contains the LTR sequences (solid box) which have a promoter-like sequence of the same 5'-3' polarity as the viral genome³⁷ and which significantly enhance the transformation efficiency of viral src genes. Clone HB-11 (ref. 18) contains the Ha-MuSV transforming sequences (cross-hatched box).

in Fig. 3f is homologous to a portion of the Ha-MuSV src region. Therefore, a 1.75-kbp segment in Ki-MuSV DNA, corresponding to segment '2' in Fig. 3c, contains the Ki-MuSV src sequences.

These heteroduplexing data have confirmed and extended the earlier RNA-DNA heteroduplexing experiments¹⁴ and have specified the location of *src* within Ki-MuSV DNA by virtue of its partial homology to Ha-MuSV *src*. In particular, our measurements of the heteroduplexes between these cloned DNA fragments can be combined with restriction endonuclease mapping data to clone a Ki-MuSV-specific fragment. To this end, we have developed a detailed restriction endonuclease map of clone KBE-2 and have compared it with the published map of clone HB-11, with the heteroduplexing data superimposed on the maps (Fig. 4). Note several features in this comparison: (1) virtually every site in the rat 30S DNA sequences (open box) of the clones is present in both Ha-MuSV and Ki-MuSV; (2) there is a complete site mismatch in the respective *src* sequences; (3) the location on clone HB-11 of the Ha-MuSV-specific (that is,

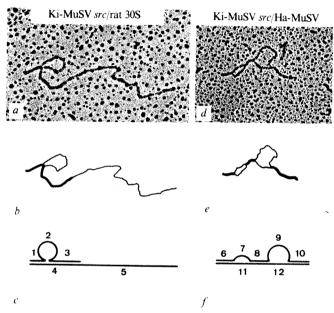


Fig. 3 Heteroduplex analyses of Ki-MuSV src, Ha-MuSV src and rat 30S DNA molecules. Heteroduplexes were prepared as described previously ^{27,38}. Briefly, a mixture of linear DNA molecules (each at a concentration of 4-6 µg ml⁻¹), freed from their cloning vectors by restriction endonuclease digestion and preparative gel electrophoresis, was denaturated by incubation in 0.1 M NaOH for 10 min at 22 °C. The solution was neutralized by the addition of 1/5 volume of 1 M Tris-HCl, pH 7.0. Deionized formamide was added to a final concentration of 50% and renaturation was permitted by incubation for 30 min at 22 °C. Heteroduplexes were mounted for electron microscopy after spreads on a hypophase of 18% formamide. The lengths of singlestranded Φ X174 (5,375 bp) and double-stranded SV40 (5,240 bp) circular DNA molecules were determined in separate experiments in similar spreading conditions to obtain a conversion factor for obtaining measurements of single- and double-stranded regions of the heteroduplexes. We calculate from these experiments that 1 μ m contour length of double-stranded DNA = 3,080 bp, 1 μ m single-stranded DNA = 3,040 bp. Electron micrographs (\times 54,000) are a, heteroduplex of Ki-MuSV fragment (clone KBE-2) and rat 30S DNA (clone 27A, which was cloned at the SacI site in λ gtWES · λ B²⁷) and d, heteroduplex of Ki-MuSV (clone KBE-2) and Ha-MuSV (clone HB-11) fragments. Interpretative tracings of the heteroduplexes are shown in b and e. Schematic representations (c, f) are based on the measurements of > 15 molecules. Contour lengths (in kb) were as follows: $1 = 0.40 \pm 0.02$, 2 = 1.75 ± 0.10 , $3 = 1.02 \pm 0.06$, 1-3 = Ki-MuSV clone, 2 = Ki-MuSVsrc, $4 = 1.50 \pm 0.08$, $5 = 3.83 \pm 0.33$, $4-5 = rat\ 30S$, $6 = 0.27 \pm 0.03$, $7 = 0.52 \pm 0.06$, $8 = 0.34 \pm 0.08$, $9 = 1.18 \pm 0.14$, $10 = 0.71 \pm 0.05$, $11 = 0.33 \pm 0.07$, $12 = 0.44 \pm 0.11$, 6-10 = Ki-MuSV clone, $7-9 = 0.00 \pm 0.00$ Ki-MuSV src, 6+8+10+11+12 = Ha-MuSV clone, 11+8+12 =Ha-MuSV src.

free of rat 30S) subgenomic fragment BS-9 (ref. 15) approximately circumscribes the region of cross-homology between Haand Ki-MuSV src. (solid box).

Pursuing this latter point, we performed Southern blot hybridization with ³²P-labelled BS-9 (Ha-specific) DNA as probe and were struck by its virtual lack of hybridization to clone KBE-2 (data not shown). We questioned whether this could be a function of differences in the relative stringency of hybridization conditions in the heteroduplexing and the Southern blotting procedures. Using the recently described formamide hybridization conditions which enable Southern blots to be hybridized under various levels of stringency²³, we found that ³²P-BS-9 and Ki-MuSV src hybridized well only in 'non-stringent' conditions (data not shown). This lack of reactivity of the two src sequences in stringent conditions suggests that a Ki-MuSV-specific probe might be isolated free not only of 30S RNA sequences but also of Ha-MuSV cross-reactivity in stringent conditions.

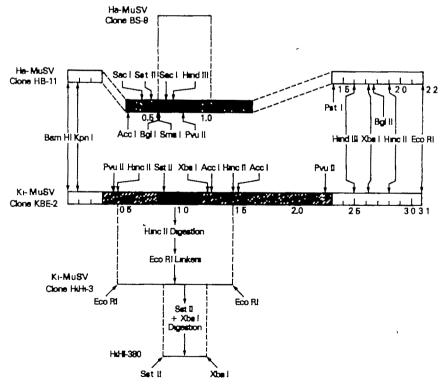
Detection of Ki-MuSV as distinct from Ha-MuSV sarc sequences in cellular DNA

Our approach to the development of a Ki-MuSV src probe depended on the probe being completely free of rat 30S sequences, insofar as these genes related to 30S are highly reiterated in rats and, to a lesser extent, in mice. Therefore, based on its lack of hybridization to cloned rat 30S DNA, a 1.0-kbp fragment of clone KBE-2 DNA was cloned in pBR322 (clone HiHi-3, Fig. 4). For use as a Ki-MuSV src-specific probe, a fragment of clone HiHi-3 was purified by preparative gel electrophoresis (HiHi-380, Fig. 4). We then used both the Ha-specific and Ki-specific DNAs as radiolabelled probes for Southern blots of high-molecular-weight DNA from cells of various species (Fig. 5). As biological controls for the specificity of the src probes, we performed hybridizations to EcoRI-digested DNAs from NIH 3T3 mouse cells infected with the

Fig 4 Detailed structure of Ha-MuSV and Ki-MuSV transforming sequences. A detailed restriction endonuclease map, on a scale demarcated in kbp, was developed for Ki-MuSV clone KBE-2. The detailed map of Ha-MuSV clone HB-11 is reproduced from the published map of the complete Ha-MuSV genome clone H-1¹⁵. Superimposed on these maps are the heterduplex data of Fig. 3. The open box represents common rat 30S RNA sequences; the solid box represents sequences shared by Ha-MuSV and Ki-MuSV but not rat 30S RNA; the hatched boxes represent sequences unique to either Ha-MuSV (lefthatched) or Ki-MuSV (right-hatched). Note the discontinuity of scale on clone HB-11 (dotted lines), drawn to compensate for the size differential between the Ha-MuSV- and Ki-MuSV-specific (hatched) sequences. details of the derivation of Ha-MuSV clone BS-9 have been published elsewhere¹⁵. To derive cloned sequences as a specific probe for KI-MuSV, we sought a fragment of clone KBE-2 which would not hybridize to rat 30S RNA sequences. Accordingly, singly and doubly restricted fragments of clone KBE-2 were electrophoround, blotted and hybridized to ³²P-labelled clone 27A DNA. A *HircII*—*HircII* fragment (map positions 0.4-1.4) was purified by preparative gel electrophoresis, and after blunt-end ligation of EcoRI linkers (Collaborative Research) to the DNA, molecules various rat-derived sarcoma viruses. The Ha-specific probe, hybridized to EcoRI-digested DNA from cells non-productively transformed by Ha-MuSV, detected an additional band compared with control cell DNA which was not detected by the Ki-specific probe (data not shown). Conversely, the Ki-specific probe, but not the Ha-specific probe, detected an extra band in EcoRI-digested DNA from cells non-productively transformed by Ki-MuSV (Fig. 5a and b, lanes 6).

A third cell line studied was NIH 3T3 cells transformed by the rat sarcoma virus (RaSV). This virus, whose entire genome is rat derived, was isolated after in vitro co-cultivation of two lines of rat cells²⁹. The only known gene product of RaSV is a 29,000-MW protein³⁰ which is closely related to Ki- and Ha-MuSV p21s in terms of common methionine-containing tryptic peptides as well as serological cross-reactivity³¹. When blots of EcoRI-digested DNA from RaSV-transformed NIH 3T3 cells were hybridized with the Ha-MuSV and Ki-MuSV src probes, an extra band relative to control cells was detected only with the Ha-specific probe, not the Ki-specific probe (Fig. 5a and b, lanes 5). These results demonstrate that the RaSV transforming sequences are more closely related to those of Ha-MuSV than to those of Ki-MuSV.

Having confirmed the specificity of these src probes, we then tested DNAs of rat, chicken, human and mouse for cellular p21 sarc sequences related to the viral src probes. It can be seen that the Ha-specific probe detected one or two bands in each of these EcoRI-digested DNAs (Fig. 5a, lanes 1-4). The Ki-specific probe detected one to three bands (Fig. 5b, lanes 1-4) distinctly different from the corresponding Ha-specific bands. The exceedingly weak hybridization of the Ki-specific probe to the 2.1- and 4.2-kbp chicken DNA fragments (Fig. 5b, lane 2) suggests that the normal cellular sarc sequences related to Ki-MuSV, having diverged more rapidly in chickens, are not as broadly conserved evolutionarily as are the Ha-MuSV sarc sequences.



were digested with EcoRI, purified free of linker monomers by Sepharose 2B (Pharmacia) chromatography and cloned at the EcoRI site of pBR322. This clone is designated Ki-MuSV clone HiHi-3. When it was used as a radiolabelled probe for Southern blots of cellular DNA sequences, innumerable bands were detected in rat DNA, even though one to three bands were detected in DNA from other species (data not shown). The origin of this redundancy with respect to rat DNA was unclear, although it could have represented rat 30S sequences present in clone HiHi-3 yet missing in the rat 30S clone which we had used to define HiHi-3. Nevertheless, we sought a fragment of clone HiHi-3, which would be 'single copy' in rat DNA, by trimming the clone HiHi-3 insert. To prepare such a fragment, the clone HiHi-3 viral insert was digested with SstII and the 0.6-kbp fragment purified by preparative gel electrophoresis. This fragment was digested with XbaI, then the 0.38 kbp fragment (HiHi-380) was purified by preparative gel electrophoresis.

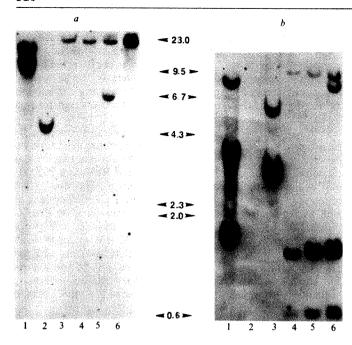


Fig. 5 Southern blot analysis of restriction endonucleasedigested high-molecular-weight cellular DNA. 50 µg of cellular DNA, prepared by established procedures³⁹, were digested for 16 h with 100 units EcoRI, electrophoresed, blotted and hybridized (in the 'stringent' conditions described in Fig. 4 legend) to either of two 32 P-labelled DNA probes (1.5×10⁷ c.p.m.). a, 32 P-labelled BS-9 (Ha-specific) DNA. b, 32 P-labelled HiHi-380 (Kispecific) DNA. Sources of high-molecular-weight DNA were: 1, FRE rat cells; 2, chicken cells; 3, human cells; 4, uninfected NIH 3T3 cells; 5, NIH 3T3 cells transformed by the rat sarcoma virus; 6, NIH 3T3 cells transformed by Ki-MuSV. The arrows indicate the positions of 32 P-labelled $\lambda/HindIII$ DNA markers (length in kbp). Panel a, lane 1 portrays the two rat cellular sarc genes related to Ha-MuSV src, as has been reported elsewhere 18. The hybridizing bands represent the intron-containing (20 kbp) and colinear (13 kbp) forms of Ha-sarc.

Discussion

The relationship between Ha-MuSV and Ki-MuSV is a more complex one than had been thought previously. Minor serological and electrophoretic differences between the viral p21s had suggested that the respective src proteins were merely variants of one another and were derived from the same cellular p21 sarc gene. This conclusion was consistent with the ability of these viruses to induce similar diseases in rodents and morphologically similar cellular transformants in vitro. The data presented here clearly demonstrate that the Ha- and Ki-MuSV src sequences are derived from distinct cellular p21 sarc genes. On the basis of the heteroduplexing, hybridization and tryptic peptide data, we deduce that there are two domains within the genes coding for the respective viral p21 molecules. One of these domains represents sequences unique to each p21 gene, sequences that are a subset of the hatched region of the viral genomes in Fig. 4. The other domain represents sequences shared by both the p21 molecules, as indicated by the solid box in Fig. 4. Assuming that these common sequences are entirely coding sequences, their length (0.35 kbp) would contain the genetic information for ~11,000 daltons of polypeptide chain, that is, one-half of the p21 polypeptide. Moreover, the ability of ³²P-Ha-specific DNA to hybridize to Ki-MuSV src in non-stringent but not in stringent conditions suggests that the Ki-common and Ha-common sequences are approximately 10-15% divergent. Most of this divergence would involve 'wobble-codon' changes and other nucleotide changes resulting in conservative amino acid substitutions, such that >50% of the tryptic peptides remain shared between the two viral p21s. It seems likely that these common sequences account for the serological cross-reactivity, guanine nucleotide-binding capacity and common biological potential (transformation) of the viral p21s. Apparently this represents the selection pressure for the conservation of the p21 amino acid sequences during the evolution of Ha-MuSV and Ki-MuSV p21 genes. Furthermore, our preliminary nucleotide sequence analysis of Ha-MuSV places these common sequences in the 5' half of the polypeptide chain (unpublished data). At this point, we can only speculate that the sequences unique to either Ha-or Ki-MuSV p21 contribute to as yet undiagnosed differences between the proteins.

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Our laboratory has demonstrated the expression of low (relative to cells transformed by Ha- or Ki-MuSV) levels of p21 in normal cells from a wide variety of species, and our Southern blots of various cellular DNAs have shown the broad evolutionary conservation of both Ha- and Ki-MuSV cellular genes. Given the presence of multiple related, yet distinct genes, are all or only some of the cellular genes being expressed in different cells? We have shown that rat DNA has two genes homologous to Ha-MuSV src: one is apparently colinear with p21 src while the other has three introns in its coding sequence (it is likely that one of these genes is the progenitor of the putatively transformation-specific p29 of the third rat-derived sarcoma virus RaSV). Both genes are fully functional, as each is capable of causing cellular transformation after DNA transfection. These transfections also have demonstrated that elevated levels of a normal cellular protein are sufficient to cause cellular transformation. With respect to homology to Ki-MuSV src, blots of EcoRI-digested rat DNA have three prominent bands, while blots of rat DNA digested with other restriction endonucleases have two to three major bands (unpublished data). These data suggest that there may be two Ki-MuSV cellular sarc genes in the rat, thereby making a minimum total of four rat genes capable of encoding a constitutively expressed p21. As there has probably been selection pressure favouring the retention of this entire family of p21 genes, it would be important to determine which of the genes is expressed in different rat cells. With respect to mouse cells, we have recently reported that a line of undifferentiated haematopoietic cells expresses higher levels of p21 than do Ha-MuSV or Ki-MuSV-transformed cells³², and we wish to determine which gene is encoding this p21. The structures of the various cellular and viral p21 polypeptides and their in vivo expression should elucidate functional differences among the various p21s. For example, there may be a tissue- or differentiation-specific pattern of expression among the genes, as in the globin gene family³³. By analogy to fetal and adult forms of globin, each distinct p21 might possess unique biochemical properties pivotal to its normal differentiation-related physiological role.

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- 1. Harvey, J. J. Nature 204, 1104-1105 (1964).

- Harvey, J. J. Pataure 204, 1104–1103 (1904).
 Kirsten, W. H. & Mayer, L. A. J. natn. Cancer Inst. 39, 311–335 (1967).
 Scher, C., Scolnick, E. M. & Siegler, D. Nature 256, 225–227 (1975).
 Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. Virolo, 31, 546–556 (1979).
 Langbeheim, H., Shih, T. Y. & Scolnick, E. M. Virology 106, 292–300 (1980).
 Spector, D. H., Varmus, H. E. & Bishop, J. M. Proc. natn. Acad. Sci. U.S.A. 75, 4102–4106
- Witte, O. N., Rosenberg, N. & Baltimore, D. Nature 281, 396-398 (1979)
- Sherr, C. J., Fedele, L. A., Donner, L. & Turek, L. P. J. Virol. 32, 860-875 (1979). Collett, M. S. & Erikson, R. L. Proc. natn. Acad. Sci. U.S.A. 75, 2021-2024 (1978)
- Witte, O. N., Dasgupta, A. & Baltimore, D. Nature 283, 826-831 (1980). Van deVen, W. J. M., Reynolds, F. H. & Stephenson, J. R. Virology 101, 185-197 (1980).
- Shih, T. Y., Papageorge, A. G., Stokes, P. E., Weeks, M. O. & Scolnick, E. M. Nature 287, 686-691 (1980).

- 586-691 (1980).
 Shih, T. Y. et al. J. Virol. 27, 45-55 (1978).
 Chien, Y. H. et al. J. Virol. 31, 752-760 (1979).
 Ellis, R. W. et al. J. Virol. 36, 408-420 (1980).
 Scolnick, E. M., Vass, W. C., Howk, R. S. & Duesberg, P. H. J. Virol. 29, 964-972 (1979).
 Tsuchida, N., Gilden, R. & Hatanaka, M. Proc. natn. Acad. Sci. U.S.A. 71, 4503-4507 (1979).
- DeFeo, D. et al. Proc. natn. Acad. Sci. U.S.A. 78, 3328-3332 (1981).
 Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. Virology 96, 64-79 (1979).
 Chang, E. H. et al. J. Virol. 35, 76-92 (1980).
- Tsuchida, N. & Vesugi, S. J. Virol. 38, 720-727 (1981)

- Griban, F. L. & van der Eb, A. J. Virology 52, 456–461 (1973).
 Gilboa, E. et al. Cell 16, 863–874 (1979).
 Chang, E. H., Ellis, R. W., Scolnick, E. M. & Lowy, D. R. Science 210, 1249–1251 (1980).

- 25. Blair, D. G., McClements, W. L., Oskarsson, M. K., Fischinger, P. J. & Vande Woude, G. F. Blair, D. G., McClements, W. L., Ssassan, S. (1980).

 McClements, W. L. et al. Cold Spring Harb. Symp. quant. Biol. 45 (in the press).

 Young, H. A. et al. Virology 107, 89-99 (1980).

- 28. Howley, P. M., Israel, M. A., Law, M.-F. & Martin, M. A. J. biol. Chem. 254, 4876-4883
- 29. Rasheed, S., Gardner, M. B. & Huebner, R. J. Proc. natn. Acad. Sci. U.S.A. 75, 2972-2976
- 30. Young, H. A., Shih, T. Y., Scolnick, E. M., Rasheed, S. & Gardner, M. B. Proc. natn. Acad.
- 31. Young, H. A. et al. J. Virol. 38, 286-293 (1981).

- 32. Scolnick, E. M., Weeks, M. O., Shih, T. Y., Ruscetti, S. K. & Dexter, T. M. Molèc, cell. Biol.
- 33. Nienhuis, A. W. & Bernz, E. J. New Engl. J. Med. 297, 1318-1328, 1371-1381, 1430-1436
- 34. Lowy, D. R., Rands, E. & Scolnick, E. M. J. Virol. 26, 291-298 (1978)

- Lowy, D. R., Kainds, E. & Gouinek, E. M. J. VIOL. 203, 231-278 (1976).
 McDonell, M. W., Simon, M. N. & Studier, F. W. J. molec. Biol. 110, 119-146 (1977).
 Hager, G. L. et al. J. Virol. 31, 795-809 (1979).
 Dhar, R., McClements, W. L., Enquist, L. W. & Vande Woude, G. F. Proc. natn. Acad. Sci. U.S.A. 77, 3937-3941 (1980).
- 38. Davis, R. W., Simon, M. & Davidson, N. Meth. Enzym. 210, 413-428 (1971). 39. Gross-Bellard, M., Oudet, P. & Chambon, P. Eur. J. Biochem. 36, 32-38 (1973)

A reinvestigation of the role of the grey crescent in axis formation in Xenopus laevis

J. Gerhart*, G. Ubbels*, S. Black*, K. Hara* & M. Kirschner*

* Department of Molecular Biology, University of California, Berkeley, California 94720, USA † Hubrecht Laboratory, Utrecht, The Netherlands

‡ Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, USA

Gravitationally induced displacements of the contents of the frog egg can predictably determine the orientation of the subsequent dorsal-ventral axis of the embryo, regardless of the original position of sperm entry or of the grey crescent. In certain conditions, these displacements in the egg can also lead to the formation of a second axis, that is, to twinning. The previously reported ability of grafts of grey crescent cortex to induce secondary axes in recipient eggs is interpreted here as an unrecognized twinning effect of gravity. Our results lead us to question the classic interpretation of the grey crescent as a dorsal determinant in amphibian development.

The unfertilized egg of most frogs and salamanders is organized with radial symmetry about its animal-vegetal axis. The embryo, however, has clear bilateral symmetry organized along anteriorposterior and dorsal-ventral axes. Early embryologists recognized the following events in the establishment of the dorsalventral axis¹⁻⁵: (1) a single sperm randomly enters the animal hemisphere, giving the egg its first bilateral symmetry; (2) before first cleavage, the egg forms a grey crescent, a region of reduced pigmentation in the animal hemisphere which, in the case of the frog egg, is opposite the side of sperm entry; (3) at gastrulation, about 15 cleavages later, the blastopore appears first at the lower limit of the surface region previously occupied by the grey crescent; and (4) dorsal structures of the embryo originate from the grey crescent region of the egg. As reported by Spemann and Mangold^{5,6}, the gastrula contains in the dorsal blastopore lip a group of cells which induce neighbouring cells to undergo dorsal development and are themselves incorporated into dorsal structures. Thus, the dorsal regions of the embryonic axis owe their location to the position of these inducer cells, the Spemann organizer, while ventral development occurs where the organizer is absent.

As the Spemann organizer region is so clearly responsible for the 'dorsalization' of the gastrula and neurula, the grey crescent, its topographical precursor, has been ascribed the capacity to generate the organizer. The most detailed proposal for the grey crescent's role is found in the cortical field hypothesis of Dalcq and Pasteels^{1,7}: the developmental fate of each egg region is said to derive from local values of two kinds of quantitative material gradients, the first a cortical gradient centred in the grey crescent at the egg surface, and the second, an internal cytoplasmic gradient oriented in the vegetal to animal direction following the concentration of yolk platelets. The blastopore and organizer would arise much later at the site where the combined value of the gradients is greatest. The two gradients were said to be distinguishable experimentally by their response to gravity or centrifugation, since the yolk platelets, but not the cortical materials, were expected to rearrange easily in the egg.

Although Pasteels¹ devised tests of this hypothesis involving rotation and inversion of eggs at the time of grey crescent formation, his results were rather ambiguous and have been generally neglected compared with the direct, dramatic support of Curtis8, who reported that the dorsalizing activity of the grey crescent cortex was transplantable. He removed a piece of grey crescent cortex from a donor egg of X. laevis shortly before first cleavage and grafted it into the equatorial surface of the prospective ventral side of a recipient egg at or close to first cleavage. In all 11, eggs receiving cortex from the crescent region double embryonic axes developed, one supposedly from the resident crescent region and one from the transplanted crescent region. Thus, the grey crescent cortex was concluded to be a dorsalizing locus, which preserved its activity on transplantion.

More recently, Nieuwkoop and co-workers^{3,9,10} obtained experimental results which contradict a strict cortical gradient hypothesis. They found that at the mid-blastula stage, the potential for dorsal development is carried by a group of cells in the vegetal hemisphere that are not topographically superimposable with the grey crescent. These vegetal cells induce the Spemann organizer to form in adjacent animal hemisphere cells, coincidentally in the grey crescent region. Furthermore, recent fate maps of X. laevis indicate that, contrary to earlier conclusions, the cells of the Spemann organizer do not derive from the grey crescent cortical area of the egg but from deeper cytoplasmic regions 11.12. Because of these discrepancies, we have repeated and extended for X. laevis several of the classic experiments on which the importance of the grey crescent rests.

Topographical relationship of sperm entry, grey crescent and blastopore in X. laevis

Shortly after fertilization, a small amount of pigment accumulates around the sperm entry point (SEP) in the animal hemisphere, producing a dark spot¹³. Approximately mid-way in the period from fertilization to first cleavage, the grey crescent appears on the side of the animal hemisphere away from the SEP, as a region of lighter and more granular pigmentation¹³. In \sim 90% of eggs, the crescent forms in the sector 135°-180° from the SEP14, but the broadness and diffuseness of the crescent in X. laevis preclude more accurate measurement of its position relative to the SEP or blastopore. As the SEP can be very accurately localized, we have marked eggs with Nile blue at an equatorial position (that is, the boundary of the pigmented and

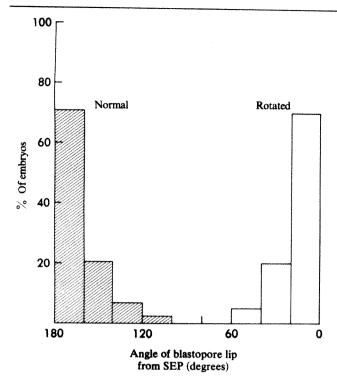


Fig. 1 The topographical relationship of the sperm entry point (SEP) to the dorsal blastopore lip in X. laevis embyros developing from normal and rotated eggs. Unfertilized eggs were squeezed from frogs into 100% MR (100 mM NaCl, 2 mM KCl, 2mM CaCl₂, 1 mM MgCl₂, 5 mM Na-HEPES, pH 7.6) and fertilized in vitro in 30% MR. After fertilization, eggs were dejellied with 2% cysteine hydrochloride, brought to pH 7.9 with NaOH, and washed extensively with 20% MR before storage until use. Procedures were as described elsewhere 28.29. Eggs were placed in 5% Ficoll-20% MR and were marked at the equatorial margin (pigmentation boundary) beneath the SEP by application of a small crystal of Nile red, a sparingly soluble form of Nile blue 16, or by injection of 1% Nile blue in 20 mM sodium phosphate or by injection of 1% Nile blue in 20 mM sodium phosphate, pH 7.4. The rotated eggs were inclined 90° to the side, with the SEP and dye mark uppermost, starting 50 min after fertilization (0.55 on a normalized scale, 19 °C), and continuing for 30 min, at which time the eggs and unrotated controls were transferred to 20% MR where the perivitelline space rehydrated and the eggs returned to gravitational alignment. After 9-15 h, when the blastopore lip first appeared, eggs were scored for the position of the lip relative to the dye mark, by inverting the egg and measuring the angle separating the lip and mark. The histogram places eggs in 20° angular spreads. Normal eggs, not rotated, cluster at the 180° angular separation, that is, the blastopore tends to be opposite the side of sperm entry. Rotated eggs group around a 0° separation, with the lip appearing on the same side as the SEP.

non-pigmented hemispheres) closest to the SEP and have recorded the position of the earliest blastopore lip 10 h later, at the gastrula stage. As shown in Fig. 1, in 70% of eggs, blastopores originate in the sector 160–180° from the dye mark (and hence the SEP of the egg), 27% in the sector 120–160° from the SEP, and 3% in the 100–120° sector. Thus, the SEP is a good but not perfect indicator of the blastopore position and the future dorsal-ventral axis of the embryo.

Dissociation of the topographical relations by gravity

We have repeated and extended with X. laevis eggs several of the experiments of Ancel and Vintemberger¹⁵ who demonstrated the strong control exerted by gravity on axis determination in eggs of Rana fusca. In our modification¹⁶ of their procedure, we have marked dejellied eggs with Nile blue at the equator near the SEP and immersed them in a solution of 5% Ficoll (Pharmacia), a hydrophilic sucrose polymer which dehydrates the perivitelline space and causes the fertilization envelope to collapse on to the egg surface. The egg can then be rotated 90° so that its animal-vegetal axis, which is normally vertical due to the dense yolk of the vegetal hemisphere, is then horizontal. In the Ficoll solution, the rotated egg is unable to return to the vertical orientation in the absence of lubrication from the perivitelline space. Inside the egg, the dense contents of

the vegetal hemisphere flow slowly downwards, driven by gravity, displacing upwards the less dense cytoplasm of the animal hemisphere^{1,15}. These rearrangements continue until the egg is returned to the vertical orientation manually or by transferring the egg to a solution without Ficoll, in which the perivitelline space rehydrates. With reference to the egg's original animal-vegetal axis, gravity causes a slight displacement of cytoplasmic contents towards one side of the egg.

The effect of brief horizontal orientation on axis determination is very striking. Shortly after fertilization, we turned eggs horizontally to a position with the SEP and blue mark uppermost, left them in this orientation at 20 °C for 30 min and then allowed the eggs to develop in the normal orientation until the gastrula stage, when we scored for the position of the earliest blastopore. To our surprise, the blastopore originated directly beneath the blue mark, that is, on the same side of the egg where the sperm had entered. Brief rotation had completely reversed the egg's selection of a dorsal-ventral axis. These data are shown in Fig. 1 on the right side of the histogram: in 70% of eggs the blastopore initiated in the sector 0-20° from the mark, 20% in the 20-40° sector and 10% in 40-60° sector. From observations of the Nile blue mark in the neurula and tailbud stages, we confirmed that dorsal embryonic structures did indeed form from the SEP side of these eggs. We also rotated eggs to horizontal inclinations in which the SEP and dye mark were to the left or right side rather than uppermost, and found that whatever region of the egg equator is uppermost during the rotation period becomes the site of dorsal development in the embryo. Clearly, the egg has selected its dorsal-ventral axis based on its relationship to the gravitational field and not on the position of the SEP. Normally the egg, in its vertical orientation. is free to rotate in the perivitelline space, protected from such gravity-induced lateral rearrangements of its contents.

Figure 2 shows the effects of gravity on eggs at various times from fertilization to first cleavage, as seen using the Ficoll method. Control eggs are shown in the right-hand part of the graph; unrotated eggs gave blastopores approximately opposite (158°) the SEP, and eggs rotated horizontally with the SEP directed downwards gave a narrower angular distribution (173°) than the unrotated controls, as if rotation in that direction reinforces the normal sperm-directed selection of an axis.

In contrast to the controls, the experimental eggs gave clear evidence of axis reversal. As the extent of reversal depends on the time at which the rotation began, the experimental results can be considered in two parts: that is, the effects of gravity in the pre-grey crescent period (time 0.0-0.5) and in the post-grey crescent period (0.5-1.0), where the scale of 0 to 1.0 represents normalized time from fertilization to first cleavage (100 min at 18 °C). The egg is very sensitive to horizontal orientation in the pre-crescent period and reversal is essentially complete. A 12-min period at 90° inclination is sufficient to reverse 100% of the eggs, and an angle of inclination of just 30°, rather than 90°, is fully effective (data not shown). Thus, the normal topographical relation of the SEP to the locus of prospective dorsal development in the egg can be easily disrupted in the precrescent period, with the egg using the gravitational perturbation for its selection of an axis, as was recognized by Ancel and Vintemberger¹⁵

In the post-crescent period, the egg becomes more refractory to the effects of gravity. As shown in Fig. 2, a horizontal inclination starting after the time 0.5 is still effective in determining the axis, although the resistance of the egg to gravity increases between time 0.7 and 0.9 and is essentially complete by 1.0, when first cleavage begins. Pasteels¹ interpreted this resistance as a reflection of the establishment of the grey crescent which, as a cortical determinant, was by definition not perturbable by gravity. Our data do not really fit this interpretation because even at times 0.7 and 0.8, when the grey crescent is fully visible in X. laevis eggs, a significant fraction of the eggs are still affected by gravity. On the other hand, we do not know when grey crescent formation is complete in X. laevis eggs, and note that in other frog species the formation continues

Table 1 Effects of gravity and centrifugal force in the period after grey crescent formation and before first cleavage

Starting time (normalized)	Duration (min)	Force (g)	Angle of inclination of egg, SEP side uppermost	No. of eggs	Position of neural groove (average angle, in degrees from SEP)
(1) —		1	0	470	138
(2) 0.40	20	1	90	109	27
(3) 0.90	20	ī	90	98	140
(4) 0.90	90	i	90	32	141
(5) 0.90	4	26	90	120	112
(6) 0.90	4+90	26 + 1	90	43	74
(7) 0.95	4	50	45	10	30
(8) 0.95	4	50	90	24	20

Eggs of X. laevis were fertilized, dejellied and embedded upright in 9% gelatin (175 Bloom, Sigma)/30% modified Ringer's solution^{28,29} in 35-mm plastic tissue culture dishes. The sperm entry side of each egg was oriented towards a marked side of the dish. The time of the start of gravitational treatment was normalized to the interval from fertilization (0.0) to first cleavage (1.0) and expressed as a fraction of this interval. The treatments were done at room temperature (from 19 to 24 °C) with cleavage times of 100 to 70 min, respectively. In the case of 2, 3 and 4, eggs in gelatin were inclined 90° with the sperm entry side uppermost, at 1 g, by turning the dish on its side. For 5, 7, and 8, eggs in gelatin were centrifuged with the sperm entry side towards the centre of the rotor, that is, uppermost in the centrifugal field. In 6, eggs were centrifuged in this orientation for 4 min and then the dishes were kept inclined at 90° for 90 min with the sperm entry side uppermost. After the gravitational treatment, the eggs were returned to the upright position and incubated at 15 °C for development. After 48 h the embryos were scored for the position of the neural groove and neural folds. The position of the neural groove is recorded as the angle between two vectors originating at the vegetal pole, one passing along the neural groove and one passing through the sperm entry point. An angle of 180° indicates that the neural groove is opposite the side of sperm entry; an angle of 0° indicates that the groove is on the same side.

from 0.5 to 0.8 (refs 17-19). Therefore, we needed to test whether forces >1g could shift the dorsal-ventral axis at still later times.

We used centrifugation to achieve more rapid and extensive movement of the egg contents in the refractory period. Eggs were first embedded and oriented uniformly in a dish of molten gelatin, which, like Ficoll, dehydrates the perivitelline space, and on solidification also holds the eggs in a fixed position. The dish was placed in the centrifuge with the SEP side of the eggs towards the centre of the rotor, so that the centrifugal force would move materials away from it. This orientation is equivalent to that of an egg turned SEP upwards in the gravitational field. After centrifugation, the embedded eggs were incubated in the normal orientation and later scored for the position of definitive dorsal structures, that is, the neural groove and folds, relative to the position of the original SEP. Data are summarized in Table 1. Uncentrifuged control embryos, left in gelatin in the normal vertical orientation, formed the dorsal side at a position averaging 138° from the SEP, a value slightly lower than the 158° found for the blastopore lip position in controls of Fig. 2, perhaps due to limited movement of the gelatin-embedded embryos after gastrulation. As a further control, embedded eggs were turned through 90° at 1g for 20 min starting at 0.4—these developed dorsal structures at a position 20° from the SEP, again indicating the strong effect of gravity on pre-grey crescent eggs. By the time 0.9, eggs turned horizontally at 1g for 20 min, or even for 90 min, in gelatin were completely refractory to gravity.

However, if eggs were centrifuged at 26g for 4 min at 0.9, while oriented with the SEP side uppermost in the centrifugal field, there was a detectable effect on the prospective axis; the dorsal side of the embryo developed, on average, 112° from the SEP. If the 4-min centrifugation at 26g was followed by a 90-min period of horizontal inclination at 1g, the effect was greater, moving the dorsal side to within 74° of the SEP. Finally, if eggs at 0.95 were centrifuged at 50g for 4 min, the dorsal-ventral axis of the embryo was essentially reversed, the dorsal side of the embryo developing within 20° or 30° from the SEP side. The latter treatment was effective until first cleavage, when the developing furrow weakened the egg so that it did not survive the centrifugation. Thus, while the post-crescent period

is characterized by an increasing resistance to gravity, the prospective axis can still be shifted as completely as in the precrescent period, provided more force is applied. Thus, the locus for dorsal development can be completely dissociated from its normal topographical relationship to the grey crescent, well after the time of crescent formation.

Double axis formation

The importance of the grey crescent in dorsalization has been most directly demonstrated by the production of double-axis embryos from eggs grafted with pieces of grey crescent cortex8 However, our data contradict such a role for the grey crescent. It seemed unlikely that the different results could be reconciled by describing the grey crescent cortex as active on transplantation while at the same time easily rearranged or replaced in the egg when exposed to gravitational force. Alternatively, we must ask whether the transplantation results could in some way be an artefact of gravity and not an effect of cortical transplantation. While exploring the effect of increasing centrifugal force on axis position, we found that double-axis embryos can indeed arise at high frequency within a narrow range of conditions of centrifugation. As shown in Table 2 (groups 1 and 3), at a force of 30g applied for 4 min in the period 0.5-0.7, 60-70% of the eggs developed into neurulae with double, opposite, partial or complete sets of dorsal structures, essentially as Siamese twins. Double axes form only if the egg is centrifuged with the SEP side oriented towards the centre of the rotor, that is, in the position uppermost in the centrifugal field. As shown in Table 2 (group 2), when the SEP is directed away from the centre, no twins are formed, and the single dorsal side is centripetal, where the crescent had resided. In this orientation, gravity presumably just

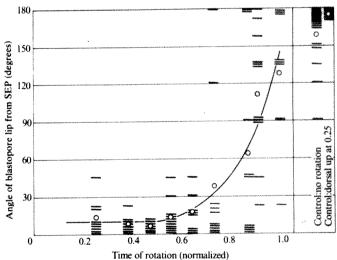


Fig. 2 Position of the dorsal blastopore lip in embryos from X. laevis eggs rotated at various times after fertilization. Eggs were fertilized and dejellied as described in Fig. 1 legend and placed in 20% MR containing 5% Ficoll. The eggs were marked at the prospective ventral equator, that is, on the side of the SEP as described in Fig. 1 legend, and were then left in a vertical position with the animal pole upwards; until the time of rotation when they were turned (90° rotation with SEP uppermost). Eggs remained in the rotated position for 60 min and were then returned to a vertical orientation and allowed to develop in 20% MR. The position of the first appearance of the blastopore lip, ~9-15 h after fertilization (16-21 °C), was scored as the angle between the lip and the dye mark, as described in Fig. 1 legend; 0 indicates superposition of the dye mark and lip, whereas an angle of separation of 180° indicates opposition of the mark and lip. The angle of separation is given on the ordinate, and the time at which the egg was rotated is shown on the abscissa. The time is normalized, with fertilization set as 0.0 and the start of first cleavage set at 1.0. This period ranges from 70 to 130 min depending on the temperature. Data for individual eggs at each time are given as horizontal dashes, while the average for that time is given as an open circle. Control results are shown at the right hand side of the figure: eggs were either left without rotation, or were turned with the dye mark (SEP side) downwards. Note that the latter controls gave an even sharper population response than unrotated controls, for the average angle of separation of the lip and mark. Note also in the experimental series the heterogeneity of the response in the time range 0.7-0.9, when some eggs were affected totally by gravity, a few were not affected at all, and others gave intermediate angles of separation.

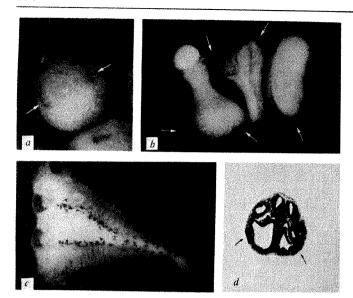


Fig. 3 Twin embryos produced from eggs removed from the fertilization envelope and turned ventral side uppermost, before first cleavage. a, Early gastrula with two blastopores, indicated by arrows; b, three late neurulae, two with double neural grooves and one a control embryo with a single neural groove. The anterior end of each groove is indicated by an arrow. c, Siamese twin at hatching, at about stage 38. One of the axes carries a less well developed head, lacking eyes, although the sucker is present and the trunk is complete. d, Section of a twin embryo, at the posterior head level. Two neural tubes are visible at the top, close together, and two mouth cavities are present at the bottom. Arrows indicate two suckers. The axes were more separated in the mid-trunk region. Paraffin sections were 7 μm thick and were stained with nuclear fast red-aniline blue-orange G.

reinforces the effects of the SEP and the grey crescent on axis determination. Furthermore, even in the orientation favourable for twinning, the extent of rearrangement is critical: at still higher centrifugal force, twins do not form and the single axis forms on the SEP side, that is, that controlled by centrifugal force. This is shown in Table 2, group 4; eggs were centrifuged with the SEP towards the centre of the rotor, as is suitable for twinning, but were subsequently inclined horizontally with the side of the SEP uppermost, to continue lateral displacements of the egg contents at 1g for 90 min. In this case, no twins were formed and the single axis derived from the SEP side, not the side of the grey crescent. Thus, twins seem to develop when the gravitational force is strong enough to create a new dorsal localization in the egg but not so strong as to eliminate the old (SEP-grey crescent-directed) one.

Reservations about cortical grafting results

As high centrifugal force produces double-axis embryos, we attempted to duplicate the conditions at 1g used by Curtis⁸ in his cortical transplantation experiments, to determine whether gravity could also act as a twinning agent there. When the egg was removed from the fertilization envelope by manual dissection and allowed to settle on to an agar surface with its SEP side uppermost, twins were in fact produced. The frequency was quite variable from one egg batch to the next and as shown in Table 2, we found two batches (that is, from two different frogs) giving 50-70% double-axis embryos, three batches giving 30-49%, eight giving 10-29% and eight giving 0-9%. Figure 3a shows an example of a double blastopore of an early gastrula from an egg turned horizontally with the SEP side uppermost starting at 0.8, and left in this orientation until the 16-cell stage. Examples of double neurulae and of a hatching-stage Siamese twin are shown in Fig. 3b and c, respectively. Histological analysis confirmed the presence of doubled internal dorsal structures (Fig. 3d).

With regard to the conditions required for twinning by this procedure, it should first be mentioned that eggs removed from the fertilization envelope lack a perivitelline space and remain in the orientation in which they are put intentionally or by chance.

Therefore, just as in the Ficoll treatment, they cannot return to the vertical orientation and are susceptible to gravity-induced displacements of the egg contents. Second, twinning has been observed by us only in eggs oriented with the SEP side, that is, the prospective ventral side, uppermost. Table 2 records no double-axis embryos for two sets of eggs oriented with the SEP directed downwards, that is, prospective dorsal side uppermost; eggs of these same batches gave 20% and 35% twins when oriented SEP upwards. This requirement is the same as found for twinning by centrifugation and implies the importance of having gravity oppose rather than reinforce the effects of the sperm and grey crescent on axis selection. Third, extreme flattening of the egg which occurs on its removal from the fertilization envelope may also be an important precondition for twinning at 1g, thereby explaining why we rarely observed twins (never more than 5%) in our experiments when eggs were rotated SEP side uppermost in Ficoll or gelatin, as these eggs largely retain their spherical shape. Thus, with the proper choice of conditions, gravity can cause the formation of double-axis embryos in \bar{X} . laevis. Double blastopores and secondary embryonic axes due to gravitational effects have been previously reported by Pasteels¹ using an axolotl egg turned with the prospective ventral side directed upwards shortly after grey crescent formation, by Kubota²⁰ with Rana nigromaculata eggs inclined horizontally at the time of crescent formation, and by Schultze²¹, Penners and Schleip²² and Penners²³ with inverted and flattened R. fusca eggs.

Because of these results, we question Curtis' interpretation⁸ that double-axis embryos in his experiments owe their origin to the implantation of grey crescent cortex. To implant the graft, Curtis had to remove the fertilization envelope of the recipient egg, which means that the egg was no longer free to control its

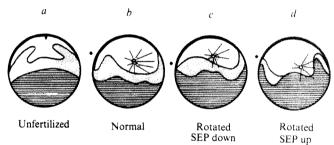


Fig. 4 Dorsal-ventral rearrangements of the cytoplasmic contents of X. laevis eggs. Eggs were fixed at various times in Bouin d'Hollande fixative, dehydrated, embedded in paraffin, sectioned at 6 µm along the prospective dorsal-ventral axis and stained with azofuchsin-aniline blue-orange G. Median sections along the prospective dorsal-ventral axis were examined for regional differences in the size and number of yolk platelets, the presence of pigment granules, aster and spindle fibres, and pronuclei. Schemes of representative sections are shown; this was necessary as only the highestquality colour reproductions preserve the regional differences seen under the microscope. Photographs of actual sections will be presented elsewhere 14. In the drawings, the region of large, closely packed vegetal platelets is indicated by cross-hatching; the intermediate size, closely packed equatorial platelets are indicated by dots, and the animal hemisphere region of small dispersed platelets and abundant cytoplasm is indicated by an open area. In all cases the egg is oriented animal hemisphere uppermost, and in the case of fertilized eggs, the SEP is at the left side of the egg, at or slightly above the equatorial level, as indicated by the asterisk. a, Drawing of an unfertilized egg, showing the radial distribution of contents. At the animal pole of the egg is the second meiotic spindle. b, A fertilized egg fixed at 0.6, well after the grey crescent became visible. The egg has been kept in the normal vertical orientation. Note the upward trail of intermediate and large yolk platelets on the prospective dorsal side of the egg. The region of the grey crescent is indicated by the raised boundary of pigment granules in the cortex. Dorsal structures will arise from this region of the egg, that is, opposite the SEP side. The sperm aster with paired pronuclei is still clearly visible. c, An egg turned horizontally with the SEP down from 0.26-0.5, fixed at 0.6. Note the trail of large and intermediate yolk on the right-hand side, occupying about the same position as in a normal (non-rotated) egg. This egg, like a non-rotated egg, would develop dorsal structures from the right side, the side opposite the SEP (asterisk). d, An egg which was turned horizontally with the SEP side upwards, starting at 0.28 and remaining until 0.5. It was subsequently fixed at 0.7. Note the trail of yolk at the left-hand side of the egg, the SEP side (asterisk). The horizontal orientation allowed gravity to displace cytoplasmic contents away from the SEP side, leaving the trail of yolk behind. The dorsal structures of the embryo will arise from the SEP side of this egg, instead of opposite the SEP.

Table 2 Secondary axis formation (twinning) by X lacous oggs perturbed by centrifugal force or gravity

•	-				
Starting time	Duration (min)	Force	Angle of inclination of egg	No. of oggs	% Embryos with double axes
(1) 0.65 (2) 0.65 (3) 0.60 (4) 0.60	.4 .4 .4 4+90	30 30 30 30+1	90°, SEP m 90°, SEP out 90°, SEP in 90°, SEP in/up	20 (1 frog) 13 (1 frog) 14 (1 frog)	70 0 6 4 0
(5) (6) (7) (8) 0.5–1.0;	Until 16-cell stage	1	45–90°, SEP up	149 (8 frogs) 159 (8 frogs) 50 (3 frogs) 26 (2 frogs)	0-9 10-29 30-49 50-70
(9) 0.5–1.0	Until 16-cell stage	1	45-90°, SEP down	34 (2 frogs)	0

Eggs were fertilized, described, and either embedded in 9% gelatin/30% modified Rmger's solution (MR)^{26,26} with the SEP towards the marked side of the dish or immersed in 6% Flooli-20% MR²⁶. The gelatin-embedded eggs (rows 1-4) were centrifuged as described in Table 1 legend at 700 r.p.m. (30g) for 4 mln, starting at 0.60 or 0.65 as indicated. During contribugation, the eggs of rows 1, 3 and 4 were oriented with the SEP side towards (SEP in) the centre of the rotor whereas the eggs of row 2 had the SEP sade away (SEP out) from the rotor centre. These orientations correspond to having the animal-vegetal axis of the egg horizontal (90°) in the centralugal field with the SEP side up or down, respectively. In the case of Ficoll-treated eggs (rows 5-9), the fertilization envelopes were removed manually using forceps, and starting in the time period 0.5-1.0 the eggs were turned SEP side uppermost (rows 5, 6, 7 and 8) or down (row 9) on an agar surface in 20% MR containing 50 µg mi⁻¹ gentamycin. Eggs were kept in this orientation until the 16- or 32-cell stage and then were transferred to hemspheric wells in agar in 20% MR-gentamycin to enhance advanced development of the embryos. The treatments were done at room temperature (19-23 °C). The number and completeness of the axes were scored at stages from late neurula to advanced tailbud when suckers were identifiable.

orientation. As shown in ref. 8, Fig. A, B, his recipient eggs were inclined 45°-90° to the side in the period of grafting or recovery, which is the orientation required for gravity to cause lateral displacements of the egg contents. We assume that eggs receiving grafts in the ventral margin were turned slightly with this side uppermost, to receive the graft; this would be the condition for twinning by gravity. Implantations in this position are the ones for which Curtis observed double-axis embryos and which led him to conclude the dorsalizing activity of the graft. The critical control for these experimental manipulations would have been a grafting of a piece of non-grey crescent cortex (for example, ventral margin cortex) into the ventral side of a recipient egg. If the source of the cortex is critical, such recipients should develop only a single axis; if unrecognized gravitational effects are responsible, the recipients should still twin. Although Curtis did not report this control, he did use several others involving grafts of grey crescent, animal pole and ventral cortex, but all were implanted in the dorsal (that is, grey crescent) side of the egg. If these eggs were turned slightly with dorsal side uppermost to receive the graft, they would not be expected to give twins, according to our results, as, in this orientation, gravity would only reinforce the normal sperm and grey crescent-dependent dorsalization of the egg. In fact, in Curtis' experiments, all implantations to the dorsal margin gave single-axis embryos. We suggest that these were not appropriate controls for the ventral implantations, all of which gave twins.

Although we cannot prove that Curtis' grafts of grey crescent cortex did not have transplantable dorsalizing activity, we question such an interpretation since we can obtain twinning without cortical transplantation, simply by exposing eggs to experimental conditions close to, if not identical, to those used by him. It seems plausible to us that the critical agent was not the cortical graft but gravity acting on an egg that was turned ventral side uppermost.

The role of the grey crescent

We suggest that the grey crescent is a manifestation of an early dorsalizing process but is not itself a transplantable dorsal determinant or source of a lasting cortical field. Although the mechanism of grey crescent formation is unknown, and may differ among various amphibia24, Lovtrup25 and Palacek et al.1 suggest that in X. laevis the crescent develops opposite the sperm entry side because a large asymmetric contraction of the egg cortex draws pigment granules of the animal hemisphere towards the sperm entry side, leaving a visibly depigmented crescent on the dorsal side. We suggest that this asymmetric contraction as a whole is an early dorsalizing mechanism important for establishing regional cytoplasmic differences along the prospective dorsal-ventral axis of the egg. The target of the contraction would not be the cortex, but deeper, movable cytoplasmic materials. Pasteels1 observed in sections of postgrey crescent eggs of R. fusca a trail of displaced vegetal yolk associated with the cortex on the dorsal side and suggested that it was pulled upwards during grey crescent formation. Klag and Ubbels²⁶ observed similar dorsal displacements in eggs of Discoglossus pictus.

Figure 4a, b shows schematic diagrams of actual sections of X. laevis eggs, in one case before fertilization when the egg contents are arranged with radial symmetry and in the other case at 0.6 when the egg has already acquired a detectable asymmetry along its dorsal-ventral axis. The displacement of yolk is discernible on the prospective dorsal side, while the ventral side has a quite different distribution of materials. Actin filaments of the cortex presumably participate in the contraction, but microtubules must also play a part since inhibitors such as colchicine and vinblastine block grey crescent formation and cytoplasmic displacements 14,18. We propose that a vectorial contraction of the cortex normally induces a rearrangement of the egg's cytoplasmic contents, forming a cytoplasmic localization necessary for the eventual induction of dorsal mesoderm.

Although in X. laevis the cortical contraction probably operates only in the time period 0.5-0.8, gravity may be able to generate a comparable dorsal-ventral asymmetry at earlier or later times, perhaps until the egg cytoskeleton or cleavage furrows interfere. Figure 4c shows a schematic section of an X. laevis egg rotated horizontally with SEP directed downwards, an orientation in which gravity reinforces normal axis formation. The cytoplasmic distribution of this egg is equivalent to that of the normal egg shown in Fig. 4b; in Fig. 4d a similar egg has been turned horizontally with the SEP uppermost, an orientation leading to axis reversal. In this case the trail of vegetal volk appears on the same side as the SEP, roughly the mirror image of the normal egg in Fig. 4b. Thus, the cytoplasmic distributions correlate with axis reversal. We have not yet sectioned eggs obtained in conditions which favour twinning, but we might expect double symmetrical trails of yolk. Cytological studies of normal, rotated and centrifuged eggs will be presented in detail elsewhere14.

Initially the sperm must in some way determine the position of the prospective dorsal-ventral axis of the egg, because the grey crescent, blastopore, and dorsal structures normally arise on the side opposite its random point of entry. Since the cortex contracts towards the side of sperm entry, the question becomes one of how the sperm orients this contraction. Soon after entering the egg, the sperm produces a large aster, organized presumably by its centriole 18,27. The astral microtubules cause a slight redistribution of cytoplasmic contents along the future dorsal-ventral axis. The antimicrotubule drugs colchicine and vinblastine block aster assembly and in their presence the cytoplasmic displacements do not occur¹⁴. Similarly, artificially activated eggs of X. laevis, lacking a functional centriole, do not have these early displacements. We suggest that this initial slight aster-driven asymmetry cues the direction of the cortical contraction, that in turn leads to the major cytoplasmic.

redistribution shown in Fig. 4b. It seems consistent that the X. laevis egg is more sensitive to the axis-orienting effects of gravity in the pre-crescent period than in the post-crescent period, since at early stages, gravity, like the sperm aster, needs only to create a small lateral displacement of cytoplasmic materials to cue the cortical contraction. In the post-grey crescent period, larger forces, afforded by centrifugation, are required to reverse the cytoplasmic distribution accomplished by the contraction and to create a new and opposite one

In summary, the important dorsal-ventral regional differences arising in the fertilized egg before first cleavage appear to reside not in the grey crescent cortex but in cytoplasmic materials reorganized normally by the sperm aster and cortical contraction, or experimentally by gravitational force.

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- 1. Pasteels, J. J. Archs Biol., Paris 49, 629-667 (1938); Folia biotheor. 3, 83-108 (1948); Adv. Morphogen, 3, 363-388 (1964)
- Brachet, J. Curr. Topics dev. Biol. 11, 133-186 (1977).
- Nieuwkoop, P. D. Wilhelm Roux's Arch. dev. Biol. 162, 341-373 (1969); 163, 298-315 (1969); Adv. Morphogen. 10, 1-39 (1973); Curr. Topics dev. Biol. 11, 115-132 (1977).
- 4. Gerhart, J. C. in Biological Regulation and Development Vol. 2 (ed. Goldberger, R.) 133-316 (Plenum, New York, 1980).
- 5. Spemann, H. Embryonic Development and Induction (Yale University Press, New Haven,
- Spemann, H. & Mangold, H. Wilhelm Roux's Arch. dev. Biol. 100, 599-638 (1924).
 Dalcq, A. & Pasteels, J. Archs Biol., Paris 48, 669-710 (1937).

- Curtis, A. S. G. J. Embryol. exp. Morph. 8, 163-173 (1960); 10, 410-422 (1962). Nieuwkoop, P. D. & Ubbels, G. A. Wilhelm Roux's Arch. dev. Biol. 169, 185-199 (1972).
- 10. Boterenbrood, E. C. & Nieuwkoop, P. D. Wilhelm Roux's Arch. dev. Biol. 173, 319-332
- Nieuwkoop, P. D. & Florschutz, P. Archs Biol., Paris 61, 113-150 (1950).
 Keller, R. E. Devl Biol. 51, 118-137 (1975).
- 13. Palaĉek, J., Ubbels, G. A. & Rzehak, K. J Embryol. exp. Morph. 45, 203-214 (1978).

While the cortex, including the grey crescent, would be part of the cellular machinery used by the egg to generate a dorsal cytoplasmic localization, it would not itself be a lasting repository of developmental information. We do not know which important materials undergo redistribution on the dorsal side of the egg, nor is it known how these materials serve in the formation of the inductively active vegetal cells of the midblastula.

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- Ubbels, G. A., Hara, K., Korster, C. H. & Kirschner, M. W. (in preparation).
 Ancel, P. & Vintemberger, P. Bull. biol. Fr. Belg. 31, 1-182 (1948); Archs Anat. microsc. Morph. exp. 38, 167-183 (1949).
 Kirschner, M. & Hara, K. Mikroskopie 36, 12-15 (1980).

- Ancel, P. & Calame, S. C. r. hebd. Séan. Acad. Sci., Paris 248, 893-895 (1959).
 Manes, M. E., Elinson, R. P. & Barbieri, F. D. Wilhelm Roux's Arch. dev. Biol. 185, 99-104
- 19. Manes, M. & Elinson, R. P. Wilhelm Roux's Arch dev. Biol. 189, 73-76 (1980).
- Kubota, T. J. Embryol. exp. Morph. 17, 331–340 (1967).
 Schultze, O. Wilhelm Roux's Arch. dev. Biol. 1, 160–204 (1894).
- Penners, A. Wilhelm Roux's Arch. dev. Biol. 116, 53-103 (1929); Z. wiss. Zool. 148, 189-220 (1936)
- Elinson, R. P. Symp. Soc. dev. Biol. 38, 217-234 (1980)
- Lovtrup, S. Wilhelm Roux's Arch. dev. Biol. 156, 204-248 (1965).
- Klag, J. J. & Ubbels, G. A. Differentiation 3, 15-20 (1975).
- Subtelny, S. & Bradt, C. J. Morph. 112, 45-60 (1963)
- 28. Kirschner, M., Gerhart, J. C., Hara, K. & Ubbels, G. A. Symp. Soc. dev. Biol. 38, 187-215
- 29. Scharf, S. R. & Gerhart, J. C. Devl Biol. 79, 181-198 (1980).

Estimates of total quantity of meteorites in the East Antarctic ice cap

Edward J. Olsen

Field Museum of Natural History, Chicago, Illinois 60605, USA

Since 1969 ~5,000 meteorite fragments have been recovered from two regions, the Yamato Mountains and Victorialand, on opposite sides of the East Antarctic ice cap^{1,2}. Based on a steady-state model for the ice cap3, and current estimates of meteorite influx4, a model is developed here which predicts that the steady-state number of meteorites being carried in and on the ice is at least 760,000. Most of these are being carried within the ice and are only exposed at peripheral regions by a combination of wind ablation and blockage of ice movement by protruding mountain barriers. The large steady-state population of meteorites does not require unusual conditions of influx. It is solely the cold, dry climate which preserves virtually all meteorites that fall except for the fragile, porous carbonaceous chondrites. The same model applied to the Greenland ice cap indicates a steady-state population of ~61,000 meteorites.

The East Antarctic ice cap has several properties that permit some simplifications in developing a model for it, without loss of generality. The area is roughly elliptical with major and minor diameters of ~4,000 km and 3,000 km, respectively. It has an area of $9.4 \times 10^6 \, \mathrm{km^2}$ and a single major centre of ice flow. The calculated circumference is 1.11×10^4 km. For the purpose of this model, the calculation is simplified if an equivalent circle is used with the same area and almost identical circumference. The equivalent circular continent would have a radius of 1,730 km (area $9.4 \times 10^6 \text{ km}^2$, circumference = $1.09 \times 10^4 \text{ km}$).

Meteorites falling randomly over the ice cap are carried in and on the ice radially coastwards. Initially, we consider that there is no blockage of movement and each meteorite reaches the coast where it is lost by calving of the ice into the sea. After some time a steady-state population, Q, of meteorites will be reached in the ice cap. A simple relationship can be derived, Q = sF/2v, where F is the influx rate (meteorites yr^{-1} for the total area, $9.4 \times$ 10^6 km^2), s is the distance travelled (m), and v is the mean velocity of travel (m yr⁻¹). The time for the steady state to be established is T(yr) = s/v. Based on a steady-state ice cap profile, as well as actual measurements in the field, the velocity near the coast is higher than in the interior.

Nagata² has developed a model for the longitudinal profile of a steady-state ice cap. In it he derives analytical expressions relating v to s and a parameter, b, the accumulation rate of ice expressed in m yr⁻¹ of water equivalent.

To determine how v varies with distance from the centre to the edge some estimates of b are needed. For East Antarctica Paterson⁵ states that accumulation ranges from $b = 0.03 \text{ m yr}^{-1}$ in the central part to $\sim 0.60 \text{ m yr}^{-1}$ near the coast. Measured velocities range from $\leq 1 \text{ m yr}^{-1}$ in the central part to over 300 m yr⁻¹ near the coast^{5,6}. These observations were used in conjunction with the Nagata model to model the velocity from the centre to the coast. A tabulation of velocities was prepared but no attempt was made to fit them by an analytical expression.

Hughes⁴ has summarized data on meteorite influx rates. The value of F to be used in the calculation of Q depends on the mass of the smallest meteorite to be considered. If dust grains are considered then Q would be very large; if ton-size meteorites are considered then Q would be very small. In Antarctica. within an area of concentration, specimens as small as 0.1-0.87 g have been collected^{7,8}. These, however, are clearly fragments from larger masses that have suffered extensive fragmentation. Thus, an estimate of the fragmentation factor is needed.

Based on the proportion of irons and pallasites to stone meteorites in the Yamato recovery region, relative to worldwide observed falls, Nagata² estimates a factor of 3. The same analysis applied to the Victorialand finds gives a value of 5. King et al. have attempted to pair many of the Victorialand specimens

based on field occurrence, macroscopic and microscopic appearances. Their suggested pairings indicate a factor between 3 and 4. Thus, a factor of 4 ± 1 seems to be indicated, which seems remarkably low. Antarctic meteorites suffer four fragmentation processes: (1) normal break-up during atmospheric passage (Hellyer¹⁰ states that about half of observed falls are multiply fragmented); (2) fragmentation on impact on ice; (3) crushing while being carried within the ice during transport; (4) frost-breakage while exposed in concentration areas near the ice periphery (dark rocks are warmed above the freezing point of water during summer months, allowing moisture within them to melt and then refreeze during the dark winter months). A better method to determine fragmentation would be to compare the distribution of recovered masses with the masses of observed meteorite falls. The data on the recoveries in Victorialand through 1979 have been tabulated by Score et al.". The distribution of masses is highly skewed. The mean mass is 1,910 g. while the median is 235 g and the mode is 15 g. Hughes¹¹ has plotted the distribution of recovered masses among 755 observed falls. The histogram shows a mode at 3 kg. If we assume that the mode of observed falls is a good measure of the highest frequency of meteorite masses that survive entry, then comparison of the mode of the Victorialand finds, 15 g, with this 3 kg figure indicates the most frequent fragmentation factor is 200. This seems to be a more reasonable number. Although iron meteorites clearly fragment less readily than stone meteorites, data on observed falls indicate that over 91% are stones. Thus, based on data for observed falls, the most frequent mass surviving entry is 3 kg. In Antarctica the most frequent size of fragment is 15 g. A 15-g meteorite is readily observed on the ice in Antarctic field conditions.

Hughes4 has fitted an equation to the observed data on recovered masses (non-Antarctic) for the mass range ≥16 kg. Extrapolation of this equation to 3 kg gives a worldwide influx rate of 3,500 meteorites yr⁻¹. This number is in good agreement with an estimate of 3,300 based on Hughes' analysis of data presented by Brown¹² and tabulated in ref. 4. Both Halliday and Hughes (personal communications) agree this is the most reasonable figure to use based on current data. In addition, Halliday¹³ has determined a latitude effect by analysis of meteorite orbits. This effect depends on the initial velocity of a meteorite encountering the Earth. Those with a velocity of 15 km s⁻¹ will be $\sim 20\%$ less frequent at high latitudes (70°-90°, N or S) relative to equatorial latitudes; those with a velocity of 25 km s⁻¹ will be 40-50% less frequent at high latitudes. ReVelle¹⁴ has developed a model for the mass-loss (ablation) of chondrites falling through the Earth's atmosphere. From it one can conclude that most meteorites that survive entry are the slower ones4. Thus, for Antarctica a reduction of 20% will be used for the latitude effect. The value of F for East Antarctica is then 48.6 meteorites of 3 kg mass or larger per year.

Tabulated mean values of v were used in each of 10 concentric subregions, of equal area, from the centre to the edge of the continent, and the respective values of Q were determined and summed. The steady-state quantity of meteorites is $Q = 529,000 \ (1.06 \times 10^8 \ \text{fragments})$. The time required for the steady state to be established is $22,000 \ \text{yr}$, which is short compared with the age of the ice cap¹⁵. In these computations it was assumed that effects due to ice surging movement average out over this time span.

These computations assume that no blockage or retardation of ice occurs. In reality $\sim 47\%$ of the East Antarctic perimeter consists of protruding mountains that cause significant retardation of ice movement. In the vicinity of the Yamato Mountains horizontal velocities of 1 to 0.5 m yr⁻¹ have been measured². Assuming 1 m yr⁻¹ in the vicinities of these barriers, and 47% of the perimeter affected, the effect is to increase the value of Q to 760,000 meteorites $(1.5 \times 10^4 \text{ fragments})$.

The computations, so far, have assumed that each meteorite lands on the ice and is carried, either in it or on it, in a straight line path to the perimeter. Clearly, this cannot be the case as ice-buried topographic highs, subsidiary flow centres on the ice

(such as the Fuji Divide near, and upstream from the Yamato Mountains), and local variations in accumulation rate cause the ice to move along on less-than-direct lines. For any path that meanders from the direct path the effect is to increase the value of Q. There is no way, quantitatively, to assess the magnitude of this effect over the whole ice cap. Thus, the value of Q determined above is a minimum.

Finally, one additional process can affect the value of Q. It has been assumed so far that v is not a function of depth within the ice at which a meteorite is carried. Clearly, as accumulation takes place over time, any meteorite that fell in an earlier time will be buried as it is carried along. Variation of velocity with depth is not believed to be a large factor. Velocity is considered almost constant from the ice surface down to about half of full depth. What happens below half depth, especially near the bottom, is not thoroughly understood and several possibilities exist. In terms of Nagata's model this factor does not seem to affect Q significantly.

Considering all the assumptions and simplifications in the foregoing model it can be concluded that the East Antarctic ice cap contains, at any given time, the order of 760,000 individual meteorites, in the mass range >3 kg, and comprising $\sim 1.5 \times 10^8$ fragments. If these numbers are applied to the Yamato Mountains, and if the velocity near the Yamato barrier (~ 40 km wide) is taken to be 1 m yr⁻¹, the ice field in front of the mountains would have a steady-state population of about 3,000 individual meteorites. This does not take into consideration the lateral convergence effect described by Nagata², which would increase this number. Even at that, this estimate is of the order of magnitude estimated by Yanai¹⁸ of < 8,000 for this region.

The mechanism by which unusual concentration fields occur, as proposed by Nagata², has permitted large numbers of meteorite fragments to be recovered within relatively small areas. On the other hand, the work of Cassidy and colleagues in southern Victorial and 1,19 shows that concentration fields are not the only way Antarctic meteorites will be recovered. Several meteorites have been encountered that were clearly moving along alone. A prime example of this is the Mt Baldr meteorite (two main fragments with missing fragments) that was found on the surface of a segment of ice cap within a few kilometres of, and moving towards, a major ice-fall onto Wright Upper Glacier at the head of one of the Dry Valleys; no barrier was present. While lateral concentration helps in recovering large numbers of specimens, the key factor in Antarcrica is the low rate of weathering at low temperatures, which preserves most of the meteorites that land on the ice cap. This is borne out by the terrestrial ages of Antarctic meteorites which are, on the average, significantly older than finds from lower latitudes²⁰ Because of the general condition of ablation by wind in peripheral regions of the ice cap (an integral part of the Nagata steady-state profile model³) meteorites carried within the ice have a high probability of being exposed in these regions. This means that bare blue ice, free from snow cover in peripheral regions, can yield meteorites with or without lateral concentration. Thus far, all meteorites found in East Antarctica, including the first finds in earlier decades of this century, have been recovered from peripheral regions.

Antarctica, however, cannot be considered a place where every meteorite is preserved. Because of the great detail in which Japanese field teams have studied the Yamato Mountains concentration area, data on the distribution of meteorite types there are quite good. Note that carbonaceous chondrites are under-represented, relative to worldwide recovery ratios, by a factor of three². This indicates that their low mechanical strength, plus their high porosity causes them to be preferentially destroyed by a combination of crushing and weathering when carried within the ice.

If this model is applied to the Greenland ice cap (without consideration of any coastal barriers) it suggests that \sim 61,000 meteorites (1.2×10⁷ fragments) are in steady-state residence. Unfortunately, most of the Greenland ice cap is snow covered. Meteorological conditions are different from those in Antarctica

because of its subpolar position. Only a couple of relatively snow-free regions in north-east Greenland seem suitable for potential meteorite recoveries.

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- 1. Cassidy, W. A., Olsen, E. & Yanai, K. Science 198, 727-731 (1977).
- Nagata, T. Proc. 2nd Symp. Yamato Meteorites 70-92 (1978). Nagata, T. Antarctic Rec. No. 60, 13-27 (1977).
- 4. Hughes, D. W. in Solid Particles in the Sclar System (eds Halliday, I. & McIntosh, B. A.) 207-210 (International Astrophysical Union, 1980).
- Paterson, W. S. B. in Dynamics of Snow and Ice Masses (ed. Colbeck, S.C.) 1-78 (Academic, New York, 1980).
- Gow, A. J. in Antarctica (ed. Hatherton, T.) 221-258 (Methuen, London, 1965).
- Yanai, K. Catalog of Yamato Meteorites, 1-188 (National Institute of Polar Research, Tokyo, 1979)
- Score, R. et al. Antarctic Met. Newslett. 4, 1–144 (1981). King, T. V. V., Score, R. Gabel, E. M. & Mason, B. Smithsonian Contr. Earth Sci. No. 23,
- Hellyer, B. Earth planet. Sci. Lett. 7, 148–150 (1969).
 Hughes, D. W. Meteoritics (submitted).
 Brown, H. J. geophys. Res. 66, 1316–17 (1961).
 Halliday, I. Meteoritics 2, 271–278 (1964).

- 14. ReVelle, D. O. Nat. Res. Council Canade, Planet. Sci. SR-76-1 (1976).
- Shackleton, J. J. & Kennett, J. P. Init. Rev. DSDP Leg 29 (1965).
 Raymond, C. F. in Dynamics of Snow and Ice Masses (ed. Colbeck, S. C.) 80-114 (Academic, New York, 1980).
- Proc. int. glaciol. Soc. (1979). Yanai, K. Mem. Nat. Inst. Polar Res. Spec. Iss. 8, 1-37 (1978)
- Lasaid, K. *Intern. Val. tras. Folia Nes.* Spec. 188. 0, 1-27 (17-6).
 Cassidy, W. A. Smithsonian Contr. Earth Sci. No. 23, 3-7 (1980).
 Evans, J. C. & Rancitelli, L. A. Smithsonian Contr. Earth Sci. No. 23, 45-46 (1980).

Measurements of diamond lattice displacement by platelet defects with electron microscopic moiré patterns

L. A. Bursill*§, J. L. Hutchison†, N. Sumida‡ & A. R. Lang‡

- * Department of Physical Chemistry, Lensfield Road, Cambridge CB2 1EP, UK
- † Department of Metallurgy and Science of Materials, Parks Road, Oxford OX1 3PH, UK
- ‡ H. H. Wills Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL, UK

Among the diverse lattice defects occurring in natural diamonds, none has engendered so much controversy as the 'platelets', those planar defects which lie on {100} planes of the diamond matrix and whose diameters commonly lie in the range 10-100 nm. For many years, the only evidence for the existence of platelets was indirect, residing in the reported anomalous 'spike' diffuse X-ray reflections1, but it was eventually accepted that these diffuse reflections could not be due to thermal vibrations but arose from static lattice disorder2. Guinier3 and Frank4 independently pointed out that the defects responsible for the 'spike' reflections must be plate-like, lying in {100}. Direct proof of the existence of platelets came with the transmission electron microscope (TEM) observations of Evans and Phaal⁵, and it has been shown that the platelet structure is extrinsic, that is, that it forces apart the diamond matrix on either side of the platelet°. We now report a new and more reliable way of measuring the magnitude of matrix displacement produced by platelets.

Moiré techniques are widely used for measuring small displacements of one periodic object relative to another of similar periodicity. If the two periodic objects are crystal plates superposed on each other in nearly parallel orientation and simultaneously diffracting from the same Bragg planes, then relative displacement of the two plates can be measured to a small fraction of a nanometre, whether electrons or X rays are being diffracted⁷⁻⁹. When studying atomic-scale displacements due to lattice defects, interpretation can be straightforward if a defectfree comparison crystal (1) is superimposed on the defectcontaining specimen crystal (2). In the case of dislocations in (2) outcropping at the interface between (1) and (2), the number of moiré fringes terminating at a dislocation outcrop is $\mathbf{g} \cdot \mathbf{b}$, where g is the reciprocal lattice vector of the active Bragg reflection and b is the Burgers vector of the dislocation^{8.9}. Furthermore, if crystal (2) contains a planar defect such as a stacking fault which involves a relative translation, f, between crystal matrices on either side of the defect, and the defect outcrops at the interface between (1) and (2), then the moiré fringe pattern will appear faulted at the defect outcrop, the fringe displacement at the outcrop being g · f fringe periods.

In our TEM studies of platelet defects in diamonds we prepared thin specimens parallel to (110). We then viewed platelets parallel to (001) edge-on and those parallel to (100) and (010) obliquely, when the electron beam is normal to the specimen surface (Fig. 5 of ref. 10 explains this geometry). The specimens are produced by sawing, mechanically polishing and ion-beam thinning. In one such specimen, cracking had occurred on the cleavage planes $(\bar{1}11)$ and $(\bar{1}1\bar{1})$ normal to the specimen plate, and on one of the cleavage planes inclined at 35.25° to its surface. Following cracking, small overthrusts had taken place, producing narrow strips of overlap in which the specimen laminae above and below were slightly rotated with respect to each other. The moiré patterns appearing in the overlap regions were usually complicated due to warping of the overlapping laminae and the mutual proximity of the platelets. However, one strip of overlap in which the moiré pattern can be straightforwardly interpreted is shown in Figs 1 and 2. This region was first observed in the high-resolution JEOL 200CX microscope at Oxford, operating at 200 kV. The set of two-beam diffraction contrast patterns reproduced here were photographed in Bristol with a Philips 400 microscope operating at 120 kV. (The corresponding electron diffraction patterns were also recorded.) The important reflections 004 and 220 were photographed by dark-field (DF) as well as by bright-field (BF) technique. In the area shown, each lamina is ~33 nm thick. The vertically oriented boundaries of the overlap strip are the cleavage planes ($\overline{1}11$). The boundary sloping up towards the right at the top of Figs 1 and 2 is parallel to $(\overline{1}1\overline{1})$. Except where the platelet strain fields influence the pattern, the moiré fringes are almost parallel and equispaced, and they lie parallel to the g vector of the Bragg reflection active. The basic pattern is thus a pure rotation moiré, the rotation measured from it being ~7 mrad.

The field in the Figs 1 and 2 contains nine platelets, four of which lie parallel to (001) and are seen edge-on giving sharp traces rotated 35° clockwise from the horizontal. The platelets in this specimen (as in many other specimens) are elongated along one of the (110) directions in the platelet plane; they tend to be lath-like, with their long pair of edges lying straight and parallel to the $\langle 110 \rangle$ direction of elongation. In a field of $\sim 1 \,\mu\text{m}^2$ surrounding the region exhibited here, roughly half of the \sim 20 platelets lying on (100) and (010) appear to outcrop on both top and bottom surfaces of the lamina containing them. This shows that their lengths parallel to the lath axis exceed 66 nm, twice the lamina thickness, as these lath axes make 60° with [110], the normal to the specimen lamina. Consequently, there is a good chance that platelets on (001) having their long axis parallel to [110] are seen with their two [110]-direction edges running straight from top to bottom of the lamina, when the direction of view of the specimen is close to [110]. The images of their edges look exactly like the images of pure edge dislocations viewed parallel to the dislocation line (as indeed they should). A platelet

[§] Present address: School of Physics, University of Melbourne, Parkville 3052, Victoria,

To whom correspondence should be addressed

image with such properties is exhibited by one of the two (001) platelets partly covered by the overlap—the platelet in the centre of the field. It can be distinctly recognized both within the overlap region (by virtue of the clear-cut displacement of the moiré fringes) and in the single lamina region to the right of the overlap; it is best seen in Fig. 1b. (The lamina on the right thus corresponds to the defect-containing crystal (2), whereas that on the left is the comparison crystal (1).)

The lower platelet in the overlap region does not give a clearly defined displacement to the moiré fringes, the confusion in its fringe pattern being especially noticeable in the higher order reflection 004. We conclude that this platelet either does not outcrop at all at the interface between (1) and (2), or that the outcrop length is small relative to the platelet diameter. The perturbation to the fringe pattern arises because electrons pass through crystal that is locally tilted due to the dislocation-type strain field at the platelet periphery.

The conditions to be satisfied so that lattice displacement can be precisely derived from the moiré fringe displacement at the image of the planar defect are that both the crystal thickness and the crystal orientation are the same on either side of the plane of the defect. The former condition certainly holds in the present

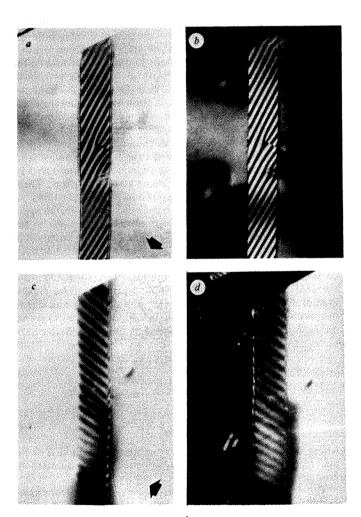


Fig. 1 Bright field and dark field electron micrographs of a platelet-containing Type Ia diamond in which moiré fringes appear where cleavage and overthrust have led to superposition of crystal laminae. The field width is 0.3 μ m, the width of overlap 66 nm. The trace of ($\bar{1}11$) is vertical. The micrograph print orientation reproduces a view of the image as seen on the fluorescent screen of the microscope; the specimen axis [110] points towards the observer, the electron beam direction is [$\bar{1}10$]. Arrowheads indicate the direction of the active g vectors. a, 004 reflection, BF; b, 004 reflection, DF; c, $\bar{2}20$ reflection, BF; d, $\bar{2}20$ reflection, DF.

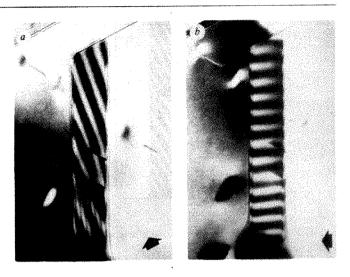


Fig. 2 Same field as in Fig. 1. Bright field images of: a, 111 reflection.

experiments. The latter condition applies when the platelet outcrop at the interface between crystals (1) and (2) is sufficiently extended, and is satisfied in the case of the upper platelet. In the 004 BF (Fig. 1a) and DF (Fig. 1b) images, a circuit enclosing the left-hand edge of the upper platelet shows the number of extra fringes to be slightly less than 15: our Lest estimate lies between 1.40 and 1.45. Figure 3 shows the way the fringes can be counted. For example, one may take a circuit up the right-hand margin of the overlap from fringes 3' to 1', then down along fringe 1'-1 to the left-hand margin where one proceeds to fringe 3, and then finally up along fringe 3 to the discontinuity at the platelet. The whole extra fringe is designated X; the fraction in excess of unity is assessed from the relative spacings at the platelet between fringes 2 and 3', and 3' and 3. The 220 BF (Fig. 1c) and DF (Fig. 1d) fringe patterns show no detectable fringe faulting due to the platelets.

Although our moiré experiments have only included Bragg reflections from planes containing [110], and are thus insensitive to translations parallel to [110], it is reasonable to take Fig. 1c and d as direct evidence confirming that the matrix displacements produced by the platelet structure are perpendicular to the platelet plane. Taking $\mathbf{g} \cdot \mathbf{f}$ for the 004 reflection to be bracketed between the values 1.40 and 1.45, and \mathbf{f} parallel to [001], gives a bracket for the magnitude of \mathbf{f} between 0.35 a_0 and 0.362 a_0 , a_0 being the face-centred cubic lattice parameter of diamond, 0.357 nm.

Although Fig. 2a and b do not provide very accurate values of the displacement, their fringe shifts are quite consistent with the displacement value obtained from Fig. La and b. Note in Fig. La and b that the fringes are deviated as they approach the middle regions of the platelet. This is predictable from the strain field of the two parallel edge dislocations which are coincident with the platelet edges, and whose Burgers vector equals the platelet displacement. The calculated change in orientation of fringes 2 and 3 relative to the average fringe direction in the overlap region is $\sim 7.5^{\circ}$. Measurement, which can only be approximate, gives between 8° and 9°, in as good agreement as can be expected. The lower part of Fig. 2a shows a second fringe system. The orientation and spacing of these fringes, together with the diffraction pattern photographs, identify them as due to the 224 reflection. The strong excitation of the 224 reflection in this region is probably a consequence of specimen bending due to local contamination. In most of the micrographs one can see fine fringes parallel to the vertical boundaries of the overlap region. These arise partly from defocus, partly from refraction at the cleavage edge and partly from a contamination layer.

What is the nature of platelets? They are known to occur in association with nitrogen impurity in the commonest natural

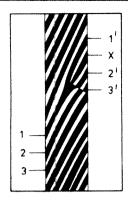


Fig. 3 Diagrammatic representation of the central part of Figs 1 and 2 showing the moiré fringe configuration as seen in Fig. 1a or b, and the numbering of fringes adopted for measuring the fringe displacement at the platelet.

diamonds (designated Type 1a). In these diamonds nitrogen is present in several non-paramagnetic aggregations, each of which has characteristic optical absorption spectra (see ref. 11 for details on optical properties and impurities in diamond). Platelets are absent in the common synthetic and the very rare natural Type 1b diamond in which nitrogen occurs in the singly substituted atomic state. Platelets are also absent in Type II diamonds, which are sufficiently pure to be free of nitrogendependent optical absorptions. The simplest interpretation of evidence from X-ray diffraction 'spikes' is that the lattice displacement due to platelets is close to 1/3 a_0 normal to the platelet plane. However, considerable complications attend the performance and interpretation of the X-ray experiments¹². A platelet structure has been proposed¹³ in which pairs of nitrogen atoms replace single carbon atoms of one of the sub-lattices of the diamond structure and form a single sheet of di-nitrogens parallel to a cube plane, the N-N bonds being perpendicular to the sheet. This model displaces the diamond matrix by about 1/3 a_0 perpendicular to the platelet plane, in accord with the X-ray evidence. Although non-nitrogen-containing platelet structures have been proposed 14,15, a platelet structure is now favoured in which nitrogen is at least an important constituent 16,17. There is scope for platelet-structure model-making, and it is important to have a strictly accurate value for the lattice displacement produced by the platelet structure. As more moiré data become available, it should be possible to narrow the uncertainty in displacement magnitude. As it stands, the present experiment provides the best and most direct measurement obtained to date.

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Raman, C. V. & Nilakantan, P. Proc. Ind. Acad. Sci. A11, 389-397 (1940).
 Lonsdale, K. Proc. R. Soc. A179, 315-320 (1942).
 Guinier, A. C. r. Acad. Sci., Paris 215, 114-115 (1942).
 Frank, F. C. Proc. R. Soc. A237, 168-174 (1956).

Frank, F. C. Proc. R. Soc. A237, 168-174 (1956). Evans, T. & Phaal, C. Proc. R. Soc. A276, 535-552 (1962). Evans, T. & James, P. F. Phil. Mag. 11, 113-129 (1965). Mitsuishi, T., Nagasaki, H. & Uyeda, R. Proc. Jap. Acad. 27, 86-87 (1951). Pashley, D. W., Menter, J. W. & Bassett, G. A. Nature 179, 752-755 (1957). Lang, A. R. Nature 220, 652-657 (1968). Lang, A. R. J. Crystal Growth 42, 625-631 (1977). Wellow, I. Pan. Page 1992 (2) 1652-1659 (1979).

Lang, A. R. J. Crystal Growth 42, 625-631 (1977).
 Walker, J. Rep. Prog. Phys. 42, 1605-1659 (1979).
 Lang, A. R. in The Properties of Diamond (ed. Field, J.) 425-469 (Academic, London, 1979).

13. Lang, A. R., Proc. phys. Soc. Lond. 84, 871-876 (1964).

14. Evans, T. Diamond Research, 2-5 (Incustrial Diamond Information Bureau, London, 1973)

17. Evans, T., Qi, Z. & Maguire, J. J. Phys. C14, L379-L384 (1981).

Woods, G. S. Phil. Mag. 34, 993-1012 (1976). Allen, B. P. & Evans, T. Proc. R. Soc. A375, 93-104 (1981)

Drag reduction in fibre suspension

W. D. McComb & K. T. J. Chan

School of Engineering, University of Edinburgh, Edinburgh EH9 3JL, UK

Spectacular reductions in turbulent friction can be obtained by adding small amounts of either long-chain polymers^{1,2}, or macroscopic fibres3,4 to a fluid. The polymer effect has been the more studied of the two. But one of the continuing puzzles is that measurements of the turbulent energy spectrum show no evidence of a direct eddy-polymer interaction, despite the fact that the influence of the polymers on the turbulence is so profound. Although some increase in energy may be observed at small wavenumbers (attributable to a decrease in the dissipation rate), inertial and dissipation range spectra in polymer solutions are unchanged from the newtonian result. In contrast with this situation, we have recently found very marked changes in energy spectra measured in fibre suspensions.

Although there are many similarities between the effects of the two kinds of additive, they are thought to involve different mechanisms. For example, use of polymers and fibres together can produce much larger reductions in drag than would be the case with either additive on its own⁵. We have previously reported measurements using laser-Doppler anemometry (LDA) which indicate that the turbulent structure in dragreducing fibre suspensions is not the same as that in polymer solutions⁶ (and both, of course, differ from newtonian fluids). Furthermore, as the fibres were degraded by repeated passage through the apparatus, we observed an apparent transition from 'fibre-like' to 'polymer-like' drag reduction, as measured by mean and r.m.s. velocity distributions across the pipe⁶. These remarks apply only to typical drag-reducing concentrations. At higher polymer concentrations, one finds changes in the spectrum at large wavenumbers7. But these seem to be due to the onset of continuum viscoelasticity and are not per se an aspect of drag reduction).

The fibres used in our experiments were chrysotile asbestos, dispersed in a 0.5% aqueous solution of Aerosol OT, at a nominal concentration of 300 p.p.m. by weight. The experimental details are given elsewhere^{6,7} and the LDA and signalprocessing arrangements were as described in ref. 8. The onedimensional spectrum E(k), where k is the wavenumber, and

$$\bar{u}^2 = \int_0^\infty E(k) \, \mathrm{d}k$$

was put in dimensionless form using the relationship

$$\tilde{E} = \frac{E(k)}{2\bar{u}^2} R$$

where \bar{u}^2 is the mean-square fluctuating velocity and R is the pipe radius. The wavenumber was made dimensionless by multiplying by R.

The LDA relies on the scattering of laser light from small particles in the flow. Before discussing our results for energy spectra, we should consider the effect of light scattered from the suspended asbestos fibres (on the LDA signal). Our normal practice (for example, when working with polymer solutions) is to add scattering particles by seeding the flow with milk at a concentration of 100 w.p.p.m. The average size of the particles of fat in milk is $\sim 0.3 \,\mu\text{m}$ and this is small enough to follow the highest frequency turbulent fluctuations. Chrysotile asbestos occurs naturally in the form of macro fibres which are made up from basic fibrils with a mean diameter of ~40 nm (refs 9, 10). These macro fibres readily break down and in the specially prepared suspensions used here, the basic fibrils are preserved

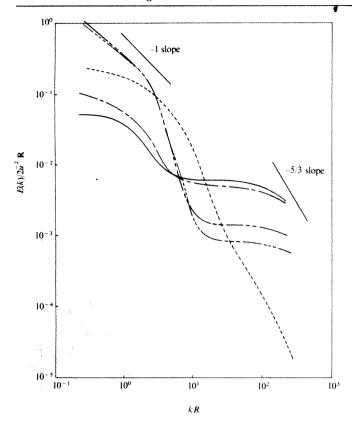


Fig. 1 One-dimensional turbulent energy spectra in a 300 p.p.m. asbestos fibre suspension at various levels of drag reduction, for $Re = 1.4 \times 10^4$: all spectra were measured at the centreline of the pipe. — Run 1, DR = 69%; ——— run 2, DR = 76%; ——— run 3, DR = 56%; ———— run 4, DR = 55%; ——— results for water.

with a very high aspect ratio⁹. It is difficult to measure the length of such fibrils directly but, although there is some disagreement between statistical methods, it is clear that the mean length-to-diameter ratio of individual fibrils in an undegraded suspension is in the range 10³-10⁴. Thus a contribution to the scattered light from individual fibrils might bias the LDA signal towards the lower turbulent frequencies. To assess this possibility, extensive tests were carried using various concentrations of milk, the surfactant and the asbestos fibres. In particular, we studied oscillographs of the 'burst' signals and spectra of the Doppler photocurrent. Details may be found in ref. 7 but we should mention two important conclusions here. First the Aerosol OT (presumably due to the naturally occurring micelles) was a satisfactory seeding material and milk was not needed. Second,

Table 1 Values of the eddy-fibre interaction length scale $l_{\rm m}$ as estimated from one-dimensional energy spectra

·				
Re	DR(%)	$I_{\mathbf{m}}$ (mm)	$l_{\rm m}/D^*\times10^{-4}$	
9.0×10^{3}	44	0.89	2.3	
1.4×10^4	69	2.1	5.3	
	58	1.4	3.5	
	46	0.9	2.3	
	38	0.84	2.1	
1.4×10^4	76	2.4	6.0	
	56	1.4	3.5	
	55	1.2	3.0	
3.2×10^4	71	1.6	4.0	
	62	1.2	3.0	
	58	0.95	2.4	
5.3×10^4	54	1.3	3.25	
	52	1.2	3.0	

D = 40 nm.

the fibrils did not contribute to the a.c. part of the LDA signal (that is, the part that gives the fluid velocity) but only to the d.c. or pedestal level (which determines the signal-to-noise ratio). From these tests we chose a nominal fibre concentration of 300 w.p.p.m. as giving large drag reductions with satisfactory signal-to-noise ratio for the LDA.

Some results for measurements at the pipe centreline are shown in Fig. 1, for several values of drag reduction (DR) and at one Reynolds number, $Re = 1.4 \times 10^4$. In each case, an experimental 'run' consisted of one pass of the whole amount of working fluid through the apparatus at a fixed Reynolds number. Further 'runs' were further passes of the same fluid at the same Reynolds number. The Reynolds number was calculated from the pipe diameter, the bulk mean velocity and the kinematic viscosity of water.

Figure 1 shows that spectra measured in the fibre suspension have a curious 'kinked' appearance; being reduced below the newtonian spectrum level at an intermediate range of wavenumbers (roughly corresponding to the inertial range). The position and extent of the 'reduced level' region was found to depend on the amount of drag reduction. This suggested that we were observing an interaction between the turbulent eddies in a particular wavenumber (or eddy-size) range and the suspended fibrils.

To test this idea the 'reduced level' region was characterized by an interaction wavenumber $k_{\rm m}$ which was calculated by noting the two wavenumbers marking the beginning and end of this region, and taking the arithmetic mean. Hence we obtained a mean interaction length scale $l_{\rm m}=k_{\rm m}^{-1}$. Values of $l_{\rm m}$ are given in Table 1 for various levels of drag reduction (DR) and at four different Reynolds numbers. These values range from $l_{\rm m}=0.84$ mm (corresponding to DR = 38%) to $l_{\rm m}=2.4$ mm (corresponding to DR = 76%). Clearly we should consider what relationship (if any) exists between: (1) $l_{\rm m}$ and some length scale associated with the individual fibrils; and (2) the amount of drag reduction and $l_{\rm m}$.

In considering the first point, note that $l_{\rm m}$ is a dynamic measurement of the length scale associated with fibrils actually interacting with the turbulent eddies. Thus there must be imponderable factors in a comparison with the length of fibrils

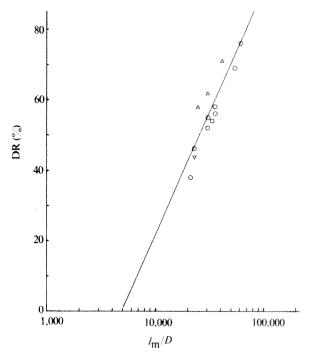


Fig. 2 Variation of percentage drag reduction (DR) with interaction length scale $(l_{\rm m})$ in a 300 p.p.m. asbestos fibre suspension. ∇ , $Re = 0.9 \times 10^4$; \bigcirc , $Re = 1.4 \times 10^4$; \triangle , $Re = 3.2 \times 10^4$; \square , $Re = 5.3 \times 10^4$.

either in a static suspension or (worse) evaporated out from such a suspension. Furthermore, there are considerable uncertainies in performing such measurements. Also the idea that the eddy interaction is with a mesh of entangled fibrils11 introduces the possibility of a length scale which is larger than any individual fibril.

Nevertheless, we may expect l_m to be related to the largest fibril lengths present in any appreciable fraction and measurements of length distribution in a similar suspension suggest a mean value of 1.4 mm for the longest fibrils 10. Thus we can reasonably claim that l_m is the correct order of magnitude for there to be a physical relationship to individual fibril length.

The second point was investigated by plotting values of drag reduction against the corresponding values of l_m . As can be seen from Fig. 2, the data points cluster quite well about a straight line. The variation with Reynolds number was not statistically significant and the single straight line was fitted using the least-squares method. The resulting relationship was found to be:

$$DR \times 100\% = \{0.70 \log_{10}(l_m/D) - 2.60\} \times 100\%$$

Finally, we previously remarked⁶ that the qualitative changes of turbulent structure in undegraded fibre suspensions agreed with a previous theoretical prediction¹². Note that the present results for spectra apparently support that theoretical picture. We seem to be observing a form of resonance phenomenon where the fibres are strongly excited by those turbulent eddies with spatial frequency roughly equal to the inverse of the fibril length. Thus, for once, a simple mechanistic model may well be relevant to the underlying physics of this intriguing effect.

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- Virk. P. S. A.I. Ch. F. I. 21, 625 (1975)
- Hoyt, J. W. Proc. 2nd int. Conf. on Drag Reduction Paper A1, Cambridge (BHRA, 1977).
- Ellis, H. D. Nature 226, 352 (1970).
- Hoyt, J. W. Naval Undersea Center Rep. TP 299, San Diego (1972)
- Lee, W. K., Vaseleski, R. C. & Metzner, A. B. A.I. Ch. E. J. 20, 128 (1974). McComb, W. D. & Chan, K. T. J. Nature 280, 45 (1979).

- McComb, W. D. & Chan, K. T. J. Nature 280, 45 (1979).

 Chan, K. T. J. thesis, Edinburgh Univ. (1980).

 McComb, W. D., Allan, J. & Greated, C. A. Phys. Fluids 20, 873 (1977).

 Atkinson, A. W., Gettins, R. B. & Rickards, A. L. Nature 226, 937 (1970).

 Thew, M. T. & Anaad, J. S. Proc. 1st int. Conf. on Drag Reduction Paper D2, Cambridge (BHRA, 1974)
- Moyls, A. L. & Sabersky, R. H. Int. J. Heat Mass Transfer 21, 7 (1978).
 McComb, W. D. Nature phys. Sci. 241, 117 (1973).

²¹⁰Pb in surface air at Enewetak and the Asian dust flux to the Pacific

Karl K. Turekian & J. Kirk Cochran*

Department of Geology and Geophysics, Yale University, Box 6666, New Haven, Connecticut 06511, USA

As part of the SEAREX programme, an air filter system and a precipitation collector were deployed at Enewetak (11°20' N, 162°20' E). A description of the air filter system and initial results on the chemical composition of the collected aerosols¹ and on the organic components² have been reported elsewhere. As part of the same programme we have measured 210Pb (and ²¹⁰Po) in air filter and integrated monthly precipitation samples during 1979 to estimate the ²¹⁰Pb collected Aux $(0.15\pm0.02~d.p.m.~cm^{-2}~yr^{-1})$ and the Asian $(38\pm20~\mu g~cm^{-2}~yr^{-1})$ at this location in the Pacific. dust flux

²¹⁰Pb (22-yr half life) is a radioactive nuclide produced in the air by decay of gaseous ²²²Rn (3.8-day half life) emanating from continental soils. As ²¹⁰Pb is produced, it is scavenged by precipitation and its mean life with respect to removal from the atmosphere is \sim 5 days (see ref. 3 for a summary). If the primary

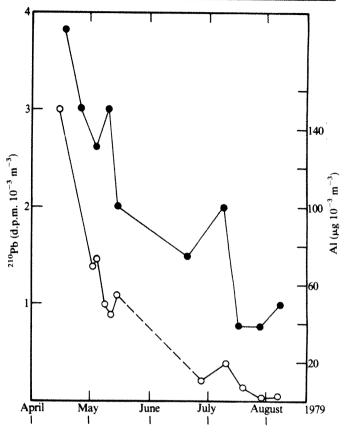


Fig. 1 210Pb concentrations (•) in air filter samples taken at Enewetak between April and August 1979. The Al data (O) from ref. 1 are also plotted.

source of ²¹⁰Pb in the aerosol is decay of atmospheric radon, then this short residence time precludes the presence of much ²¹⁰Po (138-day half life), the granddaughter of ²¹⁰Pb (the daughter being ²¹⁰Bi with a 5-day half life). Thus the ²¹⁰Po/²¹⁰Pb activity ratio in aerosols provides a way of determining the contribution of these nuclides from other sources with ²¹⁰Po/²¹⁰Pb activity ratios ≥1, such as continental dust and ocean surface and volcanic emanations. We have used 210Pb measurements to estimate the flux of other air-borne materials from the continents. Because its source is the Earth's surface rather than the stratosphere, as is the case for many cosmogenic and bomb-produced radionuclides, we believe that 210Pb is a better analogue of components injected into the troposphere.

The air filter samples were taken serially; some were used for the chemical assay and some for radiochemical analyses. The filters were dissolved for radiochemical analysis and ²¹⁰Po and ²¹⁰Pb determined using procedures described previously⁴. Our results are given in Table 1 and the ²¹⁰Pb data are plotted in Fig. 1. The monthly precipitation samples were collected as described by Benninger et al. (manuscript in preparation). Only the first seven samples of a year's collection were returned to the laboratory. The measurements (see Table 2) were made when ²¹⁰Po was virtually in equilibrium with ²¹⁰Pb. In all the air samples, the ²¹⁰Po/²¹⁰Pb activity ratios are statistically indistinguishable from 0. Thus the ²¹⁰Pb in the aerosols is derived from atmospheric 222Rn decay.

Although 210Pb is derived from atmospheric radon, there is a strong correlation between the aluminium concentration (representing the soil aerosol) and the ²¹⁰Pb concentration (Fig. 1). This relationship is also observed, to some extent, for the lipid fraction of the organic component of the air², implying that the aerosol sampled is a mixture of two parts—one derived from air rich in continental mineral and plant materials and high in ²²²Rn, and the other from air containing less of these components. Duce et al. have identified the former as dust from the Gobi Desert carried eastwards by high-level winds and

^{*} Present address: Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

Table 1 210Pb and 210Pb in air filter samples from Enewetak, April—August 1979

	²¹⁰ Pb	²¹⁰ Po/ ²¹⁰ Pb
Date of sampling	$(d.p.m. 10^{-3} m^{-3})$	activity ratio
18 April	3.82 ± 0.52	_
26 April	3.03 ± 0.25	-0.02 ± 0.08
3 May	2.61 ± 0.19	-0.02 ± 0.03
10 May	3.05 ± 0.25	-0.02 ± 0.03
15 May	1.99 ± 0.18	-0.08 ± 0.03
20 June	1.49 ± 0.05	0.14 ± 0.11
8 July	2.00 ± 0.08	0.02 ± 0.28
16 July	0.786 ± 0.035	-0.26 ± 0.04
26 July	0.778 ± 0.019	0.17 ± 0.07
4 August	1.01 ± 0.04	0.05 ± 0.07
Tower blanks (filter	carried up the sampling t	ower in exposed condi-
tions)		
10 May	0.106 ± 0.027	-0.09 ± 0.10
26 July	0.045 ± 0.008	-0.05 ± 0.18

Sampling date represents the date corresponding to the midpoint of the collection period. Errors are 1σ counting errors for all values given.

injected below the trade wind inversion east of Enewetak. The source of the air with low concentrations of all components must be the east, because of the predominantly easterly circulation.

Comparison of our Enewetak air filter results with observations made at Hawaii in 1971⁵ shows considerably higher values of ²¹⁰Pb at Hawaii. Between 15 May and 20 June 1979 at Enewetak, the concentration of ²¹⁰Pb was 1.5-2 d.p.m. 10⁻³ m⁻³. Between 30 May and 22 June 1971 at Hawaii, the values below the trade wind inversion were 3.4-19.4 d.p.m. 10⁻³ m⁻³ with a mean of 9.3, and above were 25.6-93.4 d.p.m. 10⁻³ m⁻³ with a mean of 46. Therefore the mean ²¹⁰Pb concentration in surface air at Hawaii is about five times greater than at Enewetak.

The use of ²¹⁰Pb to determine fluxes of continentally derived material requires an independent measure of the ²¹⁰Pb flux at the site. Direct measurements are not yet available for Hawaii but we have made a global model for the ²¹⁰Pb flux which can be used to estimate the flux around Hawaii³. This value is $\sim 1 \text{ d.p.m.}^{210}\text{Pb cm}^{-2}\text{ yr}^{-1}$. The ²¹⁰Pb flux at Enewetak has been measured using the monthly precipitation collections made during the year when the aerosol samples were taken. Although several precipitation samples were lost in transit we believe we can still reasonably estimate the flux from the recovered samples. Table 2a shows that although the monthly fluxes show variations, these are small and independent of season. The average of all the monthly fluxes yields an estimate of the annual ²¹⁰Pb flux of $0.15 \ (\pm 0.02) \ \text{d.p.m.}^{210}\text{Pb cm}^{-2} \text{ yr}^{-1}$ —a factor of seven lower than the Hawaii model flux estimate.

The apparent relationship between the mean concentration of ²¹⁰Pb in the air over a month's sampling and the monthly

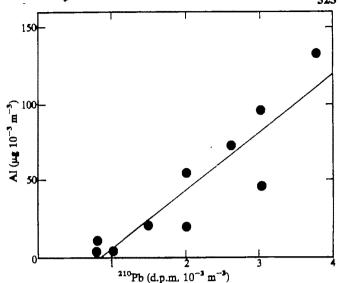


Fig. 2 Al versus ²¹⁰Pb in air filter samples from Enewetak 1979.

precipitation flux of ^{210}Pb is shown in Table 2b. The relationship can be used to estimate the mean annual concentration of ^{210}Pb (C) in the air based on the average ^{210}Pb flux (F). (The regression equation is $C[\text{d.p.m.} \quad ^{210}\text{Pb} \ 10^{-3} \ \text{m}^{-3}] = 4.7-21.4 \times F[\text{d.p.m.} \quad ^{210}\text{Pb} \ \text{cm}^{-2} \ \text{yr}^{-1}]$.) A mean ^{210}Pb flux of $0.15 \ \text{d.p.m.} \ \text{cm}^{-2} \ \text{yr}^{-1}$ corresponds to a mean air concentration of $1.5 \ \text{d.p.m.} \ 10^{-3} \ \text{m}^{-3}$.

It is evident from Table 2b that the ratio of ²¹⁰Pb flux to air concentration, which has the dimensions of a velocity (='total deposition velocity'), varies as a function of season. The dry season is characterized by a velocity of 1 cm s⁻¹ and the wet season by 4 cm s⁻¹. The total deposition velocity at Hawaii between 30 May and 22 June 1971 is ~3 cm s⁻¹. These values are comparable with that of ~4 cm s⁻¹ for the region around Bermuda (L. K. Benninger et al., in preparation) determined for the same time of year. All these oceanic sites have higher values than continental sites (ref. 3 and L. K. Benninger et al., in preparation). The air above the trade wind inversion is the source of most of the ²¹⁰Pb flux at subtropical oceanic sites because this air has trajected far over continents. When the serosols above the trade wind inversion are supplied to the inversion, they are efficiently scavenged by precipitation resulting in lower 210Pb concentrations below the trade wind Inversion than above (see ref. 5). This causes the total deposition velocity, measured at sea level, to be higher than that measured above the trade wind inversion. Strong mixing over continents tends to homogenize the lower air ²¹⁰Pb concentration, thus yielding a lower total deposition velocity. We believe that the high deposition velocity given by the 210Pb data at the ocean surface should be used to obtain fluxes of material derived from

Table 2 a, 210Pb in rain at Enewetak (April-November 1979) and b, relationship between 210Pb in air and 210Pb precipitation flux

a Collection period (1979)		Amount of rain (kg)	. ²¹⁰ Pb (d.p.m. kg	·1) (d.)	²¹⁰ Pb flux p.m. cm ⁻² yr ⁻¹)
23 April-20 May 20 May-17 June 18 June-18 July 19 July-20 August 21 August-26 September 26 September-19 October 19 October-28 November		0.138 1.045 0.762 3.795 1.301 2.491 2.378	8.72 ± 0.4 2.11 ± 0.0 3.43 ± 0.1 0.68 ± 0.0 1.20 ± 0.0 1.21 ± 0.0 1.05 ± 0.0	8 1 3 6 4	0.089 0.157 0.173 0.160 0.086 0.261 0.124
ь	Season	No. of air measurements	Mean [²¹⁰ Pb] in air (d.p.m. per 10 ³ m ³)	²¹⁰ Pb precipitation flux (d.p.m. cm ⁻² yr ⁻¹)	Total deposition velocity (cm s ⁻¹)
April 18-May 15	Dry	5 .	2.9	0.089	1.0
June 20-July 16	Wet	3	1.4	0.173	4.1
July 26-August 4	Wet	2	0.89	0.160	4.1

For the rain samples ²¹⁰Pb was calculated from ²¹⁰Po measured 13–22 months after collection, assuming no initial ²¹⁰Po and ²¹⁰Bi activity = ²¹⁰Pb activity. Area of collector for ²¹⁰Pb flux = 183.4 cm².

continents on the premise that the same processes apply to all components derived from the continental surface.

By combining the measured ²¹⁰Pb flux and the aerosol ²¹⁰Pb and Al concentrations, we can directly estimate the flux of air-borne dust at Enewetak. Comparing the Al and ²¹⁰Pb curves of Fig. 1 shows that the trends are coherent and that a correlation between the two variables exists. As the filter samples analysed for 210Pb and Al were taken serially, it is possible, by interpolation, to estimate the Al concentration corresponding to each of our ²¹⁰Pb measurements using Fig. 1. The resultant plot is shown in Fig. 2. A linear regression of Al against ²¹⁰Pb yields a slope of 38 µg Al per d.p.m. ²¹⁰Pb with a y intercept of -32 µg Al and a correlation coefficient of 0.91. We believe this represents a mixing curve between high ²²²Rn (and therefore ²¹⁰Pb), high Al air and low ²²²Rn, low Al air. The ²²²Rn/Al ratio of each of the end members is obviously different as the regression line of Fig. 2 does not go through the origin.

Using the relationship shown in Fig. 2, we can determine the atmospheric flux of aluminium if we know the flux of 210 Pb, F_{210pb} , and the mean ²¹⁰Pb concentration in the air, $\lceil ^{210}$ Pb], as

$$F_{Al} = \left(S + \frac{y}{[^{210}Pb]}\right)F_{210Pb}$$

where S is the slope (38 μ g Al per d.p.m. ²¹⁰Pb) and y the y intercept in units of μ g Al 10^{-3} m⁻³ (= -32).

Assuming that the mean concentration of ²¹⁰Pb in Enewetak air is ~ 1.5 d.p.m. 10^{-3} m⁻³ during wet and dry seasons as discussed above, the Al flux at Enewetak is 2.5 µg Al cm⁻² yr⁻¹. An aluminium concentration of the dust component of 6.5% as used by Duce et al.1 corresponds to 38 µg dust cm⁻² yr⁻¹ for Enewetak. The uncertainties in all the components of the calculation are significant. We estimate therefore that the (1σ) uncertainty could be as much as $\pm 20 \,\mu g$ dust cm⁻² yr

Duce et al. estimate the annual flux of dust to the region around Enewetak by multiplying the measured monthly flux for late April and May (4 µg cm⁻² per month) by 3-4 on the basis of dust frequency information. This yields a flux of ~12- $16 \mu g \text{ cm}^{-2} \text{ yr}^{-1}$, a factor of 2-3 lower than our estimate.

A calculation of wind-blown detrital flux for the latitude of Hawaii can be made in a similar fashion to that for Enewetak. If we use the model ²¹⁰Pb flux for Hawaii, and assume that the same air mass end members as at Enewetak are involved, a mean ²¹⁰Pb atmospheric concentration of 9.3 d.p.m. 10^{-3} m⁻³ yields a flux of 35 µg Al cm⁻² yr⁻¹, equivalent to a dust flux of 530 µg cm⁻² yr⁻¹ (assuming 6.5% Al). This is about 14 times higher than that predicted for the latitude of Enewetak and shows the dominant influence, at the latitude of Hawaii, of the westerlies carrying radon and dust from Asia. Both Enewetak and Hawaii are far enough from the land sources that the dust will be dominated by the small sizes and therefore subject to removal by the same processes as those for indigenously produced ²¹⁰Pb. Based on our calculated dust fluxes, the sediment accumulation rates should be higher at the latitude of Hawaii and northwards than at the latitude of Enewetak. This does not seem to be the general case although there are few data for this region^{6,7}, implying that either our assumptions about the broad applicability of the relationship of Fig. 2 are invalid or there has been redistribution of sediments after deposition from the atmosphere but before final accumulation. The evidence for a strong atmospheric flux from the Gobi Desert supports the latter alternative. A more detailed analysis of sediment accumulation rates in the North Pacific is needed.

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- Duce, R. A. et al. Science 209, 1522-1524 (1980).
 Gagosian, R. B., Peltzer, E. T. & Zafiriou, O. C. Nature 291, 312-314 (1981).
 Turckian, K. K., Nozaki, Y. & Benninger, L. K. A. Rev. Earth planet. Sci. 5, 227-255

- 4. Turekian, K. K., Kharkar, D. P. & Thomson, J. Final Rep. ARPA Order no. 1793 contract N0014-67-A-0097-0022
- Moore, H. E., Poet, S. E. & Martell, E. A. J. geophys. Res. 79, 5019-5024 (1974). Goldberg, E. D. & Koide, M. Geochim, cosmochim, Acta 26, 417-450 (1962)
- Cochran, J. K. thesis, Yale Univ. (1979)

Identification of polychlorinated dibenzofurans in environmental samples

Christoffer Rappe*, Hans Rudolf Buser†, David L. Stalling‡, Lawrence M. Smith‡ & Ralph C. Dougherty§

- * Department of Organic Chemistry, University of Umeå, S-901 87 Umeå, Sweden
- Swiss Federal Research Station, CH-8820 Wädenswil, Switzerland ‡ Columbia National Fisheries Research Laboratory, Columbia, Missouri 65201, USA
- § Department of Chemistry, Florida State University, Tallahassee, Florida 32306, USA

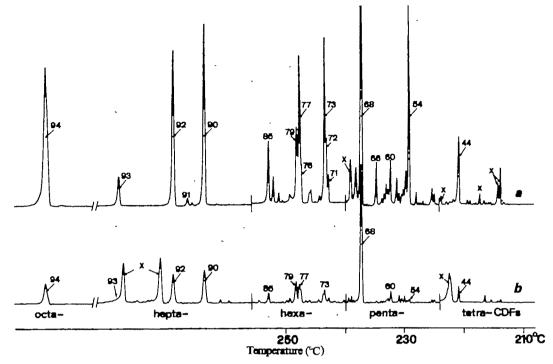
The polychlorinated dibenzofurans (PCDFs) are tricyclic aromatic compounds, which in chemical and toxicological respects are very similar to polychlorinated dioxins (PCDDs). The 2,3,7,8-tetra-, 1,2,3,7,8- and 2,3,4,7,8-penta-CDFs are extremely hazardous

compounds, they can be compared with the extremely toxic 2,3,7,8-tetra-CDD (ref. 1). PCDFs have been identified in PCBs at levels of 1-10 p.p.m., the major peaks being 2,3,7,8tetra- and 2,3,4,7,8-penta-CDF (refs 2-4). A large number of PCDFs and PCDDs occur as contaminants in chlorophenols⁵ and they have also been recognized and identified in fly ash and other incineration products at the p.p.b.-p.p.m. level⁸⁻¹¹. In 1968, 1,200 people in Japan were poisoned (Yusho disease) by a cooking oil contaminated by PCB12 and more than 50 PCDF isomers¹³. Liver samples from exposed patients were found to contain a highly reduced number of PCDFs, apparently many isomers were excreted or metabolized14. We report here the identification of a series of PCDF isomers in environmental samples.

Fat from the snapping turtle (Chelyda serpentina) obtained from the Hudson River (USA) and grey seal (Halichoerus grupus) from the Gulf of Bothnia (Haparanda, Sweden) were processed for PCDF analyses in two series of sequential chromatographic processes¹⁵. The turtle fat contained 750 p.p.m. of PCBs and the seal fat PCB concentration was $\sim 100 \text{ p.p.m.}$

Fat tissue (50-100 g) ground with sodium phosphate was applied to the first chromatographic system which contains a segment of potassium silicate and a segment of silica gel directly followed by a column containing carbon dispersed on glass fibres. The carbon adsorbs most multi-ring aromatic compounds and the major portion of biological co-extractives are not retained. The PCDFs, PCDDs and similar chemicals are then removed from the carbon by reverse elution with toluene. The toluene is removed and the sample is redissolved in hexane and applied to two columns in tandem. The first column contains both sulphuric acid dispersed on silica gel and caesium silicate. The eluate from this column passes directly into the second column that contains alumina.

Fig. 1 NCI Mass fragmentograms (m/e 306, 340, 374, 408 and 442) of a, Hudson turtle and b, Baltic seal sample showing elution of tetra, penta-, hexa-, hepta- and octa-CDFs; 50 m OV-17 glass capillary column. For peak identifications see Table 1; X denotes components other than PCDFs.



The purified extracts were directly used for GC-MS analysis. Aliquots (2 µl) corresponding to 0.1 and 0.8 g of fat of the snapping turtle and grey seal, respectively, were injected into 50-m glass capillary columns (OV-17 and Silar 10 c) leading directly into the ion source of a mass spectrometer operating in the negative chemical ionization (NCI) mode using methane as reactant gas. PCDFs exhibit intense negative molecular ions (M⁻). Some fragmentation occurs through addition of H and loss of CI with formation of (M-34) ions.

Mass specific detection (mass fragmentography) was used to detect PCDFs in these samples by selective monitoring of $(M+2)^-$ ions at m/e 306 (tetra-), 340 (penta-) and so on. More highly chlorinated PCDFs give response at the m/e values for the less highly chlorinated species due to the $(M-34)^-$ fragmentation. Quantification was based on peak height measurements and comparison with known quantities of authentic standards (2,3,7,8-tetra- and octa-CDF). Minimum detectability was 0.2 pg and 0.5 pg for 2,3,7,8-tetra- and octa-CDF, respectively. The same response for all isomers of a particular PCDF was assumed, because quantitative standards are not available. Responses for penta-, hexa- and hepta-CDFs were interpolated from the responses of tetra- and octa-CDF.

Complete NCI mass spectra (m/e 80–500, 1.4 s per scan) were recorded for the major peaks. Identification of a PCDF was based on the proper M^- ions and intensities of the ion clusters due to the Cl isotopes. Isomer identifications were based on the retention times on both OV-17 and Silar 10 c capillary columns and comparison with retention times of authentic standards.

In spite of differences in the geographical and biological origin, the samples show good correspondance. Figure 1a shows the mass fragmentograms of the turtle fat. Using the requirements discussed above, 18 different PCDF isomers could be identified (see Table 1). The total amount of PCDFs was estimated as 3 p.p.b. (Table 2). Of the individual PCDF isomers, the amount of 2,3,7,8-tetra-CDF was 45 p.p.t. and that of 2,3,4,7,8-penta-CDF was 620 p.p.t.

Figure 1b shows the mass fragmentogram of the seal fat, and the isomers identified are given in Table 1. These levels were much lower than in the turtle fat, the total amount being 40 p.p.t. The amount of 2,3,7,8-tetra-CDF was 1 p.p.t. and that of 2,3,4,7,8-penta-CDF was 15 p.p.t.

In the two samples the major PCDFs were 2,3,7,8-tetra-1,2,4,7,8- and 2,3,4,7,8-penta-1,2,4,6,7,8-, 1,2,3,4,6,7,8- and 2,3,4,6,7,8-hexa-1,2,3,4,6,7,8- and

1,2,3,4,6,8,9-hepta- and octa-CDF (see Fig. 1, Table 2). These isomers were also found to be the major PCDFs in commercial PCBs such as Aroclor 1254 and 1260 (ref. 4). However, in these commercial products some additional PCDFs were present; in the environmental samples these isomers (1,2,3,4,8- and 2,3,4,6,7-penta- and 1,2,3,4,6,7-hexa-CDF) were absent or present at much reduced levels. No such isomers were found in the study of the post-exposure tissue samples of Yusho patients, excretion or metabolism of these isomers was suggested.

The major PCDFs in commercial pentachlorophenols are 1,2,4,6,8-penta- and 1,2,4,6,8,9-hexa-CDF (refs 5-7). The former isomer was not found at all, and the latter was found at a very low level in these environmental samples. The isomer distribution of the PCDFs was also different from that reported in fly ash or PCB pyrolysates—notably differences in the

Table 1 PCDF isomers found in fat samples of the snapping turtle (Hudson River) and grey seal (Gulf of Bothnia)

PCDF-isomer	Peak no.*	Turtle	Seal
2,3,7,8-tetra-	44	++\$	+5
1,2,4,7,8-penta 1,2,3,7,8-penta- 1,2,6,7,8-penta- 2,3,4,7,8-penta-	54 60 66 68	++• (+) (+); +++•\$	(+) (+) +++
1,2,3,4,6,8-hexa 1,3,4,6,7,8-hexa- 1,2,4,6,7,8-hexa- 1,2,4,6,8,9-hexa- 1,2,3,4,7,8-hexa- 1,2,3,4,6,7-hexa- 1,2,3,6,7,8-hexa- 2,3,4,6,7,8-hexa-	71 72 73 76 77 78 79 85	#+ 	+ (+) + + (+)
1,2,3,4,6,7,8-hepta- 1,2,3,4,6,7,9-hepta- 1,2,3,4,6,8,9-hepta- 1,2,3,4,7,8,9-hepta- octs	90 91 92 93	+++\$ (+) ++++ +	++\$ ++\$

^{*} Peak no. refers to Fig. 1a, b.

⁽⁺⁾ Trace amounts possibly present. + Minor peak ++ Middle peak +++ Major peak. ++++ Dominating peak.

Identification by MS and retention times on two columns.

Identification by retention times on two columns only.

Table 2 Amounts of PCDFs found in fat samples of a snapping turtle (Hudson River) and a grey seal (Baltic)

Turtle (pg per g)	Seal (pg per g)
45	1
820	15
700	8
1,000	10
350	3
3,000	40
	45 820 700 1,000 350

hexa- and hepta-CDF range11. The 1,2,3,4,6,8-hexa- and 1,2,3,4,6,7,9-hepta-CDFs are both dominant in the fly ash samples and PCB pyrolysates while they are absent or nearly absent in these fat samples. In contrast 1,2,3,4,6,8,9-hepta-CDF, a major isomer in the fat samples, is only a minor isomer in fly ash and PCB pyrolysate. Consequently the major source of the PCDFs in the fat samples of the snapping turtle and grey seal is probably a direct contamination by PCBs.

Earlier attempts to identify PCDFs in the environment were unsuccessful due to the lack of sensitivity of the analytical techniques16 and the lack of selective contaminant enrichment procedures¹⁵. To our knowledge this is the first identification of the highly toxic 2,3,7,8-tetra- and 2,3,4,7,8-penta-CDF in environmental samples.

Received 12 March; accepted 10 June 1981.

- 1. Moore, J. A., McConnell, E. E., Dalgard, D. W. & Harris, M. W. Ann. N.Y. Acad. Sci. 320,
- 2. Bowes, G. W., Mulvihill, M. J., Simoneit, B. R. T., Burlingame, A. L. & Risebrough, R. W.
- Bowes, G. W., Mulvihill, M. J., Simonell, B. R. L., Bullingallie, R. L. & Calabara, Nature 256, 305-307 (1975).
 Albro, P. W. & Parker, C. E. J. Chromatogr. 169, 161-166 (1979).
 Rappe, C. & Buser, H. R. in Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products (ed. Kimbrough, R. D.) 41-76 (Elsevier, Amsterdam,
- 5. Buser, H. R. thesis, Univ. Umeå (1978).
- Buser, H. R. & Bosshardt, H.-P. J. Ass. Off. analyt. Chem. 59, 562-569 (1976).
- Rappe, C., Garå, A. & Buser, H. R. Chemosphere 7, 981-991 (1978), Olie, K., Vermeulen, P. L. & Hutzinger, O. Chemosphere 6, 455-459 (1977)

- Bumb, R. R. et al. Science 210, 385–390 (1980).
 Bumb, R. R. et al. Science 210, 385–390 (1980).
 Buser, H. R., Bosshardt, H.-P., & Rappe, C. Chemosphere 7-165–172 (1978).
 Buser, H. R., Bosshardt, H.-P., Rappe, C. & Lindahl, R. Chemosphere 7, 419 (29 (12 Higuchi, K. (ed.) PCB Poisoning and Pollution, 1–179 (Academic, Tokyo, 1976).
 Buser, H. R., Rappe, C. & Garå, A. Chemosphere 7, 439–449 (1978).
 Rappe, C., Buser, H. R., Kuroki, H. & Masuda, Y. Chemosphere 8, 259–266 (1979).
 Stalling, D. I., Butte, J. D., Script, L. M. Balland, C. Chemosphere 8, 259–266 (1979).

- Stalling, D. L., Petty, J. D., Smith, L. M., Rappe, C. & Buser, H.-R. Proc. Workshop on Impact of Chlorinated Dioxins and Related Compounds on the Environment, Rome, 22-24
- October 1980 (Pergamon, Oxford, in the press).

 16. Zitko, V., Hutzinger, O. & Choi, P. M. K. Envir. Hlth Perspect. 5, 47-50 (1973).

Indigenous ¹³C-NMR structural features of soil humic substances

Brian L. Worobey & G. R. Barrie Webster

Pesticide Research Laboratory, Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

13C-NMR has recently been used to characterize the structure of soild humic and fulvic acids1-7. However, these studies all used the classical NaOH extraction followed by acid precipitation (humic acids precipitate, fulvic acids are acid and base soluble) to isolate humic substances. There has been concern⁸⁻¹⁰ over the chemical alteration of such isolated acids. The studies reported here describe the effects of mild extraction techniques compared with the classical isolation procedure. Fractions were characterized by IR spectroscopy and 13C-NMR. In addition, IR and ¹³C-NMR spectra of hemicellulosic components, extracted by the classical procedure from cellulose, are compared with spectra of humic and fulvic acids isolated by the same procedure. These spectra elucidate the effect of extractants on aromaticity, paramagnetic coextractives effects on spectra quality, and spectral evidence for a cellulose component as an integral part of humic substances.

Numerous attempts have been made to devise a structural formula representative of humic and fulvic acids11. Much of the difficulty may lie in the techniques used to isolate humus substances; these are often so severe (0.5 M NaOH extraction, concentrated HCl precipitation, HCl/HF purification) as to preclude the isolation of indigenous compounds.

For complex compounds, such as humic substances, the molecules may consist of rapid and mobile regions in which only long transversal relaxation times, T_2 , predominate to be observable as broad linewidths in a high resolution spectrum. Longitudinal relaxation times, T_1 , will be even longer as T_1 is always greater than T_2 . Consequently, caution is required when deriving concentrations of carbon classes from intensities of the signals or in deducing from the absence of a signal the essence of a specific structure. Low signal-to-noise ratios are common in humic spectra due to multiple causes of line broadening.

Humic acids extracted at different pH values exhibited an increase in aromatic protons (1H-Fourier-transformed (FT)-NMR) as pH decreased from 14.0 to 4.5; however, aliphatic protons were always most abundant at any pH (65-81%)12 Additional studies have emphasized differences in ¹³C-NMR spectra based on nature of the extractant used to isolate humic and fulvic acids^{4,6,13}, and interpretation of chemical shifts especially in the 110-160 p.p.m. range. This range is classically assigned to aromatic carbon absorptions but there is evidence that alkenic carbon resonances may be present.

The isolation 17-20 of humic-like substances from plants has previously been reported¹⁸⁻²¹. Humic acids were extracted¹⁰ from humifying leaves and clover roots; IR spectra were almost identical to fulvic and humic acids isolated from soil.

Hemicelluloses are an important constituent of raw organic matter, especially in mineral soils (35% in wheat straw²²). They are typically extracted from wood with 40% NaOH followed by addition of acetic acid to the filtrate to yield the precipitate hemicellulose²³. Hemicellulose was first used to describe a product obtained from cereals with dilute alkali treatment and precipitation of the extract with acid24. The product was easily hydrolysed to give a mixture of sugars.

Cellulose contains hemicelluloses which are alkali soluble and precipitable with acid. Acid treatment can degrade the polysaccharides in hemicelluloses to furfurals. Glucuronic and galacturonic acids (associated with hemicelluloses) are degraded by 12% HCl to xylose and arabinose respectively; both of these subsequently degrade to furfural in the presence of acid21

We consider the non-destructive isolation, purification and analysis of humic acids from an agricultural soil. A precursor of humus substances was also analysed using the classical isolation procedure.

Soil (orthic black chernozem, 38% sand, 30% silt, 32% clay, 5.4% organic carbon) was extracted with 0.5M NaOH (aq) solution purged with nitrogen in a brown glass bottle and shaken for 6 h. Samples were centrifuged for 30 min at 13,000 r.p.m. The supernatant was filtered and the filtrate slowly acidified to pH 1.5 with concentrated HCl. The humic acid precipitate (I) was centrifuged; the pellet was dissolved in weak alkali and ultrafiltered on a Diaflo UM2 membrane (<1,000 molecular weight excluded) in a stirred cell to desalt and concentrate the humic solution. When the filtrate reached a pH of 6.5 and the volume was reduced to 500 ml, the retentate was shell frozen and lyophilysed. Humic acids were stored in a desiccator under vacuum. Fulvic acid was prepared from a separate soil sample using 0.5 M NaOH as described above, except that the alkali-acid soluble supernatant obtained after the humic acid centrifugation was ultrafiltered (UM2-Diaflo) and lyo-

Aqueous 0.02 M Na₄P₂O₇ was neutralized to pH 7 and used to extract the same soil to yield humic acid II. The second Na₄P₂O₇ extract was treated using acetone (1:1) to salt-out the humic acids (III) instead of acid precipitation. This precipitate (III) did not have to be dispersed with base for ultrafiltration as it was the sodium salt. Yields of humic acids were, 0.93 g (I), 0.57 g (II), and 1.47 g (III) per 500 g of soil respectively.

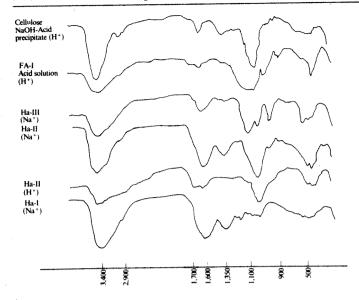


Fig. 1 Composite IR spectra of humic and fulvic acids and a cellulose component isolated in these studies (CM⁻¹).

The ratio of 1:1 acetone/water extract used for preparation of humic acid III was the minimum amount of acetone necessary for precipitation to occur. The supernatant from humic acid II did not yield any visible precipitate on addition of acetone (1:1) and humic acid III supernatant did not yield a precipitate on acidification to $pH\ 1-2$; therefore, the precipitates may be taken in each case to represent humic acids and not just a fraction of these.

IR spectra (Fig. 1) were generally diffuse and lacked coherent, well-defined resolution. However, at equal concentrations of humic acid analysed, absorption in the region of aromatic carbon (1,620 cm⁻¹ (ref. 26), and 1,690 cm⁻¹ (ref. 27)) decreased in intensity as: I>II » III. The fulvic acid IR spectra was most similar to humic acid III. This indicates the possible deformation of indigenous structure due to the acidification step.

step.

13C-FT-NMR spectra for humic acids, I, II, III, and a NaOH-extracted humic acid, precipitated, and exhaustively de-ashed humic acids (I-IV) are shown in Fig. 1. Aromatic/alkenic

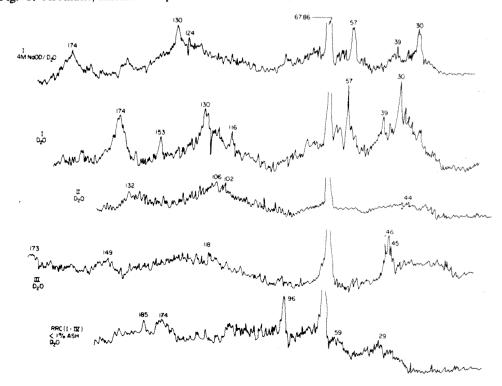
absorption in the 100-160 p.p.m. region increases (I>II>III) as the harshness of the extraction procedure increases, that is, when NaOH and HCl are used. This corroborates the IR evidence, which illustrated the same effect.

A humic acid, prepared from a Red River Clay soil (RRC I-IV, 40% sand, 16% silt, 44% clay, 4.4% organic carbon), with a very low ash content (0.1% ash) yielded a spectrum (Fig. 2) not much different in terms of broad and low intensity absorption when compared with higher ash content humic acids. The spectrum was most similar to that for fulvic acid (Fig. 3) and cation analysis showed these two humic substances to have the lowest concentration of Cu²⁺ and Fe³⁺ (Table 1). Although the concentrations were much lower than the other humic acids (I-III) they were still high enough to cause profound peak broadening (due to paramagnetic ion-induced relaxation). Concentrations as low as 6 p.p.m. of Cu²⁺ in a glycylglycine sample causes absorptions to almost disappear²⁸. Cu²⁺ concentrations were 52 and 114 p.p.m. for the fulvic acid and de-ashed humic acid respectively.

There were only slight differences between the spectra of humic acid I in D_2O solution compared with that in a 4 M NaOD solution (Fig. 2), indicating the lack of observable ($^{13}C\text{-NMR}$) alteration in the humic acid during radiation (\approx 48 h). Any alkaline degradation would have already occurred during the preparation of I.

For the humate extracted by 0.5 M NaOH, the peaks at 30.05, 38.96 and 57.52 p.p.m. fall in the range of aliphatic carbon absorption. According to Abraham and Loftus25 configurations for 30.05 p.p.m. would be R-CH₂-R or R₃C-R, while the bands at 38.96 and 57.52 p.p.m. could reflect R₂CH-R. The major peaks at 116.26 and 130.46 p.p.m. could be due either to alkenic or aromatic carbons. Early workers in ¹³C-NMR assigned the alkenic carbons to an absorption between 120 and 155 p.p.m., as opposed to a much broader aromatic region30, 13C-NMR of humic substances had generally assigned the area 110-160 p.p.m. to aromatic carbon 4,31 however, more recently the area of absorption of alkenic carbons has widened such that they cover a wider band than aromatic systems²⁹. Thus absorption in the 110-160 p.p.m. range could belong to either aromatic or alkenic carbons 32,33 Positive identification of two C-H aromatic carbons at 130 and 128 p.p.m. in a humic acid have been reported³⁴ and supported by the observation that on loss of proton decoupling the peaks split into doublets. Thus, in soil humic matter, there are some

Fig. 2 Composite of humic acid ¹³C-NMR spectra isolated by various extraction methods. ¹³Cvarious extraction methods. NMR spectra were obtained on a Brucker WS 900S Multinuclear NMR spectrometer operating at 22.63 MHz (35 °C). Free induction decays were digitized, accumulated and a Fourier transformation performed by computer. D2O lock and full proton decoupling were used during an accumulation of 80,000-100,000 scans using 90° pulse and total pulse delay and acquisition time of 1.589 s, pulse width = 14 µs, internal standard deutero-dioxane (67.86 p.p.m.), chemical shifts reported in p.p.m. resolution = ± 10 Hz(~ 5 p.p.m.).



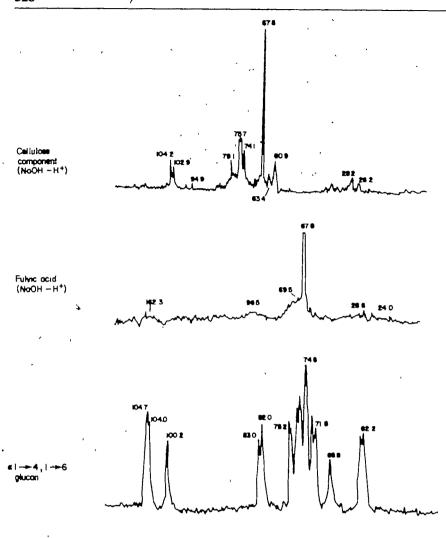


Fig. 3 ¹³C-NMR spectra of a fulvic acid, a cellulose component and a giucan, internal standard = d₆-dioxane (67.8 p.p.m.) all in NaOD/D₂O (1M).

¹³C-NMR absorptions that might be assigned to aromatic carbons.

A peak at 174.33 p.p.m. identified in our spectra agrees well with that reported elsewhere (174 p.p.m.); this can be attributed to carboxylic groups, esters and amides, but primarily to carboxylate entities.

The Na₄P₂O₇-extracted, acid-precipitated humic material (II) gave a ¹³C-NMR spectrum with a very high background noise level and very broad peaks (Fig. 2). This can be attributed to the high ash content, which include paramagnetic ions, principally Fe. There is a corresponding loss of information, with only three main absorption bands, 44.5 p.p.m. (aliphatic), 106.5 p.p.m. (alkenic or aromatic) and 132.12 (alkenic or aromatic).

The humic acid III spectrum (Fig. 2) suffered from the same defect, but sharp split peaks appeared at 40–47 p.p.m. Six-membered aliphatic rings also absorb partially in this region.

A humic acid precursor; cellulose, showed a visible precipitate on acidification of a basic extract, the yield being ~ 100 mg/per 50 g acid-washed cellulose; yields would have been higher for unwashed cellulose. Sucrose, glucose or galacturonic acid gave no precipitates using the classical extraction scheme.

The similarity of the cellulose precipitate to that of humic and fulvic acid revealed by the IR spectra (Fig. 1) is consistent with cellulose being an integral part of the innate structure of humic substances as well as a precursor.

¹³C-FT-NMR spectra revealed some similarity between the cellulose precipitate spectrum compared with the fulvic acid (Fig. 3) or humic acid IV (Fig. 2) spectra and hemicellulose component (Fig. 3)—an $\alpha 1 \rightarrow 4$, $\alpha 1 \rightarrow 6$ glucan³⁵ was reasonably similar to that of the cellulose precipitate (Fig. 3). NaOH (0.2 M) is an efficient extractant for polysaccharides from soil³⁶ and hence such hemicellulose components must be an integral part of the classical fractionation scheme.

Acid—base treatment of carbohydrates (glucose) yields aromatic compounds³⁷ even at pH 3.5, including dihydroxybenzenes and dihydroxybenzoic acids which are considered model monomers of humic substances. Although yields were low (3.5%) they may increase significantly in the presence of potential endogenous catalysts such as clays and transition metal cations. Solutions of carbohydrates in 0.63 M NaOH resulted in degradation to a large number of phenols including humic precursors³⁸.

The classical acid—base extraction scheme may artificially cause aromatic structures to form from indigenous carbohydrates. This would result in aromatic signals or absorptions in ¹³C-NMR, ¹H NMR and IR spectra. Note that the ¹³C-NMR spectrum for fulvic acid solution of polysaccharides in fulvic and humic acids extracted from sediments (Fig. 1) is very similar to that shown for the hemicellulose component isolated in our studies (Fig. 3).

Our studies suggest that humic substances may not be as aromatic as previously thought, that the extraction technique is very important in determining aromaticity that paramagnetic ions can be critical in determining spectral features, and that

Table 1 Transition metal ion contents of humic and fulvic acids studied Fe Sample Mn Cu Ζл 0.47% HA-I 1315 120 175 2.76% 275 147 HA-II 848 105 114 НА-Ш 1.96% HA-I-IV 68 ND 114 20 33 FA-I 533

Contents measured in p.p.m. unless indicated. HA, humic acid; FA, fulvic acid.

hemicellulosic compounds may be essential components of fulvic acid and humic acid structures.

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- 1. Hatcher, P. G., Berger, I. A. & Mattingly, M. A. Nature 285, 560-562 (1980).
- Villa, F. J. G., Martin, F., Saiz-Jiminez, Lentz, H. & Ludemann, H. D. Agrochimica 22, 501-506 (1978).
- Stuermer, D. H. & Payne, J. R. Geochim. cosmochim. Acta 46, 1109-1114 (1976). Wilson, M. A. & Goh, K. M. J. Soil Sci. 28, 645-652 (1977).
- Ruggiero, P., Interesse, F. S. & Sciacovelli, O. Geochim. cosmochim. Acta 43, 1771-1775
- Grant, D. Nature 270, 709-710 (1977).
- Wilson, M. A., Jones, A. J. & Williamson, B. Nature 276, 487-489 (1978).

- Wilson, M. A., Jones, A. J. & Williamson, B. Nature 276, 487–489 (1978).
 Swft, R. S. & Posner, A. M. J. Soil Sci. 23, 381–393 (1972).
 Ceccanti, B., Nannipievi, P., Cervelli, S. & Sigui, P. Soil Biochem. 10, 39, 1978.
 Konnonova, M. M. & Alexandrova, I. Va. Geoderma 9, 157–164 (1973).
 Gieseking, J. E. Soil Components Vol. 1 (Springer, New York, 1975).
 Lenz, H., Ludemann, H. D. & Ziechmann, W. Geoderma 18, 325–328 (1977).
 Sciencyalli, O. Geogeo. C. Busgier, P. A. & Tertini, C. Sci. Piol. Biochem. 13
- 13. Sciacovelli, O., Gessa, C., Ruggiero, P. A. & Testini, C. Soil Biol. Biochem. 12, 389-395
- 14. Brunow, G. & Lemmetyiner, R. Acta Chem. Scand. B32, 545-546 (1978).
- Holfe, G. Tetrahedron 32, 1431-1436 (1976). Kusumi, T., Shibata, Y., Bhitsuka, M., Kinoshuta, T. & Kakisawa, H. Chem. Lett.; Chem. Soc. Jap. 277-278 (1979)
- Visser, S. A. J. Soil Sci. 15, 202-219 (1964).
- Given, P. H. & Dickenson, C. H. Soil Biochem. 3, 123-211 (1974). Sauerbeck, D. & Fuhr, F. Soil Organic Matter No. 3-11 (IAEA, Vienna, 1968)
- 20. Swift, R. S. & Posner, A. M. Soil Organic Matter No. 211/19, 171-182 (IAEA, Vienna,
- Zhigunov, A. V. & Simakov, V. N. Pochvoverdenie 12, 59-65 (1977).
- Robertson, J. B. Topics in Dietary Fiber Research Ch. 1, 1-41 (Plenum, New York, 1978).

- Robertson, J. B. Topics in Dietary Fiber Research Ch. 1, 1-41 (Plenum, New Yor Williams, K. T. & Bevenue, A. J. Ass. Offic. analyt. Chem. 39, 901-918 (1956). Schultze, H. A. Physiol. Chem. 16, 387 (1892). Shorey, E. C. & Martin, J. B. J. Am. chem. Soc. 52, 4907-4915 (1930). Theng, B. K. G., Wake, J. R. H. & Posner, A. M. J. Soil Sci. 18, 349 (1967).
- Dyer, J. R. Applications of Absorption Spectroscopy of Organic Compounds (Prentice Hall, New York, 1965).
- Bovey, F. A. High Resolution NMR or Macromolecules (Academic, New York, 1972).

 Abraham, R. J. & Loftus, P. Proton, and C-13 NMR Spectroscopy—An Integrated Approach (Heyden, London, 1978)
- 30. Levy, G. C. & Nelson, G. L. Carbon-13 NMR for Organic Chemists (Wiley, New York,
- 31. Sposito, G., Schaumberg, G. D., Perkins, T. G. & Holtzclaw, K. M. Envir. Sci. Technol. 12.
- 32. Wilson, M. A. & Goh, K. M. Plant Soil 46, 287-289 (1977).
- 33. Villa, F. J. G., Lentz, H. & Luderman, H. Biochem. biophys. Res. Commun. 72, 1063-1070
- 34. Ogner, G. Geochem. cosmochim. Acta 43, 105-108 (1979)
- Jennings, H. J. & Smith, I. C. P. J. Am. chem. Soc. 95, 606-608 (1973).
- Cheshire, M. V. J. Soil Sci. 28, 1-10 (1977).
 Popoff, T. & Theander, O. Acta Chem. Scand. Serv. B30, 2-7 and 397-402 (1976).
- 38. Forsskahl, I., Popoff, T. & Theander, O. Carbohydr. Tes. 48, 13-21 (1976)

Land plant evidence compatible with gradual, not catastrophic, change at the end of the Cretaceous

Leo J. Hickey

Division of Paleobotany, Smithsonian Institution, Washington, DC 20560, USA

Field study of the fossil and sedimentary record across the Cretaceous-Tertiary (K-T) boundary in Wyoming and Montana has been combined here with a reassessment of the published record of terrestrial palynomorphs to test recent hypotheses that a universal biotic catastrophe caused by an asteroid1-3, cometary impact4 or a supernova5 terminated the Cretaceous. Evidence from land plants is particularly critical because plants form the base of the terrestrial food chain. Thus, massive disruptions of the Earth's vegetation by the postulated effects of a cosmic disaster (blocking of solar radiation for several years by a dust cloud¹, severe atmospheric heating^{1,4,6,7} or ionizing radiation5) would have an amplified effect on the land fauna. However, I report here that the geographically uneven and generally moderate levels of extinction and diversity change in the land flora, together with the non-synchroneity of plant and dinosaur extinctions, contradict hypotheses that a catastrophe caused terrestrial extinctions.

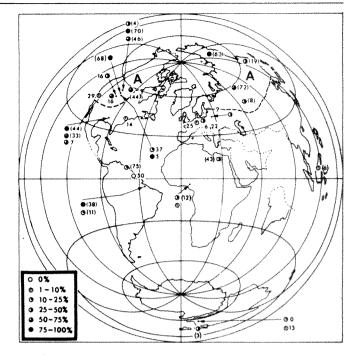


Fig. 1 Severity of plant extinction (mostly at the species level) and change in diversity across the Cretaceous-Tertiary boundary. The dashed line represents the approximate limits of the Aquilapollenites province (designated 'A') covering northwestern North America and northeastern Asia. The symbols for localities indicate the percentage of Cretaceous plants that became extinct across the K-T boundary, and are explained in the key. The figures accompanying the symbols indicate the percentage change in diversity with negative values indicated by parentheses. Levels of extinction >50% reported at two localities outside the Aquilapollenites province are attributable, in the case of the Mississippi Embayment, to facies changes 47 or restriction of the sample to the Normapolles group of pollen, which fared badly across the K-T boundary; and, in the case of northeastern Brazil, to a restricted sample of indicator species¹². (Base map for the early Palaeocene redrawn from ref. 48.)

Fossil plants, mainly palynofloras, provide a detailed picture of terrestrial change across the K-T boundary that has been sampled throughout much of the world. Unfortunately, this record does not yet offer the degree of time-stratigraphical resolution possible for the marine sequence because of the relatively few studies across the boundary, wide spacing of samples and frequent emphasis on recognition of the Cretaceous or of the Palaeocene using selected guide fossils rather than by characterizing the whole assemblage. In addition, shifts in sedimentation across the boundary are seldom documented but may strongly influence the floristic composition; megafossil remains have been extensively misidentified⁸⁻¹¹ and serious problems of correlation exist between marine and non-marine stratigraphical sections as well as between sections on different continents. Despite these difficulties, a coherent pattern of moderate floristic change can be discerned across the K-T boundary12

To standardize the extinction figures reported here, all late Maastrichtian forms not found in the Lower Palaeocene were treated as though they became extinct at the K-T boundary. These extinction figures were also based on geographically restricted floras which probably record local range changes as well as extinctions. Both these factors elevate the apparent level of extinction.

Late Cretaceous floras were dominated by the flowering plants, which appeared in the mid-early Cretaceous^{6,13-17} and achieved dominance by the Turonian^{6,18} in the Northern Hemisphere but perhaps not until the Maastrichtian¹⁸ in the Southern Hemisphere. During the mid-late Cretaceous, the pollen flora of the middle- and high-latitude Northern

Hemisphere gradually diverged into two distinct provinces^{19,20}. The area from eastern North America across Europe into western Asia was dominated by the Normapolles group. A second province extended from western North America nearly across Siberia and was characterized by forms of unusual morphology, known as the Aquilapollenites group^{21,22}, along with, for example, Wodehousia, Azonia and Proteacidites. Both pollen and leaf floras were dominated by archaic taxa with modern forms such as the palms, Nothofagus^{18,20}, Platanus^{23,24} and a few others^{20,25}, appearing in small numbers gradually through the late Cretaceous.

At the end of the Cretaceous, the most noteworthy change in the world land flora was the decimation of the Aquilapollenites province. Within its boundaries, extinction often amounted to 75% of the Cretaceous species and showed an imperfect pattern of increase towards the north (Figs 1, 2). Elsewhere the level of extinction was much less, seldom exceeding 50% of Cretaceous forms 12 . Floral diversity also decreased most markedly in the Aquilapollenites province with the level of diversity attenuation generally increasing northwards. Megafloral studies across the K-T boundary are limited to western North America but show similar levels of extinction of Cretaceous forms $(40-60\%)^{12}$ and a substantial increase in the proportion of toothed (that is, cooler climate 26,27) leaves.

For comparison, the same method of analysis was applied to extinction across the Palaeocene-Eocene boundary, where no catastrophe has ever been invoked. In North Dakota a third of the palynoflora²⁸ and one-half of the megaflora²⁹ became extinct through a section starting 10 m below the boundary. Similar levels of extinction are seen by comparing the total Clarkforkian (latest Palaeocene) with the total Wasatchian (early Eocene) megaflora of the Bighorn Basin in Wyoming (S. L. Wing, thesis in preparation, Yale University).

Given a terminal Cretaceous event of several to several hundred years duration and of overwhelming magnitude, extinctions in various terrestrial groups should appear synchronous at our resolution limits. Only in western North America have stratigraphical sections containing both the last dinosaurs and plants been carefully observed. There, the Cretaceous flora persists above the level of the highest unreworked dinosaur bone. In Alberta the floral change occurs 6 m higher³⁰. In east central Montana³¹, northwestern Wyoming (my observations and P. Gingerich, personal communication) and southeastern Wyoming³², the change occurs at a minimum of 2-3 m higher. In Colorado the change lies an unspecified distance above the last dinosaur³³. Based on a sedimentation rate of 65 m Myr⁻¹ for the Edmonton Group³⁴, the stratigraphical interval between the last dinosaur and the floral change would represent $\sim 50-90 \times 10^3$ yr. In addition, several authors note an attenuation in dinosaur

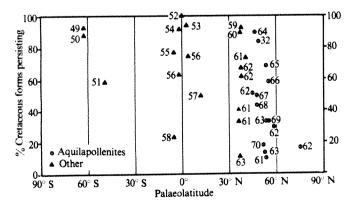


Fig. 2 Survival of Cretaceous plant species across the K-T boundary as a function of palaeolatitude. Localities falling in the Aquilapollenites province have been distinguished from those in other regions. Reference numbers appear beside the points. Note the generally high levels of floral survival from high-southern to mid-northern palaeolatitudes.

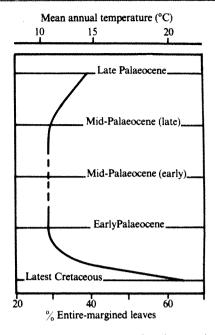


Fig. 3 Changes in the percentage of entire-margined, dicotyledonous angiosperm leaves and inferred palaeotemperature changes from the latest Cretaceous through the Palaeocene in the Bighorn Basin of northwestern Wyoming (modified from ref. 72).

abundance and diversity towards the boundary^{31,34}, a finding corroborated by my field work in north-west Wyoming.

Generally moderate, geographically variable levels of landplant extinction and the lack of synchroneity in plant-dinosaur extinctions at the close of the Cretaceous make it unlikely that a universal biotic catastrophe occurred. Despite a lower thermal gradient in the Cretaceous^{35,36} due to lack of polar ice caps³⁶ and a generally higher average Earth temperature³⁷, Northern Hemisphere floras show an increase of cooler-temperature forms with increasing latitude³⁷, though more gradually than at present. This suggests a correlative increase in the efficiency of plant dormancy mechanisms as well as chromosomal redundancy, both of which would have raised resistance to environmental stress³⁸⁻⁴⁰. The elevated levels of extinction seen at higher northern latitudes across the K-T boundary effectively contradict the assertion that the Earth's vegetation was simply able to regenerate after cosmic catastrophe^{4,5}. In addition. low-latitude floras, relatively lacking in such dormancy and carryover mechanisms, suffered least, exactly the opposite of that expected from the catastrophic model (Figs 1, 2).

Alternatively, the pattern of change in land plants, the increasingly cooler affinities of latest Cretaceous to early Palaeocene palynofloras 36,41-44, the increase in the percentage of forms with toothed leaves in the western United States11 attenuation of dinosaurs and the lack of synchroneity in extinction of plants and dinosaurs are compatible with climatic cooling. A palaeotemperature curve for Montana and northern Wyoming suggested by megafloral evidence (Fig. 3) and numerous marine palaeotemperature curves point to climatic cooling across the boundary with gradual recovery in the Palaeocene. (Recent suggestions of a very short-term temperature increase at the boundary 6.7.45.46 cannot be seen at the time scale of the floral studies reviewed here.) An attenuated dust cloud, rather than the completely opaque one of Alvarez et al., would have caused relatively less light to reach high latitudes due to the greater screening power of dust at the increasing angles of insolation that occur there. The result would have been a steepened gradient of declining temperatures towards high latitudes and an attendant increase in plant extinctions. However, the effect of such a dust cloud would have been of very short duration. Furthermore, this hypothesis fails to account for the lack of appreciable extinction or diversity decline at high southern latitudes (Figs 1, 2) or the irregular occurrence of such effects elsewhere. These are more satisfactorily explained by long-term climatic deterioration, whose influence would be far less uniform over the Earth's surface than that of a dust cloud.

The asteroid hypothesis has stimulated discussion between physical scientists and palaeontologists. However persuasive and well documented the evidence for an iridium anomaly, the connection between it and the terminal Cretaceous extinctions is tenuous at best. Before a general theory explaining these extinctions can be framed, additional data on geochemical causes of the Ir anomaly, the range of physical consequences of a postulated cosmic catastrophe, and the timing and magnitude of late Cretaceous extinctions must be gathered and evaluated.

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Alverez, L. W. et al. Science 200, 1095-1106 (1980)

    Nortit, J. & Hartogen, J. Nature 285, 198–200 (1980)
    Smit, J. & Hartogen, J. Nature 285, 198–200 (1980)
    Gagnapathy, R. Science 209, 921–923 (1980)
    Hsu, K. J. Nature 285, 201–203 (1980)
    Russell, D. & Tuokor, W. H. Nature 229, 553–554 (1971).

    Hughes, N. F. Palsacobology of Angesparan O.
    McLoan, D. M. Science 201, 401–406 (1978)
    Pachovi, B. Presia 33, 113–129 (1961)
    Wolfe, J. A. Am J. Bot. 59, 664 (1972).
    Hickey, L. J. Am J. Bot. 69, 17–33 (1973).
    Dichor, D. L. Bet. Rev. 40, 1–1547 (1974)
    Linkey, L. J. M. Chember, E. C. Berner, C. C. Berner
                                                                                                                                                                                                                                                                                                      rer Origina (Cambridge University Press, 1976)

    Dichor, D. L. Bet. Ren. 40, 1-1947 (1974)
    Hickey, L. J. in Catastepher in Earth History: The New Uniformism sense (eds Borggren, W. A. & Van Couvering, J. A.) (Princeton University Press, in the press).
    Doyle, J. A. & Hickey, L. J. in Origin and Early Esshation of Angiosperus (ed. Beck, C. B.).
    139-206 (Columbia University Press, New York, 1976).
    Hickey, L. J. & Doyle, J. A. Bet. Rev. 43, 3-104 (1977).
    Hughes, N. F. Bet. Rev. 43, 3-104, 105-127 (1977).
    Veltermen, V. A. & Estern, L. Z. Belance, 27, 1877, 101-100 (1977).

    Hughes, N. F. Bet. Rev. 43, 3-104, 105-127 (1977).
    Vakhramoov, V. A. & Kotova, I. Z. Pelsent. Zh. 1977, 101-109 (1977).
    Doylo, J. A. et al. Bestl. Aut. Rev. Expler. Prod. Eff. Aspalance 1, 451-473 (1977).
    Pomy, J. S. m. Aspects of Palynelogy (eds. Tschudy, R. H. & Scott, R. A.) 331-376 (Wiley-Internolescoa, New York, 1969).
    Doylo, J. A. J. Arnold Arises, 50, 1-35 (1969).
    Muller, J. Biel. Rev. 46, 417-450 (1970).
    Mohadhahvih, N. D. Trady VNRGRI 177, 1-342 (1961).
    Stanley, E. A. Ball. Ge Acad. Sci. 28, 1-44 (1970).
    Krasellov, V. A. Palasonia graphics B142, 105-116 (1973).
    Pacitová, B. Cour. Porach. Inst. Senchenberg 38, 70-76 (1978).
    Niklas, K. J., Tiffney, B. H. & Knoll, A. H. in Ecohationary Boology (ads. Hochit, M. K., Stoore, W. C. & Wallacs, B.) 1-80 (Pleamm, New York, 1980).
    Builey, I. W. & Simnott, E. W. Science 41, 832-833 (1915).
    Wolfe, J. A. Pref. Pap. U. S. gool Surs. 1106, 34-35 (1979).
    Hickey, L. J. Mem. gool. Soc. Am. 188, 57-71 (1977).

    Hickey, L. J. Mon. gool. Soc. Am. 186, 57-71 (1977).
    Bobout, J. W. thorn, Pennsylvania State Univ (1977).

    Larbekmo, J. F., Evens, M. E. & Beadeguard, H. Nehrer 279, 26–30 (1979).
    Clemens, W. & Archibeld, D. Mém. Soc. géol. Fr. 139, 67–74 (1980)

                            Leffingwoll, H. A. Spec. Pap. gool. Sec. Am. 127, 1-64 (1970).

Nowman, K. B. in Cremcoust-Ternery Boundary Bounds: II (eds. Christenson, W. K. &

    Nowman, K. B. in Creincesex-Terisory Boundary Events II (eds Christensen, W. K. & Birkolund, T.) 246-248 (University of Copenhagen, 1979).
    Van Valen, L. & Sloan, R. E. Brel. Theory 2, 37-64 (1977).
    Ostriner, S. in Creincesex-Terisory Boundary Events II (eds Christensen, W. K. & Birkalund, T.) 26-28 (University of Copenhagen, 1979).
    Saino, T. & Van Donk, J. Micropaleoniology 28, 152-177 (1974).
    Savin, S. M. A. Ren. Barth planet. Sci. 8, 319-355 (1977).
    Raunkiner, C. The Life Forms of Piscut and Statistical Plant Geography: Being the Collected Papers of C. Rassikiner (Chrendon Press, Oxford, 1934)
    Cain, S. A. Bet. Ren. 16, 1-32 (1950).
    Woodwell, G. M. Scient. Am 208 (6), 40-49 (1963)
    Samilovich, S. R. Res Palescolor. Polymol. 2, 127-139 (1967).
    Smiley, C. J. Geosci. Man. 4, 91-99 (1972).
    Smiley, C. J. Geosci. Man. 4, 91-99 (1972).
    Strustava, S. K. Palescopany. Palescolinstol Palescocol. 7, 221-276 (1970).

    Srivatiwa, S. K. Palacogoogy. Palacoclimatol Palacocci. 7, 221–276 (1970).
    Kramilov, V. A. Palacogoogy. Palacoclimatol Palacocci. 17, 152–172 (1975).

    Kraszilov, V. A. Pelacogosgy Pulesochmasol Pulacoscol. 17, 152–172 (1975).
    Bourans, A. et al. Intl. Rep. DSDP 43, 695–719 (1979).
    Bourans, A. & Shackteton, N. in Cresscoon.—Tentery Boundary Enems II (eds Christensen, W. K. & Birkelmid, T.) 50–53 (University of Copenhagon, 1979).
    Istreen, D. M. Pellen Spores 28, 535–553 (1978).
    Smith, A. G. & Briden, J. C. Mesorosce and Crosscole Puleocontennels Mayer (Cambridge University Press, 1977).
    Stover, L. E. & Partindge, A. O. Prec. R. Soc. Vict. 85, 237–286 (1973).
    Stover, L. E. & Evins, P. R. Spoc. Public gool. Soc. Assis. 4, 55–72 (1973).
    Couper, R. A. Pelacont. Bull., Wellington 32, 1–84 (1960).
    Leidelmeyer, P. Lind. gool. Medical. 38, 49–70 (1966).
    Muller, J. Micropalaminings 14, 1–37 (1968).
    vin Hockson-Kimbroberr, P. M. I. Laid. and Medical. 38, 37–48 (1966).

                                                                                                                                                                                                                                                                                                                                                                      escol. 17, 152-172 (1975).
                         van Hocken-Kinkenberg, P. M. Loid, geel, Meded. 28, 37–48 (1966).

Miller, H. m Prec. of the Second West African Alteropalamanogical Colloquium, Ibaden, No.

    Miller, H. in Proc. of the Second West African Micropalasmalogical Colloquium, Bucies, No. 5 (ed. van Hinto, J. E.) 123-136 (Brill, Leiden, 1966).
    Germaraad, J. H., Hopping, C. A. & Miller, J. Rev. Palasobet, Palynol. 6, 189-348 (1968).
    Kodeves, M. A.cis bet ac: Hang, 17, 371-378 (1971).
    de Boor, N. P., van der Hammon, T. & Wymstra, T. A. Goologie Mijnib. 44, 254-258 (1965).
    Krutsch, W. Z. Angew. Gool. 3, 509-548 (1957).
    Zaklinskaya, E. D. in Rasvelle Flores Operates Microscope (Ed. Vakhrameev, V. A.) 66-119 (Nanira Moccow) 1977).
```

Drugz, W. S. Pziesoniographics B128, 1-71 (1967)
 Shosmakov, R. E. Pziesoniographics B119, 54-75 (1966).
 Mamontova, I. V. m. Pziesobetenika na Dal'nem Vestoka (ed. Krasniov, V. A) 32-37 (Institute of Beology and Pedelogy, Vladivestok, 1977).
 Dorf, E. Bull geol Soc. Am. 51, 213-236 (1940), Public Carnega Intro 508, 79-159 (1942).
 Dorf, E. Public Carnegas Instit 598, 1-78 (1938).
 Vakhramoev, V. A. & Almetov, M. A. m. Razotta Flor na granita Mazozoya (ed. Vakhramoev, V. A.) 39-65 (Nanka, Moscow, 1977).
 Stenley, E. A. Rull, Am. Palaeut 48, 179-383 (1965).

Stanley, E. A. Bull. Am. Paleont. 49, 179-383 (1965)
 Norton, N. J. & Hall, J. W. Paleontographics B125, 1-92 (1969)

72 Hickoy, L. J. Contr. Mus Paleons. Unio. Mich. 24, 33-49 (1980)

Spherical phosphatic microfossils from the Silurian of North Greenland

R. J. Aldridge & H. A. Armstrong

Department of Geology, The University, Nottingham NG7 2RD, UK

We report here that during routine micropalaeontological processing of Siberian limestone samples from North Greenland, a large number of small spherical, spinose microfossils have been isolated. These are comparable with forms studied in thin section by Sannemann¹, which have been referred to the group Acritarcha, erected by Evitt² to accommodate hollow, organicwalled microfossils whose affinities are uncertain. Our material suggests that the walls of the spherical bodies were minerialized rather than organic, throwing doubt on an assignment to the

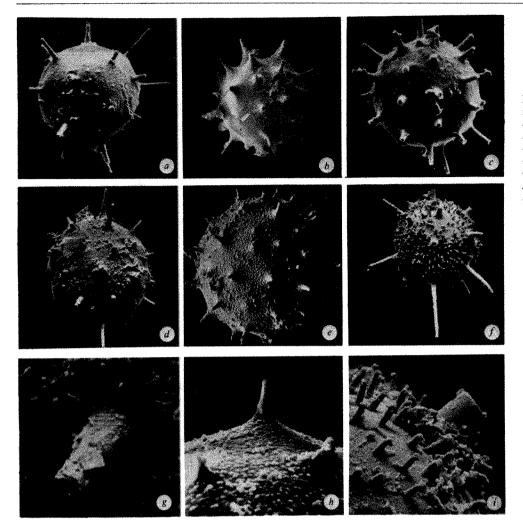
The specimens we have examined are from the upper part of the unnamed Silurian limestone formation and the unnamed Silurian black shale formation3 of Central Peary Land and their equivalents in Washington Land⁴, North Greenland. The samples were collected by field geologists of the Geological Survey of Greenland and processed in dilute acetic acid at Nottingham to recover conodonts, other phosphatic microfossils and organic microfossils. Five samples (GGU sample nos 216849, 216853, 228978, 229036 and 228926) have yielded a total of more than 3,000 microspheres; associated conodonts include Pterospathodus celloni (Walliser) and indicate a late Llandovery age³. Samples processed using standard palynological techniques (involving the use of hydrochloric and hydrofluoric acids) for the recovery of organic-walled microfossils have failed to yield any similar bodies.

The size of the microspheres varies from 100 to 400 µm in diameter, and long spines, which are broken on almost all specimens, project from the surface (Fig. 1). The density of these spines is variable and their basal insertion ranges from parallelsided on some specimens (Fig. 1a) to broadly flaring in others (Fig. 1b). The central body may be smooth (Fig. 1a), granular (Fig. 1e, h) or covered with pillars up to 10 μ m long and 2 μ m in diameter (Fig. 1f, t). Scanning electron microscope examination of broken specimens has revealed several with complex wall structures (Fig. 2b-d). Commonly, an outer, thin, single layer is separated by 5–17 μ m from a double inner layer by stout pillars. On one specimen (no. MGUH 15322) we have observed a third inner, structureless layer (Figs 2b, c, 3b). Each wall layer except the latter is composed of crystallites arranged perpendicular to the surface (Fig. 2a, c) and is less than $1 \mu m$ thick. The spines arise from the inner layer (Fig. 2d) and also commonly display a double wall; the lower portions of the spines seem to be hollow (Fig. 2a, d). Although some of our specimens show simpler wall structures, this may be the result of removal or non-fossilization of the outer layers.

Energy dispersive analysis of the spheres showed high calcium (Fig. 3c, d) and high phosphorus (Fig. 3e, f) content of the outer surface and of the inorganic wall layers, indicating that they are composed of apatite. Repeated 20-s counts of the PK α and $CaK\alpha$ peaks on two microsphere specimens provided Ca:Pratios of 1.99 and 2.12, respectively. Two apatite standards

Zarimannya, E. D. in *Parassis Pier sa Cymnius Marzanya i Kalesznya* Tachudy, R. H. *Prof. Pap. U.S. gool. Sun.* 368, 1–42 (1975).
 Zaklinskaya, E. D. in *Paleozolskie i Marzaniske Flori Ewrazi i Pisagoo* (ada Vakhrameev, V. A. et al.) 302–331 (Nanka, Moscow, 1970).

63. Tschudy, R. H. Spec Pap geel. Sec. Am 127, 65-111 (1970).



1 Variation in external morphology of the microspheres. a, Specimen MGUH 15315, ×138. b, Specimen MGUH 15316, ×165. c, Specimen MGUH 15317, ×225. d, Specimen MGUH 15318, ×150. e, Specimen MGUH 15319, ×213. Specimen MGUH 15320, ×130. g, Specimen MGUH 15318, ×1368, showing spine insertion and partial breakdown of outer layer round spine. h, Specimen MGUH 15319, ×645. i, Specimen MGUH 15320, ×515. The pillared and granular 'ornament' on these specimens may represent the remains of the pillar supports of the outer wall, which has not been preserved. All specimens are from GGU sample 216849 numbers relate Geologisk collections of the Museum, Copenhagen.

analysed at the same time yielded ratios of 1.88 and 2.10 and a coeval conodont specimen, composed of organically secreted apatite, showed a ratio of 2.19. Published analyses of apatite are mostly in the same range $^{6.7}$, although our specimens seem to fall outside the range of francolite (Ca:P 2.5–2.7), a common diagenetic carbonate apatite in sedimentary environments 8 . The third inner layer of specimen MGUH 15322 produced no peaks on energy dispersive analysis, which is sensitive to elements with an atomic number of $\geqslant 11$. It is probable that this layer is organic in composition.

In assessing the biological affinities of the microspheres it is important to determine that the phosphatic mineralization of the walls is primary, and not a result of replacement during fossilization. Unfortunately, there is no unequivocal criterion. Circumstantial evidence is provided by the fact that there is very little phosphate in our limestone samples and that calcareous crinoid and brachiopod fragments show no signs of phosphatization. A chitinozoan (with an organic wall of 'pseudochitin'), from the sample with the most abundant microspheres, shows minor silicification and no trace of calcium phosphate. Hence, if the phosphatization is secondary, it has been extremely selective and mineralogically unusual. The phosphatic wall structure of the spheres is comparable with that of other phosphate-secreting organisms, such as lingulid brachiopods9, and it is more probable that the apatite is primary. However, some features of surface sculpture may be the result of

The shape of the microspheres is very similar to that of acanthomorphic acritarchs, which are normally smaller (average diameter $30~\mu m$) and have organic walls. Sannemann¹ assigned his specimens from the Devonian of the Frankenwald, West Germany, to the genus Hystrichosphaeridium on the basis of the general similarity in morphology. Subsequently, they were

transferred to the genus Baltisphaeridium^{10,11} and were catalogued as acritarchs by Eisenack¹². He noted, however, that they were unusually large and seemed to be constructed differently from the 'true' acritarchs. These structural differences are emphasized by our material and the phosphatic walls firmly rule

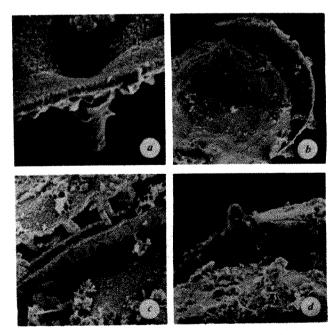


Fig. 2 Wall ultrastructure of the microspheres. a, Specimen MGUH 15321, ×800. b-d, Specimen MGUH 15322, ×215, ×860, ×1075. Both specimens from GGU sample 216849.

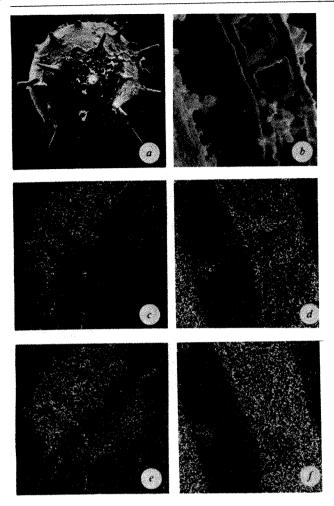


Fig. 3 Energy dispersive analysis of microsphere walls. a, Specimen MGUH 15323, ×270. b, Specimen MGUH 15322, ×860. c, d, Calcium distribution across the specimens in a, b. e, f, Phosphorus distribution across the specimens in a, b. Both specimens from GGU sample 216849.

out classification with the acritarchs. Spinose, spherical skeletons are also common among the Radiolaria¹³, but their walls are latticed, spongy or perforate, and we know of none that compare closely in structure with the microspheres. Modern radiolarians have skeletons of opaline silica, although this may be replaced by other minerals in fossil material.

Somewhat similar structures are shown by microgranular foraminifera referred to the superfamily Parathuramminacea especially the genera Parathurammina, Archaesphaera and Uralinella. These, however, lack the perfect sphericity and complexity of wall structure shown by the microspheres, and preservation in phosphate would demand replacement of the original calcium carbonate tests. Finally, there is some similarity between our microspheres and various spherical calcium carbonate microfossils that have been termed calcispheres11 particularly those referred to the radiosphaerid calcispheres16 The structure of radiosphaerid calcispheres is rather different, as they are characterized by a spinose or prismatic outer wall layer, and again, preservation in phosphate would demand diagenetic replacement. However, the calcispheres are a heterogeneous group of uncertain affinities, and it is possible that specimens comparable to the Greenland microspheres have, at some time, been assigned to that group. Even so, there is no known group that readily accommodates our specimens. Their spherical structure, multiple concentric wall layers and prominent spines are characteristic, and their probable original apatite composition sets them apart from other microfossils. For ease of reference, we informally name them mazuelloids; the name is derived from the mediaeval latin term for 'mace'.

We report the results of our preliminary studies of these microspheres in the hope that they will stimulate finds of more material. The mazuelloids are probably widely distributed, but commonly missed in micropalaeontological investigations. They would not be preserved in palynological residues as they collapse in hydrochloric and hydrofluoric acids, and their small size and delicacy may lead to them being overlooked or broken in routine microfossil searches. Indeed, since recovery of the Greenland material, we have isolated several additional phosphatic mazuelloids from the upper Silurían of Czechoslovakia.

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- 1. Sannemann, D. Senckenberg, leth. 36, 321-346 (1955)
- Evitt, W. R. Proc. natn. Acad. Sci. U.S.A. 49, 298-362 (1963).
 Christie, R. L. & Peel, J. S. Rapp. Granlands geol. Unders. 82, 1-48 (1977).
- Hurst, J. M. Bull. Grønlands geol. Unders. 138, 1-95 (1980).
 Aldridge, R. J. Rapp. Grønlands geol. Unders. 91, 7-23 (1979).
 McClellan, G. H. J. geol. Soc. Lond. 137, 675-681 (1980).
- Deer, W. A., Howie, R. A. & Zussman, J. An Introduction to the Rock-Forming Minerals (Longman, Oxford, 1974).

 McArthur, J. M., Coleman, M. L. & Bremner, J. M. J. gool. Soc. Lond. 137, 569-673 (1980).
- Hewitt, R. A. J. geol. Soc. Lond. 137, 661-667 (1980); Eisenack, A. Neues Jb. Geol. Palaont. Abh. 106, 383-422 (1958)

- Eisenack, A. Neues Jb. Geol. Palaont. Abh. 106, 385-422 (1958).
 Downie, C. & Sarjeant, W. A. S. Paleontology 6, 83-96 (1963).
 Eisenack, A. Katalog der fossillen Dinoflagellaten, Hystrichosphären und verwandten Microfossilien Vol. 3, Pt. 1 (Schweizerbartsche, Stuttgart, 1973).
 Kling, S. A. in Introduction to Marine Micropalaeontology (eds Haq. B. U. & Boersma, A.) 203-244 (Elsevier, New York, 1978).
 Loeblich, A. R. & Tappan, H. in Treatise on Invertebrate Palaeontology C (ed. Moore, R. C.)
- (University of Kansas Press and the Geological Society of America, 1964)

 15. Kaźmierczak, J. Acta palaeont. polon. 21, 245-255 (1976).
- 16. Stanton, R. I. Jr Micropalaeontology 13, 465-472 (1967)

ESR-dating of the fossil hominid cranium from Petralona Cave, Greece

G. J. Hennig*, W. Herr*, E. Weber† & N. I. Xirotiris‡

*Institut für Kernchemie der Universität zu Köln, Zülpicher Strasse 47, 5000 Köln-1, FRG

†II. Physikalisches Institut der Universität zu Köln, Abteilung für Metallphysik, Zülpicher Strasse 77, 5000 Köln-41, FRG ‡Institut für Anthropologie der Universität Frankfurt. Siesmayerstrasse 70, 6000 Frankfurt am Main, FRG

The age of the hominid cranium discovered in 1960 in a limestone cave near Petralona (Greece) is a continuing cause of controversy. The age of the skull, which was apparently encrusted by brown calcite soon after the death of the individual concerned, has been variously estimated at between 70,0001 and 700,0002 yr. Here we show using electron spin resonance (ESR) measurements of the calcite encrustation and of bone fragments that the age of the Petralona hominid lies between 160,000 and 240,000 yr. We also demonstrate, by trace element analysis, that the composition of the calcite encrustation is the same as that of the very top of the travertine floor.

Part of the difficulty in interpreting the Petralona skull stems from the circumstance of its discovery, on the floor of the cave. The cave fauna has been variously attributed to the Riss/Würm³ and Günz/Mindel4 interstadial, suggesting that the hominid might be classified as Homo sapiens neanderthaliensis or Homo erectus. It is, however, questionable, whether faunistic remains

can be related to the hominid cranium. Previous estimates of the age have been based on measurements of speleothem samples either from other branches of the cave or from deeper (and thus older) strata. We believe that only the skull itself and its encrustation should be the basis for absolute age determination.

After inspecting the calcite encrustation, which was removed from the well preserved skull by J. W. Melentis (University of Saloniki, Greece) and one of us (N.I.X.), our attention was directed to some larger speleothem samples that were collected a few years ago in the cave near where the skull was discovered. As expected, some of these travertine samples were covered by a thin, opaque, reddish-brown, non-laminated calcite layer resembling the coating on the skull. The lack of any lamination indicates only one uninterrupted depositional event. To confirm the assumption of an identical growth horizon, pieces of the skull encrustation (a) and of the calcite layer (b) covering some of the larger speleothem fragments were analysed for several trace elements by instrumental neutron activation analysis (INAA). For comparison, a calcite sample (e) from 30-40 mm beneath the surface layer of the travertine floor was included.

Comparison of the results in Table 1 clearly shows the high similarity of samples (a) and (b). Sample (e) is quite different.

The higher K-, Mn- and Na-contents of sample (b) compared with (a) can be explained by an increased fraction of clay minerals. The similarity of (a) and (b) with respect to most of the analysed elements implies that both belong to the same layer of deposition⁵. This result is significant because practically all previous age determinations⁶⁻⁹ were done on samples from deeper strata (as, for example, sample (e)), but not from the thin brown top layer (b) of the travertine floor. These earlier works on Petralona speleothems attempted to correlate the massive base calcite stratum of the floor with the skull itself. However, a reliable stratigraphy with respect to the cranium has never been established. Consequently, a definite dating of this hominid was not possible.

For the present study, five different samples with clear stratigraphical relations to the hominid skull were chosen:

- (a) Calcite encrusting the hominid skull, which should yield a lower age limit for the hominid.
 - (b) Top calcite layer covering the floor.
- (c) Bone fragments of the hominid skull, which had accidentially come loose with the calcite encrustation (a).
- (d) Calcite 3-4 mm beneath the surface layer (b), which should yield an upper limit for the hominid.
- (e) Calcite 30-40 mm beneath the surface layer (b). We considered that it was essential not to investigate only the small bone fragments of cranium (c), because although dating of animal and human bones by ESR has been reported to, bones may suffer from recrystallization and the hydroxylapatite can partially convert into fluorine-apatite. Calcite, however, is generally accepted as a stable mineral and a 'closed system'.

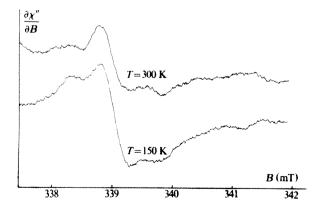


Fig. 1 ESR-spectra of bone fragments (c) of the Petralona hominid cranium, taken at 300 K and 150 K. $\nu = 9.5$ GHz, p = 3.7 mW, m = 50 mg.

Table 1 Trace element contents of calcite samples (a), (b) and (e), related to the hominid skull from Petralona Cave

Element	(a) (p.p.m.)	(b) (p.p.m.)	(e) (p.p.m.)
As	0.5	2.8	7.9
Co	1.7	1.3	0.02
Cr	13.3	14.3	0.32
Cs	1.9	1.1	0.01
Eu	0.09	0.08	0.004
Fe	4,700	4,600	140
K	87.4	377	≤10
Mn	42.3	163	0.44
Na	30.3	164	18
Rb	10.3	9.6	≤1
Sb	0.93	0.65	0.05
Sc	1.17	1.01	0.05
Th	1.84	1.39	<0.03 ≤0.1

Trace element concentrations determined by instrumental neutron activation analysis (INAA). There were two successive neutron irradiations of 3 min and 12 h: thermal neutron flux was 8×10^{13} neutrons cm⁻² s⁻¹. Sample weights were ~150 mg, and average errors $\pm 10\%$.

Of the modern techniques such as thermoluminescence $(TL)^{11,12}$, electron spin resonance $(ESR)^{13-15}$ and uranium series dating 16-18, which are suitable for dating Quaternary calcites, ESR seemed to be the most advantageous for the following reasons: (1) The presumed ages of all samples are beyond the limits of radiocarbon dating. (2) Only milligramme amounts of most samples were available, so that the low U content (<1 p.p.m.) precludes the use of 230 Th/ 234 U-dating. (3) The high opacity of the bone and of some calcite samples is disadvantageous for TL-dating. The presence of a small clay fraction (of the order of a few parts per thousand) would cause strong interference with the TL-signal 11. The principle of ESR-dating has been described in detail elsewhere 10,13-15. In brief, ESR measures the concentration of paramagnetic electrons in defects of the crystal lattice, created by interactions with the natural radiation since the time of (speleothem) deposition. In the present study, ESR measurements were performed by means of a 'Bruker' spectrometer (BER 420) at a microwave frequency of 9 GHz (X-band) and a typical microwave power of 2 mW. The weighed samples were positioned in a variable, homogeneous magnetic field of $\sim 300 \text{ mT}$.

For ESR-dating, ~ 1 g of each sample was cleaned from the adherent clay or bone impurities, ground, etched for 10 min in 0.1 M HCl and separated from the fine-grain fraction by decanting off the supernatant, milky solution. Portions of identical weight (generally 50–150 mg) were exposed to different γ -doses between 10 and 100 krad (1 krad = 10 J kg⁻¹) using a calibrated 6,000 Ci (1 Ci = 3.7 \times 10¹⁰ s⁻¹) ⁶⁰Co γ -source. One portion of each set of samples was left unirradiated to obtain the ESR-signal of the natural radiation dose, that is the 'archaeological' dose. The γ -irradiated samples were either left to stand for at least 1 day or were kept at 100 °C for 1 h to remove the interfering (unstable) ESR signal, which presumably corresponds to the \sim 80 °C TL glow peak.

The specific ESR-signal (proportional to the radiation dose) was sometimes subject to interference from other resonances which did not increase linearly with γ -doses (that is, it showed strong saturation effects). This problem was overcome by lowering the temperature of the sample to $\sim 150 \, \text{K}$ using a helium gas-flow cryostat. At this temperature, not only was there better discrimination, but also a significant enlargement of the radiation-induced ESR signal. This signal enhancement is shown in Fig. 1 for the bone fragments (c).

To ensure that the radiation-induced ESR-signal (used for dating) had been stable for at least some 10^6 yr, annealing experiments were carried out. The initial disappearance of the ESR signal between $200\,^{\circ}$ C and $250\,^{\circ}$ C indicates a correlation with the $230\,^{\circ}$ C TL glow peak of natural calcite. This TL signal, which shifts up to $\sim\!275\,^{\circ}$ C when using a fast heating rate, has already been studied by Wintle¹⁹. Isothermal annealing experiments performed on ESR active defects chosen for dating

resulted in exponential signal decays with time constants τ according to an Arrhenius law $\tau = \tau_0 \exp(E_a/kT)$ with a pre-exponential $\tau_0 = 3.8 \times 10^{-14} \, \mathrm{s}$ and a trap depth $E_a = 1.65 \pm 0.1 \, \mathrm{eV}$. This allows us to deduce mean defect annealing times of $\tau = 9 \times 10^7 \, \mathrm{yr}$ and $3 \times 10^8 \, \mathrm{yr}$ for storage temperatures of 15 °C and 10 °C, respectively. These data are in good agreement with the mean lives given by Wintle for TL-dating¹⁹. Thus speleothem dating should be possible using ESR techniques up to a few million years without significant corrections for annealing, particularly as no anomalous fading is observed in the TL of calcite¹².

The heights of the radiation-induced ESR signals were recorded as well as one of the six absorption lines of Mn^{2+} , which are present in practically all ESR spectra of natural calcites. It has been suggested that these lines be used instead of sample weight for the calibration of the radiation-induced ESR-signals. Where possible, we applied both methods of calibration to evaluate the γ -equivalent archaeological doses (EDs).

In the same way the γ -equivalent archaeological doses (EDs) were determined for the other four samples (b)–(e). Results are listed in Table 3.

To obtain the formation ages in years, the EDs are divided by the corresponding radiation doses per year. To determine these annual doses requires that for each sample (see Table 2): (1) the U, Th, K and Rb contents have to be analysed; (2) posssible disequilibria in the natural decay series have to be considered; (3) the efficiency of α -radiation (relative to an identical γ -dose) with regard to the radiation-induced ESR signal has to be determined; (4) the external γ -dose rates at the original sites of the samples must be measured.

U-contents were established by fission-track analysis, using NBS reference glass standard SRM 614. Th, K and Rb contents were determined by INAA and K was independently measured by flame photometry.

An α -spectrometric determination of the $^{230}\text{Th}/^{234}\text{U}$ -disequilibria was not possible for the most useful samples (a)-(d) (directly related to the skull) because of the small amounts and U-content (see Table 2). U-series dating of the massive base travertine (e) yielded an age of $\geq 300,000\,\text{yr}$. The growth of ^{230}Th (which is initially absent in speleothem) and its short-lived daughter nuclides (which are high-energy emitters) towards equilibrium results in a continuously increasing annual dose. Hence, the average annual dose was calculated iteratively with an equation derived by Wintle⁸. ^{222}Rn losses are taken to be negligible for all samples.

For ESR-dating of calcites, there have been no data on the efficiency of an α -radiation dose (relative to a β - or γ -dose) for the generation of unpaired, ESR-active electrons. We thus carried out this calibration on the calcite encrustation of the skull (a) by means of a standardized ²⁴¹Am α -source. The α -efficiency determined for sample (a) was $30 \pm 6\%$ and this value was also used for sample (b) in the same growth horizon.

The external γ-doses were measured by CaSO₄: Dy TL-dosimeters (Harshaw TLD control 4378-32) (ref. 5). Dose rates

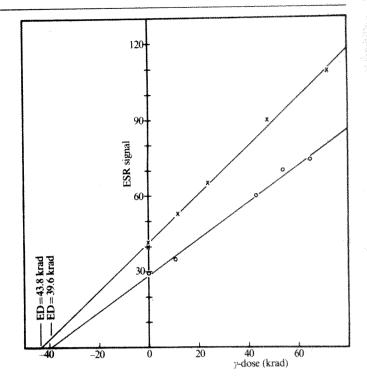


Fig. 2 Determination of the archaeological doses equivalent to ⁶⁰Co γ-irradiation (EDs) of the calcite encrustation (a) of the Petralona hominid skull from two sets of samples. ×, 150 K, 70 mg, calibration by Mn²⁺-signals; ○, 300 K, 150 mg, calibration by weight.

in Petralona Cave varied between 30 mrad yr⁻¹ in some 20-30cm deep boreholes in pure calcite floors and up to ~200 mrad in clay-rich areas. At the site of discovery of the skull an external dose rate of 190 ± 20 mrad yr 1 was recorded on the surface of the travertine floor. The 1 mm polyethylene walls of the dosimeters containing the CaSO₄: Dy partly absorb lowenergy y-rays and thus do not allow total y-dose determinations. In addition, α - and β -radiation from airborne radionuclides (for example, radon and daughter products), which are not recorded by the CaSO₄: Dy monitors, might have also affected the top layers. Hence, for the top layers (a) and (b) the given total dose-rate values may even be underestimated. The external dose rate 3-4 mm beneath the surface (as for samples (c) and (d)) was estimated to be 170 mrad yr⁻¹ because of shielding which was confirmed by laboratory experiments. At various locations, 5-10 cm deep in the travertine floor dose rates varied between 70 and 110 mrad yr⁻¹. Thus a value of 90 ± 20 mrad vr⁻¹ was taken for sample (e).

Although it cannot be proved for any ESR- or TL-dating, the external dose rate is commonly assumed to have remained

	Table 2 Dose rates	per year for the investig	gated samples (a)-(e)		
	(a)	(b)	(c)	(d)	(e)
U-content (p.p.m.)	0.066	0.119	0.418	0.261	0.231
Mean* annual dose (mrad yr 1)	5.0 ± 0.4	9.0 ± 1.4	39.3 ± 16.0	24.5 ± 10.0	35.5 ± 14.5
Th-content† (p.p.m.)	1.84	1.39	0.3	≤ 0.1	≤ 0.1
Annual dose (mrad yr ⁻¹)	14.70	11.1	2.4	≤ 0.8	≤0.8
K-content (p.p.m.)	87	377	139	15	≤ 10
Annual dose (mrad yr ⁻¹)	0.9	4.0	1.2	0.1	≤ 0.1
F intermed done note (mand us-1)	Σ 21 ± 1	Σ 24 ± 1	Σ43±16	$\Sigma.25\pm10$	Σ 36 ± 15
Σ = internal dose rate (mrad yr ⁻¹) External dose rate (mrad yr ⁻¹)	190±20	190±20	170 ± 20	170 ± 20	90±20
Total dose rate (mrad yr ⁻¹)	211 ± 21	214 ± 21	213 ± 36	195 ± 30	126 ± 35

Internal dose rates are calculated from the U-, Th- and K-contents on the basis of the tables of Bell^{21,22}. Dose rate contribution by $Rb \le 0.2$ mrad yr⁻¹ throughout.

*The mean annual dose contributions by U-series nuclides take into account ²³⁴U/²³⁸U- and ²³⁰Th/²³⁴U-disequilibria of ~1.1 and ~0.85, respectively, for samples (a)-(d), according to an age of ~20,000 yr. Secular equilibrium was determined for sample (e) by α -spectrometry. The α -efficiency of $30 \pm 6\%$ evaluated for sample (a) was also used for (b) of the same growth horizon. For samples (c), (d), and (e) the total range of α -efficiencies of 20% to 60% was applied, which have been evaluated in TL-dating of various stalagmites by Wintle⁸ and Bangert (personal communication).

tα-contributions to dose rates are neglected for Th-series, because the bulk of Th is in detrital aggregates (for example, in clay particles).

Table 3 Archaeological y-equivalent doses (EDs), annual doses and ESR age date evaluated for the bone fragments from the Petralona hominid cranium (c) and interrelated calcite strata (a), (b), (d) and (e)

Sample	ED* (ESR) (×10³ rad)	Annual dose (mrad yr ⁻¹)	ESR age (kyr)
(a)	$41.7^{+} \pm 4.2$	211 ± 21	198 ± 40
(b)	$41.8^{\dagger} \pm 4.2$	214 ± 21	195 ± 40
(c)	27.1 ± 2.7	213 ± 36	127 ± 35
(d)	38.6 ± 3.9	195 ± 30	198 ± 50
(e)	$81.7^{+} \pm 12.3$	126 ± 35	\sim 650 ± 280

^{*}Temperature of ESR-measurements is 150 K; linear regression coefficients of ESR growth curves ≥0.99 throughout.

constant since the time of speleothem deposition. For the Petralona cranium this is probably true, because the place of discovery is at the head of a 'dead-end' branch of the cave², where there will be hardly any air movement.

The y-dose rates monitored in Petralona cave by a portable NaI-scintillometer yielded smaller variations in the annual γ -doses (57-91 mrad yr⁻¹) (ref. 20) than those recorded by CaSO₄: Dy-monitors (~30-200 mrad yr⁻¹) (U. Bangert, personal communication). This is due to a higher spatial resolution of the CaSO₄: Dy-dosimeters in recording the γ -dose rates which generally show considerable variations (on a centimetre scale) vertical to the speleothem growth horizons.

From the total dose rates given in Table 2, we deduce absolute ages from the y-equivalent archaeological doses (EDs) (see Table 3).

The ESR-ages of samples (a)-(d) are concordant within their limits of errors and sample (e), which is obviously much older, is not related to the hominid skull. The lower age of the bone fragments (c) compared with (a), (b) and (d) may be due to a partial recrystallization of the apatite crystral lattice of the bone.

Our ESR ages in Table 3 consistently indicate an age of ~200,000 yr for the Petralona cranium. Taking into account the uncertainties of location, the technical difficulties and the controversial interpretations of the cranium, this result may clarify the phylogenetical position of the Petralona hominid, which lies between 160,000 and 240,000 yr BP.

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- 1. Poulianos, A. N. Archaeology 24, 6-11 (1971)
- Poulianos, A. N. Anthropos 7, 7-11; 13-29 (1980). Sickenberg, O. Geol, Geophys. Res. Inst. Athens 9, 1-16 (1964).
- Sickenberg, O. Ann. Geol. Pays. Hellen. 23, 230-264 (1971). Hennig, G. J. thesis, Univ. Cologne (1979).

- Hennig, G. J. thesis, Univ. Cologne (1979).
 Ikeya, M. Anthropos 7, 143-151 (1980).
 Schwarcz, H. P., Liritzis, Y. & Dixon, A. Anthropos 7, 152-173 (1980).
 Hennig, G. J., Bangert, U., Herr, W. & Poulianos, A. N. Anthropos 7, 215-241 (1980).
 Liritzis, Y. Anthropos 7, 215-241 (1980).
 Ikeya, M. Science 207, 977-979 (1980).
 Bangert, U. & Hennig, G. J. PACT J. 3, 281-289 (1979).
 Williah C. Cont. Engl. 5: 15, 1977, 1986 (1978).

- Wintle, A. G. Can. J. Earth Sci. 15, 1977-1986 (1978).
 Zeller, E. J., Levy, P. W. & Mattern, P. L. Proc. Symp. Radioactive Dating and Low Level Counting, 531-540 (IAEA, Vienna, 1967) 14. Ikeya, M. Nature 255, 48-50 (1975).
- Ikeya, M. Archaeometry 20, 147-158 (1978).
 Harmon, R. S., Thompson, P., Schwarcz, H. & Ford, D. Nat. Speleol. Soc. Bull. 27, 21-33
- 17. Hennig, G. J., Bangert, U. & Herr, W. Br. Mass. Occas. Pap. 21, 73-78 (1980).
- Schwarcz, H. P. Archaeometry 22, 3-24 (1980).
 Wintle, A. G. J. Electrostat. 3, 281-288 (1977).
 Liritzis, Y. & Poulianos, A. N. Anthropos 7, 252-259 (1980).
- Bell, W. T. Archaeometry 18, 107-111 (1976).
- 22. Bell, W. T. Archaeometry 21, 243-245 (1979).

Isotopic evidence for prehistoric subsistence change at Parmana, Venezuela

Nikolaas J. van der Merwe*, Anna Curtenius Roosevelt† & J. C. Vogel‡

- * Department of Archaeology, University of Cape Town, Rondebosch 7700, South Africa
- † Museum of the American Indian, Heye Foundation New York, New York 10032, USA
- ‡ National Physical Research Laboratory, PO Box 395, Pretoria 0001. South Africa

Recent archaeological results from Parmana, Venezuela, suggest that maize became a staple along the Orinoco River between about 800 BC and AD 400, contrary to prevailing views that maize was not important in prehistoric Amazonia. In maize, carbon dioxide is initially fixed as a C4 carboxylic acid, rather than as phosphoglyceric acid as in C₃ plants. The organic carbon constituents of C₄ plants have a different ¹³C/¹²C ratio from C₃ plants, and this difference is transmitted to animals dependent on maize as a major carbon source. Thus, the 13C/12C ratio of animal remains is a reflection of their diet. Stable carbon isotope measurements on human skeletons from Parmana reported here show that a dramatic shift in the prehistoric diet from dependence on C₃ plants to C₄ plants (which include maize) did take place.

Students of Greater Amazonian cultural development commonly assume that the 'tropical forest' system was the major subsistence system of prehistoric times. In this system most calories are provided by cultivated manioc (that is, cassava) and most protein by hunting and fishing. In the Amazonian forests, where soils are poor and game scarce, tropical forest culture is associated with small, autonomous villages. The development of large, populous chiefdoms in the Amazon and Orinoco mainstreams in late prehistoric times is usually attributed to the higher productivity of the floodplains for tropical forest subsistence1

An alternative argument holds that manioc cultivation and animal capture could not have supported dense populations in floodplains and that larger populations were based on the cultivation of seed crops, particularly maize⁴. Although maize is a minor crop in present Amazonian tropical forest societies, several ethnohistoric accounts⁵⁻⁸ describe it as an important staple in floodplain subsistence. Recent archaeological fieldwork in the Parmana area of the Orinoco basin, Venezuela, suggests that maize may have replaced manioc as the major staple along the Orinoco River during the Corozal phase, between about 800 BC and AD 400, and that the population increased 15-fold during this period.

To assess the archaeological and isotopic evidence for a change in prehistoric diet it is necessary to know the potential human food sources in the Parmana ecosystem, their carbon isotope ratios, and the potential productive capacity of the local tropical forest system as compared with one based on seed crop cultivation. The plants relevant to the discussion are all C₃ or C₄ plants, which have markedly different 13C contents9-11. Cumulative assessments of ¹³C/¹²C ratios in plant foliage, expressed as per mil (%) deviations in 13 C (δ^{13} C) from the Chicago PDB standard 12 , yield mean values of -26.5% for C_3 and -12.5% for C₄ plants, with no overlap between their ranges of values¹³⁻¹⁵ When animals and humans eat plants, these characteristic isotope ratios are registered in their tissues¹⁶, with further fractionation occurring during tissue formation^{17,18}. A fractionation value of +5.1% in human bone collagen, established for prehistoric hunter-gatherers in the North American woodlands 19, is used here for calculations.

Maize (Zea mays) is a C_4 plant. A suitable average $\delta^{13}C$ value for maize kernels based on multiple measurements is not avail-

Mean value of 2 EDs (calibration by weight and Mn²⁺ signals, respectively).

Table 1 δ^{13} C values of some manioc (Manihot esculenta) root products

Laboratory no.	Sample description	δ ¹³ C (%)
UCT-480	Manioc cake purchased in New York City; probable origin the Dominican Republic	-25.6
UCT-481 UCT-483	Same as UCT-480, another example Manioc root, Nkhata Bay, Malawi	-26.0 -25.0

Precision of δ^{13} C is $\pm 0.1\%$.

able, so the average value for C₄ foliage (-12.5%) will be used here. Manioc (Manihot esculenta) is a C₃ plant, as shown by the δ^{13} C values in Table 1. The average for three samples from the Dominican Republic and Malawi is -25.5%, near the C₃ plant average. The samples were presumably cultivated in open fields, however, where the air is well mixed and the CO_2 has the $\delta^{13}C$ value of atmospheric CO₂ at -7%. In dense forests, restricted air circulation and CO2 from rotting leaf litter combine to deplete the 13C content of CO2 available for photosynthesis20. In the Amazonian rain forest, this canopy effect results in leaves of the upper canopy having δ^{13} C values of about -30%, while the undergrowth averages about -35% (ref. 21). The severity of the canopy effect can be expected to vary with forest density. Thus, the effect would be slightly less marked in tropical gallery forests, and less marked still in small forest clearings like swidden fields. No measurements are available for these situations, but it can be assumed that forest plants will have δ^{13} C values more negative than the C₃ plant average of -26.5%.

There are two major components to the Parmana ecosystem-the terrestrial habitats of gallery forest and savannah, and the seasonally fluctuating aquatic habitats of river, stream and lagoon (for detailed refs see ref. 4). Significant human food sources in the natural environment include fauna, a few tree and shrub products, and root plants. Plant material in the ecosystem is predominantly C₃ and forest derived, with the exception of floating meadows, which are C₃ but grow in the open, and savannahs, which have some C4 grasses. The terrestrial fauna include a few grazers which may eat C4 grasses, but are small in biomass and of relatively slow turnover. The aquatic fauna, especially the fish, make up the only substantial intensifiable human food source; they feed on forest-derived plant detritus. Thus, most carbon available to humans from wild sources is derived from C₃ plants and is strongly ¹³C depleted; only a miniscule proportion of the carbon could come from C₄ plants.

The archaeological sequence consists of three ceramic traditions: La Gruta, Corozal and Camoruco^{4,22-26}. Excavations by Roosevelt in 1974 and 1975 provided information on prehistoric subsistence and population⁴. For La Gruta (2100-800 BC), tropical forest subsistence is indicated by ceramic griddles, small stone chips possibly from manioc graters, projectile points and poorly preserved faunal remains. Excavation produced carbonized plants apparently of wild species only (S.G. Stephens and H. Pinkley, personal communication). Population of the 500 km² area was small throughout the period, estimated at 120 or 0.24 per km² (based on a refuse area of 1.5 ha and comparison with ethnographic settlements). The subsistence of Corozal (~800 BC-AD 400) is characterized by the introduction of maize and transition to intensive maize cultivation, with continuation of tropical forest subsistence. Carbonized maize appears for the first time early in the period, becoming common by its end. Possible domestic legumes, as yet unidentified, occur in small quantities throughout Corozal, and possible maize-grinding tools appear. Estimated population for early Corozal is 560 or 1.1 per km² and 1,750 or 3.5 per km² for late Corozal (based on refuse areas of 7.5 and 23.3 ha, respectively). The Camoruco period (AD 400-1500) is characterized by intensive maize cultivation, with manioc cultivation and hunting and fishing probably making a small contribution to calories and protein. Maize remains were abundant, and Canavalia, a tropical bean, was identified (L. Kaplan, personal communication). During this period population stabilized at about 1,800 or 3.6 per km², increasing to about 2,500 or 5 per km² between AD 1100 and 1500 (based on refuse areas of 24.1 and 34 ha, respectively).

The remains suggest a shift during Corozal from tropical forest subsistence, based on manioc, fish and game, to a system based primarily on maize, associated with a sizeable population increase. Maize is conclusively identified, based on carbonized plant remains (W. Galinat, personal communication), but identification of manioc is not conclusive, being based on presumed food-processing tools that could have been used for other foods²⁷. Manioc's presence will be unproven until direct botanical evidence can be found. Moreover, the archaeological finds do not establish the proportion of maize in the diet, due to poor preservation of other foods, especially manioc.

A shift from forest-based subsistence to a diet based on maize should produce a substantial change in the 13 C/ 12 C ratios of human tissues and should be detectable in bone collagen. Given a food web based primarily on plant material from the forest (δ^{13} C more negative than -26.5%) and enrichment in 13 C of 5.1% during collagen formation, human bone collagen should have a δ^{13} C value more negative than -21.4%. A hypothetical diet based entirely on maize or other C₄ plants (such as sorghum and millet) should yield bone collagen with a δ^{13} C value of -7.4%; mixed diets should produce intermediate values.

Stable carbon isotope measurements were made on the five human skeletons recovered from the Corozal site (Table 2); two specimens dated to the inception of Corozal (~800 BC) and three to the Corozal/Camoruco interface (~AD 400). Collagen was extracted from the postcranial remains and converted to CO2 in the Archaeometry Laboratory, University of Cape Town. The procedure involved slow decalcification in 2% HCl, washing in distilled water, vacuum drying and combustion. The bones were badly decomposed by the acid tropical soil, their appearance chalk-white and friable. All the postcranial remains of the child burial (UCT-267) had to be used to obtain the required 0.1 g of dried collagen. Mass spectrometric measurements of the ¹³C/¹²C ratios were made at the Pretoria Radiocarbon Laboratory and related to the Chicago PDB standard through a secondary standard. The results show a substantial isotopic difference between the early and late Corozal skeletons, the two early skeletons having an average δ^{13} C value of -26.0%, and the late group averaging -10.3%; the individual values in each group are closely similar, presumably the result of stable subsistence systems.

The average δ^{13} C values of early and late Corozal diets can be calculated by subtracting the collagen enrichment value of 5.1% from the results in Table 2. The early Corozal diet averaged -31.1%, considerably more negative than the C_3 plant average.

Table 2 δ¹³C values for collagen from human skeletons excavated at Corozal in the Parmana area

Laboratory no.	Provenence	δ ¹³ C (%)
	Late Corozal, ~AD 400	
UCT-259	Skeleton 2 (adult), Exc. 3, level 18- 22, prov. 543	-9.7
UCT-258	Skeleton 3 (adult), Exc. 3, level 18- 22, prov. 544	-10.5
UCT-286	Skeleton 4 (adult), Exc. 3, level 18- 22, prov. 544	-10.8
	Average $(n=3)$	-10.3
	Early Corozal, ~800 BC	
UCT-260	Adult skeleton, centre burial, Exc. 2, level 16-17, prov. 293	-26.1
UCT-287	Child's skeleton, Exc. 2, level 18, prov. 545	-25.8
	Average $(n=2)$	-26.0

Early Corozal skeletons are associated archaeologically with a tropical forest subsistence system (probably manioc, fish and game), Late Corozal skeletons with maize, legumes and probably less manioc and animal food. Precision of $\delta^{13}\mathrm{C}$ is $\sim 0.1\%$.

This result could only have been achieved through a food web which was based primarily on forest-derived plant material, probably to the near exclusion of anything else. Local fauna and manioc raised in the forest would produce such a result, although we cannot prove that manioc was involved. By contrast, the late Corozal diet had an average δ^{13} C value of -15.4%, heavily dominated by C₄ plants. The most likely explanation for this change is the introduction of maize cultivation. No other C4 staple crops are known for the area, nor can a massive increase in the human diet of animals raised on C4 plants be invoked to explain the results, as the faunal remains are primarily of fish (E. Wing, A. Garson and E. Simons, personal communication). If one adopts the explanation that maize cultivation was involved, the proportion of maize in the diet can be calculated. This yields a range of possible results, depending on the food web constructed and the δ^{13} C values used. If the diet consisted entirely of seed crops raised on the floodplain, where normal atmospheric CO2 was available for photosynthesis, it needed to include 80% maize and 20% legumes (δ^{13} C = -26.5%) to achieve the observed results. If some forest-derived plants or animals were included (average δ^{13} C value -31.1%), the maize component needed to be slightly more. Populations depending on maize components >80% are common in the modern Third World²⁸

We conclude that the prehistoric peoples of Parmana maintained a forest-derived subsistence system, probably based mainly on manioc calories and animal protein, until about 800 BC or a little later. By ~AD 400 their diet had changed dramatically to include a maize component of perhaps 80% or more. This change allowed for a 15-fold increase in population, which then stabilized at the higher level. These conclusions help to explain how large concentrations of people and complex societies could have developed in Amazonia.

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- Gross, D. R. Am. Anthrop. 77, 526-549 (1975).
 Morey, R. & Marwitt, J. in War, Its Causes and Correlates (eds Nettleship, M. A., Givens, R. D. & Nettleship, A.) 434-450 (Mouton, The Hague, 1975). Lathrap, D. W. The Upper Amazon (Praeger, New York, 1970).
- 4. Roosevelt, A. C. Parmana: Prehistoric Maize and Manioc Subsistence along the Amazon and Orinoco (Academic, New York, 1980).
- 5. de Acuna, C. Collecion de Libros que Tratan de American Raros o Curiosos Vol. 2 (Garcia, Madrid, 1891).
- 6. de Heriarte, M. Faksimile-Ausgabe Mss 5880 und 5879 der Osterreichischen National-Bibliothek, Vien (Academische Druck und Verlaganstalt, Graz 1964).
 7. Gumilla, J. El Orinoco Ilustrado, Historia Natural, Civil y Geografica de este Gran Rio
- (Biblioteca de la Presidencia de Colombia, No. 8, Editorial ABC, Bogota, 1955 8. Meggers, B. J. Amazonia: Man and Culture in a Counterfeit Paradise (Aldine, Chicago,
- Hatch, M. D., Slack, C. R. & Johnson, H. S. Eiochem. J. 102, 417-422 (1967). 10. Park, R. & Epstein, S. Geochim. cosmochim. Acta 21, 110-126 (1960)
- 11. Smith, B. N. & Epstein, S. Pl. Physiol. 47, 380-384 (1971). 12. Craig, H. Geochim. cosmochim. Acta 3, 53-92 (1953).
- 13. Silberbauer, F. B. thesis, Univ. Cape Town (1979
- Siberbauer, F. B. Hiesis, Univ. Cape Town (1979).
 Vogel, J. C., Fuls, A. & Ellis, R. P. S. Afr. J. Sci. 75, 209-215 (1978).
 Troughton, J. H. in Photosynthesis and Photorespiration (eds Hatch, M. D., Osmond, C. B. & Slatyer, R. O.) 124-129 (Wiley, New York, 1971).
 Vogel, J. C. & van der Merwe, N. J. Am. Antia, 42, 238-242 (1977).
 De Niro, M. J. & Epstein, S. Geochim. cosmochim. Acta 42, 495-506 (1978).
 Vogel, J. C. & Af. J. Sci. 74, 209-201 (1979).

- Vogel, J. C. S. Afr. J. Sci. 74, 298-301 (1978).
 van der Merwe, N. J. & Vogel, J. C. Nature 276, 815-816 (1978).
- Vogel, J. C. Oecol. Pl. 13, 89-94 (1978).
- Medina, E. & Minchin, P. Oecologia 45, 377-378 (1980).
- 22. Howard, G. Excavations at Ronquin (Yale University Publications in Anthropology, No.
- Roosevelt, A. C. in Unidad y Variedad: Ensayos Antropologicas en Homenaje a J. M. Cruxens (eds Wagner, E. & Zucchi, A.) 173-207 (Instituto Venezolanode Investigaciones Científicas, Caracas, 1978).
- Rouse, I. in Unidad y Variedad: Ensayos Autropologicas en Homenaje a J. M. Cruxent (eds Wagner, E. & Zucchi, A.) 203-229 (Instituto Venezolanode Investigaciones Científicas, Caracas, 1978).
- 25. Rouse, I. & Cruxent, J. M. Venezuelan Archaeology (Yale University Press, 1963)
- Vargas Arenas, I. in Proc. 6th Int. Congr. Study of Precolumbian Cultures of the Lesser Antilles, Guadeloupe, 123-124 (Université de Montreal, Centre de Recherches Caraibes,
- De Boer, W. Am. Antiq. 40, 419-433 (1975).
- 28. UN Food and Agriculture Organization Maize and Maize Diets: a National Survey (United Nations, 1953).

Relationship of aardvark to elephants, hyraxes and sea cows from α -crystallin sequences

Wilfried W. de Jong*, Anneke Zweers* & Morris Goodman†

*Department of Biochemistry, University of Nijmegen, 6500 HB Nijmegen, The Netherlands †Department of Anatomy, Wayne State University, School of Medicine, Detroit, Michigan 48201, USA

One of the great enigmas of mammalian phylogeny is the genealogical relationship of the aardvark (Orycteropus afer), the only living representative of the order Tubulidentata. Although now generally grouped close to the ungulates and paenungulates. 4, and thought to be derived from a common , and thought to be derived from a common condylarthran stock⁵⁻⁷, it still holds an isolated position among eutherian mammals. The evidence of the evolutionary relationships of the aardvark may be obscured at the morphological and anatomical level by the pronounced adaptations to its ant-eating and burrowing habits, and may be better preserved in its protein molecules, as direct copies of the genetic material. We have therefore now studied the amino acid sequence of the eve lens protein lpha -crystallin A of the aardvark, and compared it with the corresponding sequences from species representing 15 mammalian orders. Unique similarities were observed among the α -crystallin A sequences of aardvark and the paenungulates manatee, hyrax and elephant, suggesting that the closest relatives of the Tubulidentata are found in the paenungulate orders Sirenia (sea cows), Hydracoidea (hydraxes) and Proboscidea (elephants). Moreover, these orders do not seem to be derived from a common stem group with the ungulates, but might well be one of the first offshoots of the placental mammalian line.

α-Crystallin is a high molecular weight protein aggregate, essentially composed of two types of polypeptide chain, α A and αB, each about 175 residues long8. α-Crystallin occurs in considerable quantities in the eye lenses of all vertebrates9, and thus can readily be isolated and analysed. Sequence analysis of the α -crystallin A chain has previously been used to study phylogenetic relationships between 11 mammalian orders¹¹ The results mostly agreed with prevailing opinions about mammalian phylogeny, and prompted the investigation of the αA chains from species representing orders of more disputed affinity, like Edentata (sloths and anteaters)11, Pholidota

Phylogenetically informative positions in aardvark, manatee and other published mammalian α -crystallin A sequences^{10,11} Table 1

POSITION NR	3	4	3	Ē	÷	5	58	6	7	?	?	9	9	9	0	100	3	į	3	3	4.56	147	3 6	149		-502	145145	CACH	1	-47g	168	100
WHALE HORSE RHINOCEROS PIG OX DOG, CAT PANGOLIN HEDGEHOG RAT, TUPAIA RABBIT HUNAN MONKEY ELEPHANT HYRAX MANATEE 2-TOE SLOTH TAMANDUA KANGROO OPOSSUM	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		A I'm the summerment for fee fee management and the second	O O		A William A W Commenceron and a commenceron and	C)	recommendation of the property	K	> ····································	F	لے لیے لیے استسسست لیے لے لیے سسسسست	E		S	-	Z AAA	10 for for any and any and any and any	had part severes and anti-construction of the construction of the	()	. And had were bod announcemental maken had bed bed announcement	400	C)	The same of the sa	V	0 - 0 - 0	000000000000000000000000000000000000000	S more and the first management of the second	(1) A C C C C C C C C C C C C C C C C C C	A	S C Transport Control of the Control	(A)

Only those positions are shown at which substitutions have occurred in at least two of the investigated species. Full species names can be found in refs 10 and 11. Vertical lines indicate where residues are identical to the topmost sequence. Positions which determine the paenungulate branching pattern as shown in Fig. 2 are marked by an asterisk. The one-letter notation for amino acids is: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr; (-) indicates a deletion.

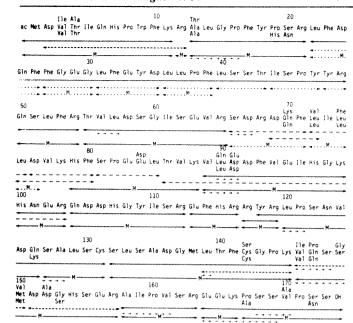


Fig. 1 Summary of sequence analysis of aardvark and manatee α -crystallin A chains. The central line represents the proposed sequence of aardvark α A. Residues which are different in the bovine α A chain¹⁸ are shown above the aardvark sequence and those residues in which the manatee α A chain differs from the bovine chain are given below the aardvark sequence. Positions 40-49 and 77-78 of the manatee sequence could not be determined, and a substitution Ser - Asn has occurred either at position 172 or 173. From three aardvark lenses and six manatee lenses, 36 mg and 12.5 mg, respectively, of α A chains could be isolated. The isolation and sequence analysis of the α A chains followed previously detailed methods¹⁹. The procedure is largely based on amino acid analyses of small peptides, which are compared with corresponding ones of the completely sequenced bovine αA chain¹⁸. When amino acid compositions of homologous peptides are found to be the same, it is assumed that their sequences are also. Peptides which differ in composition from corresponding bovine ones are, if possible, subjected to dansyl-Edman degradation to localize the positions of substitutions. The inaccuracy in the sequences so deduced is very small²⁰. This figure shows by different underlinings the peptides from which satisfactory amino acid analyses could be obtained. Peptides and residues within these peptides are mainly ordered by homology with the known bovine sequence18. The positions of a number of residues were determined by dansyl-Edman degradation $(\rightarrow \rightarrow)$. Aardvark αA chain: Tryptic peptides $(\leftarrow \rightarrow)$ were isolated by peptide mapping and gel filtration¹⁹. The large tryptic peptide 22-49 was further digested with thermolysin and the resulting small peptides (←···→) isolated and analysed. From a tryptic digest of citraconylated cyanogen bromide fragment (residues 2-138) was isolated peptide 2-12 --→) and a large peptide containing residues 66-104. The latter peptide was decitraconylated and digested with chymotrypsin (-which resolved the sequence 66-88. Cyanogen bromide fragments corresponding to residues 139-150 and 151-173 were digested with trypsin. The resulting peptides $(\leftarrow ---\rightarrow)$ covered the region 146-157. Manatee α A chain: Soluble tryptic peptides (\leftarrow M \rightarrow) were isolated by peptide mapping. A thermolytic digest of the water-insoluble tryptic core yielded peptides (← · · · M · · · →) which resolved residues 22-39 and the important region 66-76

(pangolins)¹¹ and now the Tubulidentata. Although the relationship of Sirenia to the other paenungulates has not been questioned¹⁻⁷, we, nevertheless, report here the α A sequence of the Brazilian manatee (*Trichechus inunguis*) because it increases the body of relevant data on which to base our phylogenetic inferences.

 α -Crystallin was isolated from three aardvark lenses and six manatee lenses, and the sequences of the αA chains were analysed as shown in Fig. 1. Comparison of the proposed aardvark and manatee αA sequences with the data set of other αA chains (Table 1) clearly reveals that both sequences share three or four apparently derived substitutions with hyrax and elephant: $70 \cdot \text{Lys} \rightarrow \text{Gln}$, $72 \cdot \text{Val} \rightarrow \text{Leu}$ (not present in elephant), $74 \cdot \text{Phe} \rightarrow \text{Leu}$ (also in the edentate Tamandua) and $142 \cdot \text{Ser} \rightarrow \text{Cys}$ (also in man). No substitutions are uniquely shared with any other particular eutherian order. The aardvark and manatee αA chains each have in addition a relatively large number of unique substitutions: four in the aardvark (84 Glu, 149 Ser, 152 Asp, 170 Val) and five in the manatee (19 His, 20 Asn, 126 Lys, 167 Ala, 172 or 173 Asn).

To assess the sequence relationships of the aardvark and manatee a A chains more objectively and quantitatively, trees were constructed for the available a A sequences, using a maximum parsimony approach 12,13. The fewest possible number of nucleotide replacements (NRs) to arrange the 23 mammalian sequences, and the chicken and frog (Rana esculenta) sequences¹⁰ as outgroups, in an ancestral branching order, was found to be 133. Several equally parsimonious trees could be constructed which, however, all agreed in the positioning of the aardvark and the place of the paenungulates in relation to the other mammalian orders (Fig. 2). These most parsimonious trees clearly suggest a monophyletic origin of aardvark and paenungulates. Indeed, to separate the aardvark from the paenungulate branch, and place it at the base of the edentate line or as a separate branch next to edentates and paenungulates. costs an additional 4 NRs. All other positions are even less parsimonious. The most parsimonious solutions depict the elephant as the first offshoot of the paenungulates, due to the substitution 72 Val → Leu in hyrax, manatee and aardvark (Fig. 2). There is good morphological evidence to place elephant and manatee closest together¹⁻⁶. This would require one additional substitution, most probably a back substitution 72 Leu → Val in the elephant line. Faster evolving proteins, like myoglobin and haemoglobin, might provide better evidence on the phylogenetic relationships among the living paenungulate orders.

The suggestion that the Tubulidentata should be included in a monophyletic superorder Paenungulata is supported by some independent osteological and immunological findings¹⁴. Significant morphological resemblances of aardvark to hyraxes and elephants has already been noted¹⁵. Interestingly too, Tubulidentata, Hyracoidea, Sirenia and Proboscidea all seem to be indigenous African orders, which came into existence during the early Tertiary isolation of the continent⁵⁻⁷.

In agreement with our previous results ^{10,11}, the Edentata and Paenungulata are consistently placed as the oldest offshoots from the main eutherian line (Fig. 2). This is mainly due to the absence in these orders of the substitutions 91 Asp → Glu and 153 Ser → Gly, shared by almost all other eutherian orders (Table 1). Such an early divergence of the edentates is in agreement with recent opinions about their phylogenetic position^{3,16}. More surprising is the ancient separation of a paenungulate branch, far from the ungulates. It would in fact cost at least 5 additional NRs to place the paenungulates and aardvark in the ungulate—cetacean region of the tree. Although the paenungulates are usually grouped among the ungulate orders, Romer⁵ states that it is "far from certain that they form a single natural group".

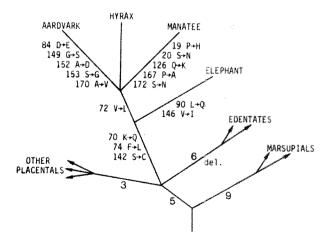


Fig. 2 The aardvark-paenungulate region of the most parsimonious phylogenetic trees constructed on the basis of α -crystallin A sequences from 23 mammalian species (Table 1), and from chicken and frog as outgroups. Several equally parsimonious trees are possible, which differ in the precise branching pattern of the other placental orders. The inferred amino acid substitutions in the aardvark and paenungulate lines are indicated. Numbers of nucleotide replacements are given in neighbouring branches. A deletion of three residues occurs in the edentate α A chains.

No other protein sequence data are available from aardvark, hyrax and manatee to corroborate our proposals. However, the sequences of elephant β -haemoglobin, myoglobin and fibrinopeptides are known and also fail to group the Proboscidea with the ungulates 17

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- Simpson, G. G. Bull. Am. Mus. nat. Hist. 85, 1-350 (1945).
- Van Valen, L. Evolution 25, 420-428 (1971)
- McKenna, M. C. in Phylogeny of the Primates (eds Luckett, W. P. & Szalay, F. S.) 21-46 (Plenum, New York, 1975).
- Szalay, F. S. in Major Patterns in Vertebrate Evolution (eds Hecht, M. K., Goody, P. C. & Hecht, B. M.) 315-374 (NATO Advanced Study Institute, Plenum, New York, 1977).
 Romer, A. S. Vertebrate Paleontology 3rd edn (University of Chicago Press, 1966).
 Thenius, E. Phylogenie der Mammalia (de Gruyter, Berlin, 1969).
- Patterson, B. in Evolution of African Mammals (eds Maglio, V. J. & Cooke, H. B. S.) 268-278 (Harvard University Press, 1978).
- Bloemendal, H. (ed.) Molecular and Cellular Biology of the Eye Lens (Wiley-Interscience, New York, 1981).

- New York, 1981).
 Clayton, R. M. in The Eye Vol. 5 (ed. Davson, H.) 399-494 (Academic, New York, 1974).
 De Jong, W. W., Gleaves, J. T. & Boulter, D. J. molec. Evol. 10, 123-135 (1977).
 De Jong, W. W., Zweers, A., Joysey, K. A., Gleaves, J. T. & Boulter, D. in The Evolution and Ecology of Sloths, Anteaters and Armadillos (ed. Montgomery, G. G.) (Smithsonian Louising Page Westlemen DC in Armadillos (ed. Montgomery, G. G.) Institution Press, Washington DC, in the press).
- 12. Moore, G. W., Barnabas, J. & Goodman, M. J. theor. Biol. 38, 459-485 (1973)
- 13. Moore, G. W. in Molecular Anthropology (eds Goodman, M. & Tashian, R. E.) 117-137 (Plenum, New York, 1976)
- 14. Shoshani, J., Goodman, M. & Prychodko, W. Am. Zool. 18, 601 (1978)
- Shoshani, J., Goodman, M. & Prychodko, W. Am. Zool. 18, 601 (1978).
 Le Gros Clark, W. E. & Sonntag, D. F. Proc. zool. Soc. Lond. 30, 445-485 (1926).
 Engelmann, G. F. in The Evolution and Ecology of Sloths, Antesters and Armadillos (ed. Montgomery, G. G.) (Smithsonian Institution Press, Washington DC, in the press).
 Dene, H., Goodman, M. & Romero-Herrera, A. E. Proc. R. Soc. B207, 111-127 (1980).
- Van der Ouderaa, F. J., De Jong, W. W. & Bloemendal, H. Eur. J. Biochem. 39, 207-222
- De Jong, W. W. & Terwindt, E. C. Eur. J. Biochem. 67, 503-510 (1976).
 Van Druten, J. A. M., Peer, P. G. M., Bos, A. B. H. & De Jong, W. W. J. theor. Biol. 73, 549-561 (1978).

Experience required for pheromone recognition by the apple maggot fly

Bernard D. Roitberg & Ronald J. Prokopy

Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003, USA

Remarkable progress has been made during the past 20 yr in the identification of chemical components and receptor sites of pheromones of various insects. However, our understanding of the physiological and ecological parameters which affect the response of individual insects to pheromones remains rather limited. Here we report that in apple maggot fly, Rhagoletis pomonella, females require experience with oviposition-deterring pheromone (ODP) before they are able to discriminate between ODP-marked and unmarked host fruit. This seems to be the first conclusive evidence in an insect that pheromone recognition may depend on previous contact with the particular

After egg laying in the fruit flesh, a R. pomonella female deposits ODP by dragging its ovipositor on the fruit surface One bout of dragging on a hawthorn (Crataegus sp.) fruit of ~5 mm diameter deters the same or other females from ovipositing in that fruit in the field, although the degree of deterrence is reduced in the laboratory. Prokopy suggested that the ODP elicits uniform egg distribution among larval food resources, a character which has high survival value in so far as only a limited number of larvae can mature in a single fruit.

We found that recently matured females deprived of host fruit until testing did not discriminate between ODP-marked and

unmarked hosts. In contrast, females that were allowed access to ODP-marked host fruit 1 day before testing generally rejected ODP-marked fruit (Table 1, expt 1). To determine whether these results stemmed from a difference between females in oviposition deprivation or to a difference in previous contact with ODP, we placed newly matured wild flies singly in cages and treated each fly in one of two ways.

In treatment A, each fly was presented with and allowed to oviposit in one clean (no ODP), uninfested hawthorn fruit. Immediately after oviposition, the fly was transferred using a small triangular piece of clean filter paper to a clean uninfested apple, where it was allowed to deposit ODP. We directed the fly's course of dragging on this much larger fruit so that it never contacted its own pheromone trail with its pheromone receptors (tarsal D-hairs)2.3. We repeated this procedure twice more at 45-min intervals on day 1. On day 2, we again repeated the procedure twice. Forty-five minutes after the second oviposition, we presented the fly with a 5-mm diameter hawthorn fruit marked with ODP from two dragging bouts by other females.

Treatment B flies were also allowed to oviposit in three hawthorn fruits and drag on three apples on day 1. However, the second and third hawthorn fruits offered were marked with ODP from three previous (~1 h earlier) dragging bouts. If the flies rejected the marked fruit, they were presented with clean fruit for oviposition. The procedure followed on day 2 was the same as for treatment A flies.

Treatment B (experienced) flies generally rejected ODPmarked fruit and accepted clean ones. Treatment A (naive) flies readily accepted ODP-marked fruit (Table 1, expt 2a). In addition, significantly more treatment A than treatment B flies rejected clean fruit presented to them after their rejection of a marked fruit. This experiment was repeated, with similar though less dramatic results (Table 1, expt 2b).

Prokopy4 reported that barometric pressure may affect the level of activity and ODP discrimination ability of apple maggot flies. In expt 2a and b we found that more flies completed the experimental protocol on days with moderate or high barometric pressure (≥29.7 mbar). When we reexamined data for expt 2b including only data from days in which we were able to test to completion at least four naive and four experienced flies, 71% (n = 14) of the naive flies accepted ODP-marked fruit compared with 40% (n = 10) which accepted on days when we were unable to test four naive and four experienced flies.

To determine whether flies could obtain necessary pheromone experience by contacting their own ODP trail while dragging, we repeated the experimental protocol, but did not transfer the flies to apples for dragging. All flies contacted their own trail an average of 4.4 times (per fruit) while dragging. In this case, treatment A flies rejected ODP-marked fruit as readily as did treatment B flies (Table 1, expt 3).

In a final test, we withheld experienced flies from ODP for 96 h (flies were allowed to oviposit and ODP drag, but not contact any ODP, three times on each of the 4 days) and found that they rejected ODP-marked fruit as readily as did recently experienced flies, thus demonstrating the retention of ability to recognize ODP for at least 4 days (Table 1, expt 4). Klomp et al. reported that Trichogramma embryophagum, a hymenopteran parasite of lepidopteran larvae, forgets and must relearn to discriminate between parasitized and non-parasitized hosts. However, they suggested that their results could be explained by the increasing tendency of the wasps to oviposit at increasing time of deprivation from oviposition sites.

Alcock's definition⁶ of 'restricted learning', that is, 'an animal acquires a limited piece of information from the environment that changes the behaviour of the animal in a precise manner', can be applied to the ability to recognize ODP by apple maggot flies. Electrophysiological tests show that the ODP receptors on the tarsi fire the first time they contact ODP (L. Bowdan, V. Dethier and R.J.P., unpublished data). However, our results show that such a message is not translated into a rejection response in naive flies. This mechanism may provide flies with a means of reducing the cost of continually maintaining an unused

Table 1 Response of apple maggot flies to ODP-marked hawthorn fruit before and after experience with ODP

Ex	periment	Treatment	n	% Rejection of ODP-marked fruit*	n	% Rejection of clean fruit after rejection of an ODP-marked fruit
	1	No previous host or ODP experience	17	$17.6 (P \le 0.001)$	5	40.0 (NS)
	1	Previous fruit and ODP experience	20	60	14	14.3
	2	Treatment A (naive)	29	$13.8 (P \le 0.001)$	17	$76.5 \ (P \le 0.001)$
	2 <i>a</i>	Treatment B (experienced)	28	64.3	20	10.0
	21		24	$41.6 \ (P \le 0.08)$	17	$41.2 (P \le 0.07)$
	2b	A	27	66.7	21	14.3
	•	B	16	75.0 (NS)	23	$47.8 \ (P \le 0.02)$
	3	A	18	88.8	18	11.1
		B	16	62.5 (NS)	10	20.0 (NS)
	4	96-h ODP deprivation No ODP deprivation	16	56.3	9	22.2

^{*} If a fly rejected ODP-marked fruit, we presented it with a clean fruit. If the fly also rejected the clean fruit we disqualified it from this analysis. NS, not significant.

information processing system, because flies may not encounter ODP-marked fruit in conditions of high fruit density, low fly population or when immature. However, once mature flies have oviposited in a single small hawthorn fruit (= native host fruit) they may gain, through tarsal contact with their own ODP trail, the pheromonal experience needed to activate the system.

We are carrying out further experiments to determine why naive apple maggot flies reject clean fruit more often after encountering ODP than do experienced flies.

We suggest that restricted learning of pheromone recognition may be more widespread than is believed. For example, Cammaerts-Tricot7 and Le Moli and Passetti8 suggested, but did not prove, that perception of pheromones by Myrmica and Formica ants, respectively, depends on experience, and Vinson et al.9 demonstrated associative learning of kairomonal ovipositional cues by Bracon mellitor, a parasitic wasp. van Lenteren and Bakker10 first demonstrated that Pseudeucoila bochei, a

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Prokopy, R. J. Envir. Ent. 1, 326-332 (1972).

Prokopy, R. J. & Spatcher, P. Ann. ent. Soc. Am. 70, 960-962 (1977).

Crajar, R., Dethier, V. G. & Prokopy, R. J. Proc. N.Y. ent. Soc. 86, 283-284 (1978).

Prokopy, R. J. in Management of Insect Pests with Semiochemicals (ed. Mitchell, E. R.) (Plenum, New York, in the press).

hymenopteran parasite of Drosophila larvae, must 'learn' to discriminate against parasitized hosts. Their results strongly suggest that the key component in the learning process is the marking pheromone deposited by P. bochei after oviposition. However, because the parasite marks its hosts internally, van Lenteren and Bakker were unable to demonstrate pheromonal contact. Our study parallels their pioneering work and provides the first unequivocal evidence for pheromone learning in

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- Klomp, H., Teerink, B. J. & Wei Chun, M. Neth. J. Zool. 30, 254-277 (1980).
 Alcock, J. Animal Behaviour (Sinauer, Sunderland, 1979).
 Cammaerts-Tricot, M. C. Insectes soc. 21, 235-248 (1974).

- Le Moli, F. & Passetti, M. Boll. Zool. 45, 389-398 (1978)
- Vinson, S. R., Barfield, C. S. & Henson, R. D. Phys. Ent. 2, 157-164 (1977).
- 10. van Lenteren, J. C. & Bakker, K. Nature 254, 417-419 (1975).

Behavioural and microspectrophotometric measurements of colour vision in monkeys

G. H. Jacobs*, J. K. Bowmaker† & J. D. Mollon‡

* Department of Psychology, University of California, Santa Barbara, California 93106, USA

† Department of Zoology and Comparative Physiology, Queen Mary College, Mile End Road, London E1 4NS, UK

‡ Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK

Inherited abnormalities of colour vision are most commonly attributed to abnormalities of the photosensitive pigments in the cone cells of the retina^{1,2}. Here, we describe direct microspectrophotometric measurements on the eyes of two squirrel monkeys whose colour vision had been shown to differ behaviourally. Our results are consistent with classical explanations of abnormal colour vision.

Some colour-deficient human observers are dichromats, which means that they are able to match any colour with a mixture of two primary lights, whereas the normal, trichromatic observer requires three variables. It is exactly two hundred years since von Gentilly3-5 advanced the most natural explanation of this condition, that the dichromat lacks one of the three types of retinal receptor enjoyed by the normal trichromat. It is also one hundred years since Lord Rayleigh described a different abnormality of colour vision, anomalous trichromacy. Like the normal, the anomalous observer requires three variables in colour matching, but in matching a mixture of red and green to a monochromatic yellow light (the 'Rayleigh match') he needs either more red (protanomalous) or less red (deuteranomalous) than the normal. This has been explained by supposing that the anomalous retina contains three types of photopigment but that the absorbance curve for one of them is displaced along the spectrum from its normal position 1,2,7-11. Despite much work, the exact relationships between the various types of colour vision and retinal photopigments are far from settled. Two recent developments have led to the present report. First, it has been shown that among a population of squirrel monkeys (Saimiri sciureus) there occur clear variations in colour Second, the technique of microspectrophotometry¹⁴⁻²⁰, in which a narrow, monochromatic, measuring beam is passed through isolated photoreceptors, has advanced to a stage where it is possible to characterize the types of receptor within an individual primate retina and thus relate the results to earlier behavioural measurements on the same subject.

The squirrel monkeys examined in the present study were both adult females of the subtype exported through Iquitos, Peru²¹; they were drawn from a larger group trained on colour vision tests in Santa Barbara, California. The behavioural tests 12,13 were all conducted in a forced-choice discrimination apparatus in which the monkeys viewed three circular, transilluminated panels. They were taught to touch one of the three panels which was illuminated differently from the other two, in order to receive a 97-mg banana-flavoured food pellet. Which of

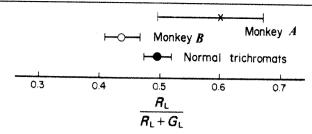


Fig. 1 Rayleigh match results from squirrel monkeys and humans. The match values are expressed as the ratio of the luminance of a red (624 nm) light ($R_{\rm L}$) to the sum of the luminances of the red and green (536 nm) lights ($R_{\rm L}+G_{\rm L}$) needed in a mixture to match an equiluminant yellow (585 nm) light. The solid circles give the average values for 20 normal human trichromats, so categorized by their performances on the Farnsworth–Munsell 100-hue test. The horizontal line shows the total range of matches made by this sample of subjects. The results for the two monkeys were obtained from tests in which they were forced to discriminate between a red/green mixture and a yellow. Across repeated test sessions, 21 different mixture combinations were tested. The large symbols represent the midpoints of the range of mixtures over which they were unable to discriminate various red/green combinations from yellow; the horizontal bars enclose the mixture range over which their discrimination performances were not significantly different from chance (95% confidence level).

the three panels was positive (that is, differently illuminated) varied randomly from trial to trial.

Results from three tests of vision are reported. First, detection thresholds were measured for 540 and 640 nm monochromatic test lights superimposed on achromatic backgrounds (3 cd m⁻²). One animal (A) was 1.27 log units less sensitive to the 640 nm light than to 540 nm, whereas the other (B) was much more sensitive to the long-wavelength light, showing a threshold difference of only 0.36 log units. Sensitivity variations of this magnitude are characteristic of this species¹².

Two further tests specifically examined colour vision. The first was a Rayleigh match assessing the relative proportions of mixed red (625 nm) and green (536 nm) light that the animal was unable to discriminate from a yellow (585 nm) (Fig. 1). Human subjects were tested in the same apparatus and the matches (mean and total range) obtained from 20 normal trichromats are shown in Fig. 1 for comparison. For the monkeys, the symbols represent the midpoints of the range over which they were unable to discriminate the red/green mixture from the yellow and the horizontal bars show the mixture range over which their discrimination was not significantly different from chance. It will be seen that monkey B required somewhat more green light in the mixture than any of the normal human trichromats whereas monkey A required much more red. Note also that the mixture range that could not reliably be discriminated from yellow was very much larger for subject A.

In another experiment we measured, at each of 10 spectral locations, how much the wavelength of a test light had to be shifted along the spectrum for the animal to discriminate a wavelength difference correctly. The abilities of the two monkeys to distinguish wavelengths were very similar for values from $450 \text{ to} \sim 540 \text{ nm}$ (Fig. 2). At longer wavelengths, monkey B continued to show relatively good discrimination, but A's discrimination worsened strikingly.

If we categorize the animals in terms of human vision, then monkey A was a protan; we leave open the question of whether she was an extreme protanomal or a true dichromat. Monkey B requires significantly more green light in the Rayleigh match than the normal human trichromat, and we describe her as deuteranomalous, but she is less aberrant in this regard than the typical human deuteranomalous observer^{1,2}.

The behavioural results were not known to the microspectrophotometrists (J.K.B. and J.D.M.), nor the microspectrophotometric data to G.H.J., until the two sets of results had been handed to an independent third party.

The monkeys were flown to Britain, where microspectrophotometric measurements were made with a modified Liebman microspectrophotometer^{16,22} under computer control. The preparation of tissue was as described for *Macaca fascicularis* by Bowmaker, Dartnall and Mollon¹⁹. Several samples were

taken from each retina. The microspectrophotometer was programmed to step from 700 to 390 nm in 2-nm steps, taking wavelengths with even values, and to return taking the interleaved wavelengths. A total of 34 individual records were obtained from animal A, and 47 from B.

Figure 3 shows for each animal (1) the mean absorbance curve for each class of photoreceptor, excluding short-wave cones, which were too few to provide mean spectra, and (2) the distribution of peak sensitivities for all individual records. From animal A we recorded 2 violet-sensitive receptors (mean λ_{max} = 431.3 nm), 13 rods (mean $\lambda_{\text{max}} = 496.4$ nm, s.d. = 4.69) and 19 green-sensitive receptors (mean $\lambda_{\text{max}} = 535.4 \text{ nm}$, s.d. = 3.45); the longest λ_{max} estimated for any cell of this animal was 542.5 nm. The mean and standard deviation for the P535 receptors closely resemble those for the middle-wavelength receptors of macaques¹⁸⁻¹⁹. Because animal A seems to have only one photopigment in the red-green range, its behavioural sensitivity at 640 nm relative to that at 540 nm should be directly predictable from the microspectrophotometric measurements. From the mean absorbance values at the two wavelengths we estimated log absorptance¹⁹ for an axial beam, assuming an outer segment length of 30 µm and a pigment density of $0.015~\mu\text{m}^{-1}$ and assuming that absorption by the ocular media is identical at 540 and 640 nm. This calculation predicts a difference in log sensitivity of 1.23, very close to the obtained value of 1.27

From monkey B we recorded one short-wave receptor ($\lambda_{\text{max}} = 427 \text{ nm}$), 24 rods (mean $\lambda_{\text{max}} = 501.7 \text{ nm}$, s.d. = 3.58), no receptors in the vicinity of 535 nm and a broad range of receptors with peak sensitivities between 546 and 577 nm. There is no overlap between the long-wavelength receptors from this animal and the P535 receptors from the protan, a highly significant difference (z = 5.63, Mann-Whitney U-test).

The long-wave receptors of animal B, taken as a whole, have a higher standard deviation (8.17 nm) than we should typically expect for a single class of photoreceptor in an individual primate; and prima facie they fall into two groups, with a gap in the distribution at 562 nm (Fig. 3d). The two groups have means of 552.2 nm (n = 16) and 568.2 nm (n = 9). The latter value can be compared with the mean of 567.0 nm obtained for the long-wavelength receptors of Macaca fascicularis. Our confidence in the existence of more than one long-wavelength pigment in this animal is reinforced by the fact that the mean absorbance spectra for the two subgroups (Fig. 3c) show very similar absorbance at short wavelengths and seem to have a constant separation; we should not expect this to be the case if, say, the large variation in λ_{max} arose from variations in optical

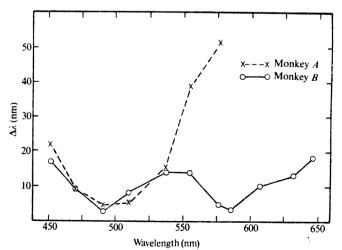


Fig. 2 Wavelength discrimination functions for two squirrel monkeys. The wavelength discrimination values $(\Delta\lambda)$ indicate the magnitude of the wavelength change required at each spectral location to support discrimination between two equiluminant spectral lights at a criterial level of 70% correct. The values are averages for differences in both spectral directions except at 452 nm where the change could only be measured towards the longer wavelengths.

scattering or in the presence of photoproducts at short wavelengths, which would distort the absorbance spectrum.

As in macaques and man^{19,20}, the short-wave receptors seem to be rare in squirrel monkeys. In the case of the rods there is a 5-nm difference between animals in λ_{max} , a difference which is associated with a small difference in absorbance at short wavelengths; we do not know whether the latter difference reflects short-wave contaminants or a true difference in the absorbance spectrum.

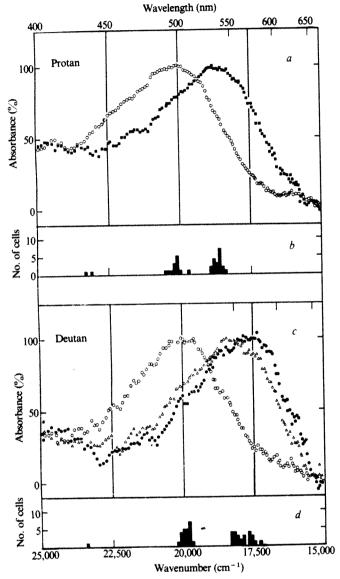


Fig. 3 a, c, Mean absorbance spectra for different classes of photoreceptor in two individual squirrel monkeys. Each datum point corresponds to the average of values obtained at two adjacent wavelengths, one value recorded in the descending scan (see text) and the other recorded in the ascending scan. The absorbance spectra for individual receptors were not normalized before averaging. The mean spectra have been normalized to have a maximum of 100%. b, d, Distribution of values of peak sensitivity of individual receptors from the protan (b) and deutan (d) animals. The bin size is 100 cm⁻¹. With the exception of the three short-wavelength receptors, values of peak sensitivity were derived as follows. The raw absorbance values for individual wavelengths were smoothed using an 11-nm running average The approximate peak of this curve was estimated and each of 50 individual (smoothed) absorbance values on either side of the provisional peak was referred to an appropriate nomogram to estimate the wavenumber of peak sensitivity; this operation amounts to finding where the nomogram must be located on a wavenumber abscissa to yield the absorbance value under consideration. The mean of the many separate estimates for a given cell is the value entered in the histogram. This method resembles that described by Bowmaker et al. 19, except that the smoothing and subsequent analysis are carried out by the computer and more individual estimates are used. For the three short-wavelength records we estimated wavenumber of peak sensitivity directly from the 2-nm averages (see above) in the restricted range 400-475 nm, using the frog green-rod nomogram²².

This first microspectrophotometric study of behaviourally different conspecifics is consistent with classical explanations of colour deficiency and anomaly. Within the limits of our sampling, the severely protan animal lacks entirely the long-wave receptors found in macaques^{18,19}. The deuteranomalous animal lacks the P535 receptors found in the protan but probably has more than one type of receptor in the range 546-577 nm.

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- Boynton, R. M. Human Color Vision (Holt, Rinehart & Winston, New York, 1979)
- Boynton, R. M. Human Color Vision (Holt, Kinehart & Winston, New York, 1979).
 Pokorny, J., Smith, V. C., Verriest, G. & Pinckers, A. J. L. G. Congenital and Acquired Color Vision Defects Grune & Stratton, New York, 1979).
 Voigt, J. H. Gothaisches Magazin für das Neueste aus der Physik und Naturgeschichte Vol. 1 (ed. Lichtenberg, L. C.) 57-61 (Ettinger, 1781).
- 4. Palmer, G. Théorie de la Lumière, Applicable aux Arts et Principalement à la Peinture
- (Hardouin & Gattey, Paris, 1786). Walls, G. L. J. hist. Med. 11, 66-96 (1956). Rayleigh, Lord Nature 25, 64-66 (1881).
- Rayleigh, Lord Nature 25, 64-66 (1881).
 von Kries, J. in Handbook of Physiological Optics Vol. 2 (von Helmholtz, H.) (translated by Southall, J. P. C.) (Optical Society of America, 1924).
 Hurvich, L. M. & Jameson, D. Documenta Ophth. 16, 409-442 (1962).
 Alpern, M. & Torii, S. J. gen. Physiol. 52, 717-737. 738-749 (1968).
 Rushton, W. A. H., Powell, D. S. & White, K. D. Vision Res. 13, 2017-2031 (1973).
 MacLeod, D. I. A. & Hayhoe, M. J. opt. Soc. Am. 64, 92-96 (1974).
 Jacobs, G. H. Science 197, 499-500 (1977).
 Jacobs, G. H. Science 197, 499-500 (1977).

- Jacobs, G. H. & Blakeslee, B. Invest. Ophth. vis. Sci. Suppl. 136 (1980).
 Marks, W. B., Dobelle, W. H. & MacNichol, E. F. Science 143, 1181-1183 (1964).

- Marks, W. D., Double, W. H. & Macivicino, E. F. Science 143, 1101-1103 (1904).
 Brown, P. K. & Wald, G. Science 144, 45-51 (1964).
 Liebman, P. A. & Entine, G. J. opt. Soc. Am. 54, 1451-1459 (1964).
 Dobelle, W. H., Marks, W. B. & MacNichol, E. F. Science 166, 1508-1510 (1969).
- 18. Bowmaker, J. K., Dartnall, H. J. A., Lythgoe, J. N. & Mollon, J. D. J. Physiol., Lond. 274,
- 19. Bowmaker, J. K., Dartnall, H. J. A. & Mollon, J. D. J. Physiol., Lond. 298, 131-143 (1980).
- Bowmaker, J. K. & Dartnall, H. J. A. J. Physiol., Lond. 298, 501-511 (1980).
 Cooper, R. W. in The Squirrel Monkey (eds Rosenblum, L. A. & Cooper, R. W.) 1-29 (Academic, New York, 1968).

 22. Knowles, A. & Dartnall, H. J. A. The Photobiology of Vision (Academic, New York, 1977).

Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque

Robert Shapley, Ehud Kaplan & Robert Soodak

The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA

Study of parallel processing in the visual pathway1 of the cat has revealed several classes of retinal ganglion cells which are physiologically distinct and which project to various locations in 3. Two classes have been studied most extensively: X cells, which sum neural signals linearly over their receptive fields, and Y cells, in which the spatial summation is nonlinear 1.4. In the cat's lateral geniculate nucleus (LGN) cells also can be classified as X or Y, a result of the parallel projection of retinal X and Y inputs to different geniculate neurones 5-9. We report here our study of parallel signal processing in the LGN of the macaque monkey. We find that (1) monkey LGN cells can be classified as X or Y on the basis of spatial summation; (2) X-like cells are found in the four parvocellular and the two magnocellular laminae, whereas Y-like cells are found almost exclusively in the magnocellular laminae; and (3) the cells of the magnocellular laminae have high sensitivity and the parvocellular cells low sensitivity for homochromatic patterns. This implies that in macaque monkeys the magnocellular cells and their cortical projections may be the neural vehicle for contrast vision near threshold. The cells of the parvocellular laminae seem to be primarily concerned with wavelength discrimination and patterns of colour. As the human visual system is similar to that of the macaque in structure and behavioural performance, our findings are probably also applicable to man.

In macaque monkeys, as in man, the LGN is a highly organized, layered nucleus. There are four dorsal layers of small cells (parvocellular) and two ventral layers of large cells (magnocellular). Previous investigators 10-12 concluded that there was a complete segregation of X and Y cells in the monkey's geniculate nucleus, the X cells residing in the parvocellular laminae and the Y cells in the magnocellular laminae. These conclusions depended on identifying X and Y cells by the time course of response to visual stimulation and the latency of response to electrical stimulation of the optic chiasm. Although such characteristics have been used to classify X and Y cells in the cat LGN^{6,7}, a correlation between response time course and linearity of spatial summation has not been established for neurones of the monkey's LGN.

In our experiments we used nine cynomolgus monkeys (Macaca fascicularis) anaesthetized with urethane (20 mg per kg per h) and paralysed with Flaxedil. The activity of single cells was recorded extracellularly using glass micropipettes (tip resistance, $10-30~\mathrm{M}\Omega$). In about half the penetrations the pipettes were filled with a saturated solution of Fast Green dye in Ringer's solution to mark the position of the recording electrode. Electrode tracks were reconstructed from histological sections by using the dye marks and distances traversed along the track. This was important for assigning a given cell to a particular geniculate lamina because laminar boundaries are often irregular and the layer 3-layer 2 boundary, which is the critical parvocellular-magnocellular boundary, is not accompanied by an eye change. Visual stimuli were produced electronically on the face of a CRT¹³ which subtended 10° by 8° in

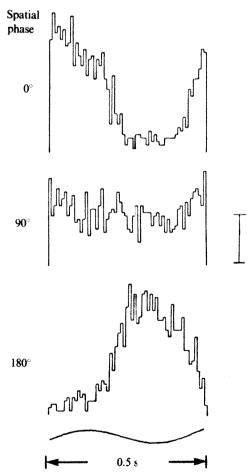


Fig. 1 Response of an X cell from a magnocellular layer at three different positions (spatial phases). The cell was recorded in layer 2 of the macaque's LGN. The stimulus was a sine grating of 1.5 cycle deg⁻¹, 50% contrast, contrast reversed with a sinusoidal time course at 2 Hz as indicated at the bottom of the figure. 0° and 180° were spatial phases at which the stimulus produced maximal responses; 90° was the spatial phase of the null response. The vertical calibration, drawn close to the middle histogram, is 30 impulses per second.

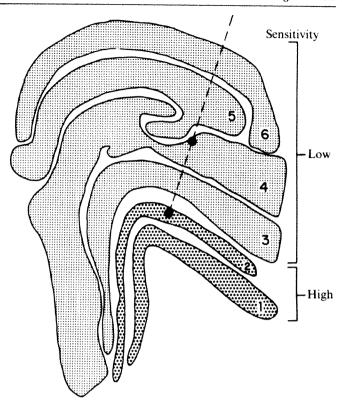


Fig. 2 Contrast sensitivity in the macaque's LGN. This drawing is traced from a single 100-μm thick section of the LGN. The parvocellular laminae are numbered 6, 5, 4 and 3 and the magnocellular laminae 2 and 1. The parvocellular cells all had lower contrast sensitivity than did the X and Y cells of the magnocellular laminae. A reconstructed electrode track is shown; the two dark circles represent green dots left by the electrode at sites where (1) a typical parvocellular X cell of low sensitivity was recorded in layer 4 and (2) a typical magnocellular X cell of high contrast sensitivity (>50) was recorded in layer 2. The latter was the first cell of high contrast sensitivity found on this track.

visual angle. In some experiments a CRT with a P31 (green) phosphor was used, in others a CRT with a P4 (white) phosphor with no difference in results. The mean luminance was 20-60 cd m⁻², in the low photopic range. The pupils were dilated to ~ 6 -mm diameter and the eyes protected with clear plastic contact lenses. In several experiments Wratten colour filters (no. 26, red; no. 61, green; no. 48A, blue) were placed in front of the CRT to check whether linearity of spatial summation depended on the colour of the screen.

Cells were classified as X or Y by means of their response to sine gratings, patterns in which the luminance varied sinusoidally with position in one dimension. The contrast of the gratings was reversed with a sinusoidal time course^{4,8,9}. A cell was called X if it responded to low and high spatial frequency patterns as if it received input from a single linear receptive field mechanism. This means that an X cell would respond to contrast reversal of the grating at the temporal modulation frequency, and its response would exhibit a sinusoidal dependence on the position (or spatial phase) of the grating^{4,8,9}. A cell was called Y if it responded to low spatial frequencies approximately like an X cell, but revealed only nonlinear behaviour at higher spatial frequencies. The nonlinear behaviour looked for, based on our experience with cat Y cells, was frequency doubling of responses to contrast reversal. In monkey Y cells, as in the cat, a frequency-doubled response was present at all positions of the grating. Classification of cells as X or Y did not depend on the colour of the screen in the experiments with Wratten filters. That retinal ganglion cells in the monkey can be classified as X or Y has been previously reported14. Thus, in the monkey, as in the cat, retinal ganglion cells confer their characteristic properties on their geniculate targets.

Cells classified as X by above criterion were found in all laminae of the macaque's LGN. Figure 1 shows the responses of a magnocellular X cell to the stimulus of a 1.5 cycle deg⁻¹ sine grating undergoing contrast reversal with a sinusoidal time course at 2 Hz. Responses at three different positions or spatial phases are shown: at 0° spatial phase there was a maximal response, at 90° no response, and at 180° the response peaked again.

Almost all the parvocellular cells recorded were X cells (225 out of 226 cells recorded and assigned to the parvocellular laminae by histological reconstruction of tracks); only one was a Y cell. Most of the magnocellular cells recorded were also X cells (59 out of 77 cells assigned to the magnocellular layers); the rest were Y cells. Thus, our results show that magnocellular cells do not form a homogeneous class with respect to spatial summation properties. Similar results have been reported in preliminary form elsewhere 15-18.

We next investigated functional differences between parvocellular and magnocellular cells by comparing their contrast sensitivities. Contrast sensitivity was determined as the reciprocal of the contrast required to give a modulated discharge with an amplitude of 5 impulses per second in response to a drifting sine grating. The spatial frequency of the grating was varied from 0 to 10 cycle deg⁻¹, and the peak contrast sensitivity for each cell was determined. The parvocellular cells had much lower peak contrast sensitivity than the magnocellular cells. The parvocellular cells had a peak contrast sensitivity of 10. Both X and Y cells in the magnocellular laminae had a peak contrast sensitivity of approximately 75. The contrast sensitivity of magnocellular cells was high enough that it might account for the behavioural contrast sensitivity of Macaca fascicularis 19. Figure 2 shows that the magnocellular layers are the site of high contrast sensitivity and the parvocellular laminae contain cells which are relatively insensitive to luminance contrast. Our discovery that parvocellular neurones have low contrast sensitivity is consistent with previous evidence that these cells analyse colour 10,12,20,21. Note that peak contrast sensitivity did not show a strong dependence on the colour of the CRT screen in the experiments with Wratten filters.

The contrast sensitivities of cells in the magnocellular laminae of the monkey are comparable with those of cells in the A and A1 laminae of the cat LGN. As the parvocellular X cells were far less sensitive to contrast, the proposed homology between the X cells in the parvocellular laminae of the monkey and X cells in the cat LGN¹⁰⁻¹² is not compelling. A closer homology exists between the X and Y cells of the magnocellular laminae and the X and Y cells of the cat LGN. The parvocellular laminae of the monkey's LGN, and their retinal inputs, seem to form a visual neural pathway which is not present in cats.

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- Enroth-Cugell, C. & Robson, J. G. J. Physiol., Lond. 187, 517-552 (1966). Rodieck, R. W. A. Rev. Neurosci. 2, 193-225 (1979). Lennie, P. Vision Res. 20, 561-594 (1980).

- Rodieck, K. W. A. Rev. Neurosci. 2, 193-223 (1979).
 Lennie, P. Vision Res. 20, 561-594 (1980).
 Hochstein, S. & Shapley, R. M. J. Physiol., Lond. 262, 237-264 (1976).
 Shapley, R. M. & Hochstein, S. Nature 256, 411-413 (1975).
 Cleland, B. G., Dubin, M. W. & Levick, W. R. J. Physiol., Lond. 217, 473-496 (1971).
 Hoffmann, K. P., Stone, J. & Sherman, S. M. J. Neurophysiol. 35, 518-51 (1972).
 So, Y. T. & Shapley, R. M. J. Neurophysiol. 45, 107-120 (1981).
 Dreher, B., Fukada, Y. & Rodieck, R. W. J. Physiol., Lond. 258, 433-452 (1976).
 Sherman, S. M., Wilson, J. R., Kaas, J. H. & Webb, S. V. Science 192, 475-477 (1976).
 Schiller, P. H. & Malpeli, J. G. J. Neurophysiol. 41, 788-797 (1978).
 Shapley, R. M. & Rossetto, M. Behau. Res. Meth. Instrum. 8, 15-20 (1976).
 De Monasterio, F. J. Neurophysiol. 41, 1394-1417 (1978).
 Kaplan, E. & Shapley, R. M. Invest. Ophthalmol. vis. Sci. suppl. 19, 41 (1980).
 Shapley, R. M. & Kaplan, E. Neurosci. Abstr. 6, 583 (1980).
 Blakemore, C. B. & Vital-Durand, R. Trans. ophthalmol Soc. U.K. 99, 363-368 (1980).
 Blakemore, C. B. & Vital-Durand, R. Trans. ophthalmol vis. Sci. suppl. 20, 14 (1981).
 DeValois, R., Morgan, H. & Snodderly, D. M. Vision Res. 14, 75-81 (1974).
 Wiesel, T. & Hubel, D. H. J. Neurophysiol. 29, 1115-1156 (1966).
 DeValois, R. & DeValois, K. in Handbook of Perception Vol. 5, 116-168 (1975).

Two genes interact to control development of a lymphoid/erythroid hyperplastic disorder of mice

Luisa DeGiorgi, Arpi Matossian-Rogers & Hilliard Festenstein

Department of Immunology, London Hospital Medical College, Turner Street, London El 2AD, UK

We have previously established the presence of a gene in mice (called Lus)1 which is dominant for the suppression of cytostasis, an experimental assay of cell-mediated immunity2. Here we describe the definition of a gene (segregating independently of Lus) that, in combination with the dominant gene Lusa, is homozygous recessive for a lymphoid hyperplastic disorder of T and B cells. We have called this gene Arp. The disease involves abnormalitities of lymphopoietic and other organs, and could be model system for regulatory defects of the immune system.

Cytostasis' is an in vivo/in vitre assay of cell-mediated immunity3. In our experiments mice were sensitized by injections of allogeneic lymphoid cells4. After 14 days we tested lymph node cells from sensitized mice in an in vitro cytostasis assay, using tumour cells as target cells.

Cytostasis is assayed by the uptake of radioactive thymidine by the tumour cells and is positive if uptake is inhibited by the effector cells. Both T and B cells (A. M.-R., L. DeG. and H. F., in preparation) are capable of causing cytostasis. Allogeneic cytostasis following sensitization across the H-2 barrier usually gives a positive result. However, we noted that whereas immunization of CBA/H (H-2k) mice with BALB/c spleen cells led to the production of cytostatic effectors against SL2 (H-2^d) tumour cells (a spontaneous Thy-1-positive DBA/2 lymphoma), immunization with B10.D2 (also H-2^d) spleen cells failed to generate anti-H-2^d effectors in the CBA/H (H-2^k) recipients. Mating B10.D2 with BALB/c mice produced F1 hybrids whose spleen cells also did not stimulate the production of cytostatic effector cells, suggesting the presence of a dominant suppressor gene in the B10.D2 mice. This finding was further studied and confirmed using (B10.D2×BALB/c)F₁×BALB/c backcross mice up to the fourth generation2 and beyond. Approximately 50% of backcross mice from each generation failed to stimulate the production of cytostatic effectors and were therefore carrying the cytostasis suppressor gene Lusa (ref. 1 and Tables 1, 2). These were selected for further backcrossing to BALB/c, to develop a strain congeneic with BALB/c at the suppressor locus. This was done up to the tenth generation

After the fourth backcross mating, we first noted the appearance of the hyperplastic disorder with abnormalities in the lymphopoietic and other organs in ~50% of 3-4-week old mice carrying the suppressor gene (Table 2), that is, cytostasisnegative mice. The disorder was manifested by gross hyperplasia of the thymus, spleen (Fig. 1), Peyer's patches and lymph nodes. There were concomitant histopathological changes in these

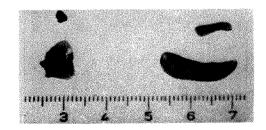


Fig. 1 Spleen and thymus from hyperplastic (right) and nonhyperplastic (left) cytostasis-negative littermates. Note the approximate 10-fold difference between the size of the organs.

Table 1 Data demonstrating the effects of the suppressor gene on the generation of cytostasis in CBA/H (H-2k) mice

	c.p.m. ± s.d. of SL2 (H-2d) tumour targets cultured with:	
Immunizing Bc Mice	Normal lymphocytes	Immune lymphocytes	% Inhibition of tumour growth
5th generation Cyts(+)	$28,727 \pm 2,618$	$10,383 \pm 648$	64
5th generation Cyts(-)	$28,724 \pm 2,618$	28.828 ± 1.680	0
5th generation Cyts(-) with hyperplasia	$28,724 \pm 2,618$	29.379 ± 1.131	0
6th generation Cyts(+)	$56,582 \pm 840$	20.378 ± 811	64
6th generation Cyts(-)	$56,582 \pm 840$	57.054 ± 3.188	0
6th generation Cyts(-) with hyperplasia	$56,582 \pm 840$	$64,123 \pm 6,047$	0
7th generation Cyts(+)	$33,060 \pm 1,472$	17.558 ± 1.416	47
7th generation Cyts(-)	$56,582 \pm 840$	$54,665 \pm 2,196$	4
7th generation Cyts(-) with hyperplasia	$56,582 \pm 840$	$53,053 \pm 2,728$	7

CBA/H mice were immunized intraperitoneally with 10^7 spleen cells obtained by biopsy from each backcross mouse, which was earmarked. 10–20 days after immunization, spleen cells were removed from the immunized mice under anaesthesia and cultured at a ratio of 10:1 with (SL2) tumour targets of DBA/2 origin in RPMI medium containing 5% fetal calf serum and antibiotics. Normal CBA/H lymphocytes were used as controls. The cultures were maintained for 48 h at 37 °C in a 4% CO₂, 10% N₂, 86% O₂ atmosphere. Tritiated thymidine was then added and the cultures were incubated for a further 16 h. The uptake of isotope, which was largely that of the tumour targets, was then measured and the inhibition of tumour growth calculated according to the following formula

% Inhibition =
$$100 - \frac{\text{c.p.m. of tumour cells + immune lymphocytes}}{\text{c.p.m. of tumour cells + normal lymphocytes}} \times 100$$

Cyts(+) and Cyts(-) are, respectively, mice producing and failing to produce cytostatic effector cells.

tissues (Fig. 2a, b) as well as lymphocytic and metamyelocytic accumulations in the liver (Fig. 3) and hyperplasia of the bone marrow. There was marked general thymic hyperplasia and thymic medullary hypercellularity ('thymitis') in the T lymphoid system, accompanied by paracortical hyperplasia of the lymph nodes. Evidence of germinal centre development was also seen in spleens and lymph nodes of affected mice, and plasma cells were abundant in the lymph node medullae.

The hypercellularity of bone marrow and spleen was due to two components: haemopoiesis, both erythroid and myeloid, and enlargement of malpighian follicles with occasional germinal centre formation (spleen). The presence of excess stainable iron was particularly evident in spleens of mice affected with the disease. There was no evidence of lymphoid or haematopoietic malignancy.

These lesions therefore represent a dyshaematopoiesis associated with lymphoid hyperplasia of both T and B lymphoid components (T more marked than B). Aspects of this pathological picture are consistent with the disorder being due to a primary haemolytic process or autoimmune disorder—with



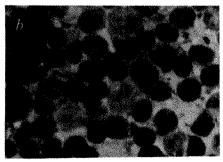


Fig. 2 Splenic impressions from hyperplastic and non-hyperplastic cytostasis-negative littermates. Note the presence of large numbers of blast cells with nucleoli (a) compared with predominantly mature leukocytes in the smears made from the non-hyperplastic spleens (b).



Fig. 3 Section of liver showing focal infiltration with metamyelocytes.

secondary hyperplasia as in NZB mice⁵. Although this disease is debilitating, fatality in affected mice observed up to 6 months was not greater than in controls.

As this lymphoproliferative syndrome was found in approximately half of the cytostasis-negative mice (those with the Lus^a cytostasis suppressor allele) from all backcross generations (Table 2), the involvement of a second gene in the control of the disorder was suggested, and was designated Arp. The syndrome occurs only in Lus^a-positive mice and so presumably Lus^a interacts epistatically with Arp. Because the disease occurs in 50% of the Lus^a-positive mice, the genotypes of the diseased

Table 2 Unigeneic segregation of the cytostasis suppressor gene allele Lus^a in (B10.D2×BALB/c)F₁×BALB/c backcross mice

Backcross* generation	No. of mice tested	No. of Bc mice Cyts(+)	No. of Bc mice Cyts(-)	No. hyperplastic
1	8	3	5)	
2	12	6	6 (2.000
3	18	9	9 (NT
4	16	9	7)	
5	50	21	29	14
6	46	22	24	10
7	20	9	11	6
8	21	10	10	5
9	17	9	8	4
10	12	6	6	3
Total	220	47%	53%	(47%)†

Segregation was assayed by the capacity of the backcross mouse $(H-2^d)$ spleen cells to inhibit stimulation of cytostatic effectors in CBA/H recipients $(H-2^b)$ against SL2 $(H-2^d)$ tumour target cells) and with the second gene (Arp) producing the hyperplastic disorder in Cyts(-) backcross mice. NT, not tested.

the hyperplastic disorder in Cyts(-) backcross mice. NT, not tested.

*The same BALB/c male was mated with (B10.D2×BALB/c)F₁ females and four healthy Cyts(-) females selected from each backcross generation through to the tenth backcross.

†47% of Cyts(-) mice from the fifth and subsequent backcross generations presented a lymphohyperplastic syndrome described in the text.

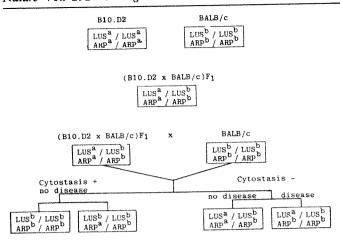


Fig. 4 Proposed genotype of B10.D2, BALB/c, (B10.D2× BALB/c)F₁ and backcrosses to BALB/c mice, with respect to the allelic expression of Lus^a (suppressor) and Arp^a protector (or Arp^b/Arp^b recessive hyperplastic) genes. Lus^a : cytostasis cytostasis (heterozygote co-dominant); Lus^b: suppressor enhancer; Arp^a : protector against hyperplastic disorder (heterozygote co-dominant); Arp^b : inducer of hyperplastic disorder (recessive homozygote). Genotype $Lus^a/Lus^b Arp^b/Arp^b$ suggests that epistasis of recessive Arp^b/Arp^b and co-dominant Lus" is needed to produce the hyperplastic disorder, or that protector Arp^a is a co-dominant allele preventing the development of the hyperplastic disorder.

mice with respect to the second gene must by Arp^b/Arb^b , and Lus^a and Arp genes segregate independently. Figure 4 shows the segregation of these genes and the proposed phenotypes of the parental strains and the crosses. (The disease is produced when the Arp b gene from BALB/c is homozygous and associated with Lusa.) There is no evidence of linkage of these genes to sex chromosome genes (data not shown).

We found that the disease was present in several mice of the first generation backcross, but was missed in the early matings of the first backcrossing programme. If female cytostasis-negative mice were randomly selected for backcrossing, there would be a probability of 1/16 that after four generations Arpa would still be segregating. However, these odds are increased because we selected for healthy mice (Arpa/Arpb heterozygotes) for backcrosses. Also, we selected for coloured coats, and Arpa might be linked to a coat colour gene. When the disease appeared, only healthy non-hyperplastic mice that failed to stimulate the production of cytostatic effectors were then selected for further matings. Two genetic traits were thus simultaneously selected, one associated with Lusa (cytostasis suppressor) and the other with Arpa (a healthy mouse). This selection procedure allowed for segregation of these two genes in all successive backcross matings, resulting in the same proportional representation of sick and healthy mice carrying Lus" at each future generation.

The way in which a genetic marker for a gene silent in its original context can, on transfer to a different genetic background, bring about a hyperplastic syndrome is intriguing. One can envisage that a human analogue of the suppressor gene may be transmitted to offspring with defective regulatory genes and thus promote neoplastic or hyperplastic cellular transformation.

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- 1. DeGiorgi, L. thesis, London Univ. (1979).
- DeGiorgi, L., Matossian-Rogers, A. & Festenstein, H. Immunology 36, 711-718 (1979).
- Chia, E. & Festenstein, H. Eur. J. Immun. 3, 483-487 (1973). DeGiorgi, L., Biasi, G. & Festenstein, H. J. Immunogenet. 5, 49 (1978).
- Holmes, M. C. & Burref, F. M. Ann. int. Med. 59, 265-273 (1963)

Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted

Barry Jones & Charles A. Janeway Jr

Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510, USA

To produce antibody, bone-marrow-derived (B) lymphocytes generally require the synergistic action of a thymus-derived (T) lymphocyte, termed a helper T cell (Th)1. The interaction of Th with B cells is antigen specific and requires that both the T and the B cell be specific for physically linked antigenic determinants2. More recently it has been demonstrated in numerous systems that the interaction of Th and B cells is governed by antigens encoded within the major histocompatibility complex (MHC), such that Th and B cells must be identical in the I region of the MHC in order to cooperate efficiently3-5. This finding has been termed MHC restriction. However, studies using populations of T cells as a source of Th could be influenced by the presence of alloreactive T cells, suppressor T cells and other T-cells subsets, thus making interpretation difficult. Moreover, it has been proposed that MHC restriction governs not the interaction of Th with B cells, but rather the interaction of Th cells with antigen-presenting cells (APCs), whereafter the interaction of Th with B cells is said to be unrestricted. We have now attempted to clarify this situation by examining the interaction of soft agar colonies and clones of antigen-specific, MHC-restricted proliferating T cells 7.8 with purified B cells using several different assays. We conclude that the antigenspecific cooperative interactions between Th and B cells are themselves MHC restricted, and that B cells bearing the wrong MHC-encoded antigens cannot be activated by addition of APCs matched to the Th cell but differing from the B cell at MHC.

As a source of Th cells, we have used BALB/c (H-2^d) and BALB·B (H-2b) T cells primed with hen's egg albumin (OVA), cloned in soft agar and propagated in vitro according to Sredni et al.7. Evidence that these cells were antigen specific and MHC restricted in their proliferative responses is presented elsewhere8. The cells were then tested for their interaction with B cells in three different assays.

When antigen-specific, MHC-restricted T-cell colonies are treated with mitomycin C and added to B cells purified by elution from goat anti-mouse immunoglobulin (GaMIg) plates or by treatment of spleen cells with anti-Thy-1.2 antiserum and complement, intense B-cell proliferation ensues in the presence of specific antigen, peaking 48 h after initiation of the culture. This is not classically an assay for T-cell help, but seems rather to measure the polyclonal activation of B-cell proliferation by T cells. The data in Table 1 show that this response is antigen specific (furthermore, the response did not occur in the presence of 100 µg ml⁻¹ keyhole limpet haemocyanin), MHC restricted between T and B cells, and cannot be overcome by addition of mitomycin C-treated spleen cells as a source of APCs syngeneic to the Th but allogenic to the B cells (Table 1b). Figure 1 shows a second experiment in which the Th colony cells are titrated. In this instance, some induction of allogeneic B-cell proliferation is seen in the presence of mitomycin C-treated syngeneic spleen cells, but it is much less than that observed when Th and B cells are MHC matched, and is relatively little influenced by Th-cell dose. Although allogeneic B cells can be stimulated to proliferate in this manner, the response is meagre at best and occurs only in some experiments. Thus, we conclude that Th cells will induce B-cell proliferation in the presence of antigen, and that this interaction is MHC restricted largely or entirely between the Th and B cells themselves.

Table 1 Induction of B-cell proliferation by OVA-specific T-cell colonies and clones is antigen specific and MHC restricted

A RUIC	i induction of D-cen pro	increation by O 771-spec			igon specific and with to	
a, BALB/c B-cell proliferat	tive response* (△ c.p.m.) v	vith colony† number:				
Antigen added	A3a	A4a	C5b	D2b	Cla	Normal BALB/c T cell
0	(7,700)‡	(9,500)	(4,400)	(7,200)	(3,800)	(3,400)
100 μg ml ⁻¹ OVA	111,200	120,800	83,400	76,100	69,800	900
, B-cell proliferative respon	nse to OVA $(\triangle \overline{c.p.m.})$ usi	ng B cells from:				
Cloned cells	BALB/c (H-2d)	BALB·B (H-2	')	BALB·B+	BALB·K (H-2k)	$BALB \cdot K +$
tested				BALB/c (mito)§		BALB/c (mito)
Cla	58,500 (17,400)	4,800 (22,000)		4,900	-1,900 (31,000)	3,000
Cla	19,900 (2,000)	1,500 (4,300)		NT	3,200 (3,700)	NT
CIGI	63,400 (48,600)	NT		NT	5,400 (54,600)	NT

^{*} Normal T cells (the non-adherent fraction collected after incubation of spleen cells on goat anti-MIg antibody-coated dishes) or T-cell colonies were incubated (30 min at 37 °C) with mitomycin C (30 μ g ml⁻¹), which effectively blocked DNA synthesis, and 2-4 × 10⁴ cells were added to 4 × 10⁵ B cells prepared by adherence to goat anti-MIg antibody-coated dishes (a, A3a, A4a, C5b, D2b and normal T cells; b, experiments of lines 2 and 3), or by treatment with anti-Thy 1.2 and complement (a, Cla; b, experiment of line 1). Cultures were pulsed with 1 μ Ci ³H-thymidine (60 Ci mmol⁻¹) after 48 h, and collected 3 h later.

Table 2 Induction of B-cell proliferation by normal T cells or T-cell colonies and clones mediated by rabbit anti-mouse brain antiserum a, B-cell proliferative response* $(\triangle \overline{c.p.m.})$ with colony number: Normal BALB/c T cells Serum added A3a C5b D2b Cla (2.900)(3,800)NRS (7,700)†(9,500)(4,400)(7,200)45,000 116 500 24.100 RaMBr 70.600 122,400 110,000 b, B-cell proliferative response (\(\Delta \) c.p.m.) using B cells from: BALB·B (H-2b) BALB·K (H-2k) Cloned cells tested: BALB/c (H-2d) 16,200 (2,000) 50,200 (4,300) 13,900 (3,700) Cla 83,800 (31,900) NT 38,400 (42,00) CIG

^{*} Mitomycin C-treated T cells were incubated with B cells and pulsed with ³H-thymidine after 48 h as described in Table 1 legend, but in these experiments, instead of OVA, normal rabbit serum (NRS) or rabbit anti-mouse brain (RaMBr) antiserum absorbed with mouse liver (×3) were added to the cultures at a final dilution of 1:100. † Background c.p.m. with NRS are shown in parentheses and have been subtracted from the responses with RaMBr to calculate \triangle c.p.m.

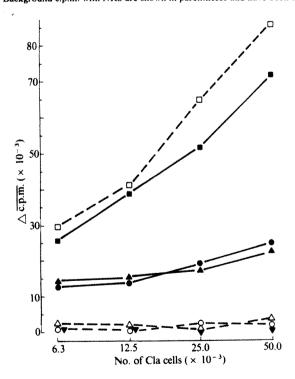


Fig. 1 BALB/c T_{OVA} colony cells (Cla) were treated with mitomycin C and titrated for their ability to induce proliferation in purified, non-immune, spleen B cells either in the absence (open symbols ——) or presence (closed symbols ——) of 2×10⁵ mitomycin C-treated BALB/c spleen cells added with OVA (100 µg ml⁻¹) as a supply of syngeneic antigen-presenting cells. 4×10⁵ B cells of the following strains were tested: BALB/c (□), BALB/K (○) or BALB/B (△). A control in the absence of B cells was performed (▼). After 48 h the cultures were pulsed with ³H-thymidine and the results are expressed as △ c.p.m., the difference between the response stimulated by OVA and controls in the absence of antigen.

Two objections could be raised to these experiments. The first is that the Th cells might in some way be suppressing the response of allogeneic B cells, and the second is that this response does not represent help, which involves both B-cell proliferation and differentiation to antibody-forming cells 10. We have therefore performed two further sets of studies to deal with these potential objections. In the first, Th- to B-cell interactions were initiated not with antigen but with rabbit anti-mouse brain antiserum (RaMBr), which activates T-cell proliferation in the presence of B cells in a non-MHC-restricted fashion¹¹. We have subsequently shown that it can also induce B-cell proliferation and polyclonal antibody production 48 h after initiation of culture in the presence of mitomycin C-treated Lyt-1⁺, 2⁻ cells. Because the T-cell colonies we have prepared are surface Ig⁻, Thy-1⁺, Lyt-1⁺, 2⁻, we treated them with mitomycin C and added them to purified B cells in the presence of normal rabbit serum (NRS) or RaMBr. Both normal BALB/c T cells and antigen-specific, MHC-restricted T-cell colonies induce a potent, polyclonal B-cell proliferative response in the presence of RaMBr but not NRS (Table 2), and this response is both T-cell dependent and unaffected by the MHC genotype of the responding B cell (Table 2b). Thus, these studies demonstrate that both normal T cells and MHC-restricted T-cell colonies can activate allogeneic B cells via RaMBr. It therefore seems unlikely that the failure of allogeneic B cells to respond to antigen in the presence of cloned T cells is due to some inhibitory effect of the T cells, but rather that it represents MHC-restricted activation of B cells by T cells.

To examine this question further, the ability of B cells to be induced to produce specific antibody by such Th cells was examined in an *in vitro* response to the hapten phosphorylcholine (PC) coupled to OVA, a system previously used by Bottomly and Jones¹² to explore the regulation of idiotype expression. In the present experiments, two colonies, one derived from BALB/c (H-2^d) and the other from the MHC-

[†] BALB/c OVA-specific T-cell colonies were prepared as described by Sredni et al.⁷ with the following modifications: T cells were prepared by passage over Ig-anti-Ig columns¹⁵, and the clones were stimulated with 5×10⁵ mitomycin C-treated spleen cells every 5-7 days. Colonies were tested 2-3 months after cloning. By immunofluorescence tests all cloned cells were Thyl.2⁺, Lyt-1.2⁺, Lyt-2.2⁻.

[‡] Background c.p.m. in the absence of antigen are shown in parentheses, and have been subtracted from responses. NT, not tested.

 $[\]S 2 \times 10^5$ mitomycin C-treated BALB/c spleen cells were added to the cultures.

Colony Cla was grown in bulk culture on B10·G spleen stimulator cells plus 20% T-cell growth factor (TCGF) (v/v) in the absence of OVA or H-2^d stimulators for 3 weeks, and then recloned by limiting dilution on BALB/c mitomycin C-treated spleen cells with 20% TCGF and OVA (200 μ g ml⁻¹). After 2-3 weeks six subclones were isolated, of which ClGl is an example.

Colony preparation was as described in Table 1 legend.

congeneic line BALB·B (H-2b), were tested for their ability to help BALB/c or C57BL/6J (H-2b) B cells. Furthermore, to test critically whether Th to B interactions were MHC restricted, we mixed purified B cells from the two strains with T-cell colonies of one type, cultured for 5 days with antigen, and assayed the plaque-forming cells (PFCs) before and after treatment with a monoclonal anti-H-2.5 antibody and rabbit complement. H-2.5 is present on H-2b but not H-2d cells, and thus the genotype of each PFC could be determined. If restriction operates at the level of Th to APC, and the genotype of the B cell is irrelevant as has been proposed6, then in these mixtures half of the PFCs should be H-2.5 positive and half negative, independent of the MHC restriction of the Th cells. In fact, although there is some nonspecific killing of PFCs by the antibody, as shown in the syngeneic mixtures, it is clear that there is little or no cooperation between Th cells and non-self B cells in this experiment (Table 3). One cannot argue, in this instance, that we have not added the necessary APCs, as a perfectly good PFC response is obtained in both cases. This experiment thus demonstrates conclusively that the MHC-restricted interaction in cooperation between Th and B cells in the response to soluble hapten-carrier conjugates occurs between Th and the B cells themselves, and does not involve non-MHC-restricted activation of B cells by Th

B cells bear large amounts of Ia antigens on their surfaces¹³. The interaction of Th with B cells is known to be restricted by Ia antigens^{3,14}. We interpret the present studies as showing that the function of these Ia molecules is to promote the interaction of Th cells with their appropriate target cells, the B cells. APCs also have Ia on their surface, and it is clear that Th-like cells can also interact with antigen in the context of APC Ia. Perhaps APCs, being non-antigen specific, are especially important for the initial activation of Th cells, after which Th cells preferentially interact with B cells bearing both the specific antigen bound by the B cell's immunoglobulin receptor and the appropriate Ia antigen. Thus, MHC restriction is seen as providing the Th cell with a mechanism of finding antigen only on the surface of those cells with which it can functionally interact, namely, B cells and APCs. If Th- to B-cell interactions were not themselves MHC restricted, this interpretation would not make sense. However, the present studies show that this interaction is indeed MHC restricted between Th and B cells.

The present report also describes two convenient and rapid methods for measuring Th- to B-cell interactions in proliferation assays. The polyclonal B-cell response to antigen in the presence of both Th colonies and cloned Th is not fully understood. Because it requires 1,000 times as much antigen as the in vitro specific anti-PC PFC response to PC-OVA, it may be that all B cells acquire a small amount of antigen at high antigen dose, independent of their immunoglobulin receptor, and thus can be

Table 3 Cooperation between OVA-specific T-cell colonies and B cells is MHC restricted in the response to PC-OVA

			ti-PC-PFCs per cells with Th col	
B cells	Treatment to PFCs	0	II 2 B6d (H-2 ^d)	II 2 D4b (H-2 ^b)
BALB/c (H-2 ^d)	0	3	112	10
BALB/c	Y - 3 + C		75 (33%)	
C57B1/6 (H-2b)	0	1	8	106
C57B1/6	Y - 3 + C			2 (98%)
BALB/c+C57BL/6	0		153	191
BALB/c+C57BL/6	Y-3+C		102 (33%)	18 (91%)

B cells were normal spleen cells treated with anti-Thy-1.2+C followed by anti-Lyt-1.2+ anti-Lyt-2.2 plus C. 3×106 viable cells placed in 1 ml culture with 0.1 µg ml⁻¹ PC-OVA and 10⁵ helper T cells. Five days later, plaque-forming cells (PFCs) were evaluated on PC-coupled sheep red blood cells. Values represent means of triplicate cultures. After culture, cells were treated with rabbit complement, or with monoclonal anti-H-2.5 antibody Y-3, which was raised in a BALB/c anti-H-2b immunization and kills 100% of H-2b spleen cells to a titre of 1:108, but does not react with H-2d. Helper T-cell colonies from BALB/c or BALB·B mice were prepared as described in Table 1 legend. Numbers in parentheses give per cent reduction in PFC response after treatment with anti-H-2b monoclonal antibody and complement.

induced to respond by the Th cells. If this is the case, it might imply that the immunoglobulin receptor is not involved in this form of B-cell proliferation. We are now investigating the role of the immunoglobulin receptor in this response, and also whether such cells subsequently secrete immunoglobulin. The assay involving RaMBr is reported in detail elsewhere. We forsee a role for this assay in assessing T- and B-cell function in man, as RaMBr-mediated T to B interactions do indeed lead to immunoglobulin production (J. P. Tite and B.J., unpublished), and because the interaction is not MHC restricted, the assay could be used between any two individuals.

We conclude that the interaction of Th cells with B cells is antigen specific and is itself MHC restricted. This conclusion supports the notion that Ia on B cells is critically important in promoting appropriate interactions with Ia-restricted helper T cells.

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Mitchell, G. F. & Miller, J. F. A. P. J. exp. Med. 128, 821-837 (1968).

Mitchison, N. A. Eur. J. Immun. 1, 18-27 (1971). Katz, D. W., Graves, M., Dorf, M. E., DiMuzio, H. & Benacerraf, B. J. exp. Med. 141,

Sprent, J. Immun. Rev. 42, 108-137 (1978).

- Sprent, J. Immun. Kev. 42, 108-13 (1978).
 Schreier, M. H. & Tees, R. Int. Archs Allergy appl. Immun. 61, 227-237 (1980).
 Singer, A., Hathcock, K. S. & Hodes, R. J. J. exp. Med. 149, 1208-1226 (1979).
 Sredni, B., Tse, H. Y. & Schwartz, R. H. Nature 283, 581-583 (1980).
 Janeway, C. A. & Conrad, P. J. Behring Inst. Mitt. (submitted).

- Wysocki, L. J. & Sato, V. L. Proc. natn. Acad. Sci. U.S.A. 75, 2844-2848 (1978). Schimpl, A. & Wecker, E. Transplantn Rev. 23, 176-188 (1975).

- Jones, B., Dockrell, H. & Janeway, C. A. in T and B Lymphocytes: Recognition and Function (eds Bach, F. H., Bonavida, B., Vitteta, E. S. & Fox, F. C.) 175-180 (Academic,
- 12. Bottomly, K. & Jones, F. in 2nd Int. Conf. B Lymphocytes in the Immune Response (eds Klinman, N., Mosier, D. E., Scher, I. & Vittetta, E.) 415 (Elsevier, Amsterdam, 1981).

 13. Sachs, D. H. & Cone, J. L. J. exp. Med. 138, 1289–1304 (1973).

- 14. Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H. & Benacerraf, B. J. exp. Med. 138, 734-739 (1973).
- 15. Wigzell, H. in In vitro Methods in Cell Mediated and Tumour Immunity (eds Bloom, B. R. & David, J. R.) 245-253 (Academic, New York, 1976).

Distinct genes for fibroblast and serum C1q

J. Skok*, Ellen Solomon*, K. B. M. Reid† & R. A. Thompson‡

* Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields,

London WC2A 3PX, UK † MRC Immunochemistry Unit, Department of Biochemistry,

University of Oxford, Oxford OX1 3QU, UK

‡ East Birmingham Hospital, Regional Department of Immunology, Bordesley Green East, Birmingham B9 5ST, UK

Complement component C1 is composed of three subcomponents, C1q, C1r and C1s (refs 1, 2). The problem of which cells produce C1 in vivo has been controversial, and many cell types³⁻⁹ have been reported to synthesize these molecules. Material antigenically similar to C1q was reported10 to be synthesized and secreted by human and rat fibroblast cell lines. Reid and Solmon¹¹ subsequently showed that C1 haemolytic activity was secreted into the medium of cultured human fibroblast cell lines, and that the form of the C1q was unusual in that it had an apparently higher molecular weight than did C1q isolated from normal serum. As fibroblasts are an abundant cell type, this observation suggested that they might be a major contributor to serum C1q, secreting C1q precursor, pro-C1q. We describe here a genetic defect of serum C1q. Homozygotes for this defect do not produce functional plasma C1q, although they seem to produce normal fibroblast C1q. Heterozygotes produce both normal and defective serum C1q. This suggests that different genes code for the chains of C1q produced by fibroblasts and for those produced in serum.

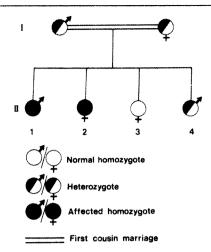


Fig. 1 Family tree showing the inheritance of the gene responsible for the production of the non-functional serum C1q. This condition appears to behave as an autosomal recessive in which both affected siblings, II 1 and II 2, are homozygous for the defective gene, and both parents and sibling II 4 are heterozygous. Skin biopsies were obtained from II 1 (SSF) and II 2 (RKF).

A family has been described¹² in which two siblings are totally deficient in serum C1q activity. The eldest child, a boy, suffers from a lupus-like syndrome and glomerulonephritis. The younger sister shows no evidence of nephritis clinically, but has cutaneous vasculitic lesions of the face and hands. Both children have a non-functional, but immunologically cross-reacting serum C1q. The normal and defective C1q in the family can be distinguished on Ouchterlony double-diffusion gels¹². The defective serum C1q is slightly less mobile on SDS-polacrylamide gel electrophoresis (PAGE) in non-reducing conditions than is normal C1q (K.B.M.R., unpublished results), as shown in Fig. 3a. In addition, the patients' serum C1q behaves anomalously compared with normal C1q on a Sephadex G-200 column, and can be separated in non-dissociating conditions. Of two other siblings, one is entirely normal, and the other, apparently heterozygous, has both functional and nonfunctional C1q. The parents are first cousins, and they also have functional and non-functional C1q. Figure 1 shows the family

To determine whether the fibroblasts of these patients produce the haemolytically inactive C1q that is found in their serum, we established skin fibroblast cultures, designated SSF (male) and RKF (female), from each of the two affected homozygous siblings. Human fibroblasts, designated Bu, from a C1q normal individual were used as the control culture. Cells were grown for 7 days in RPMI 1640 medium in the presence of 10% inactivated fetal calf serum (FCS), and the samples from days 0 and 7 compared. The FCS was inactivated by heating at 56 °C for 45 min, which destroys most, but not all, of the bovine C1 haemolytic activity. As seen in Fig. 2, SSF and RKF cultures gave the same titres of C1 haemolytic activity as did the control fibroblasts. Assay conditions indicated that these fibroblasts were producing C1r and C1s, in addition to active C1q, a finding consistent with previous work 11.

To characterize the structure of the fibroblast C1q produced by the serum C1q-deficient patients, immunoprecipitates derived from the supernatants of labelled cultures of SSF, RKF and Bu were compared on SDS-PAGE (Fig. 3a, b). These cultures were also assayed for haemolytic activity and found to be positive.

Cold carrier serum C1q was added to each sample of labelled fibroblast medium before immunoprecipitation. The immunoprecipitates were run on SDS-PAGE in reducing and non-reducing conditions. Figure 3a shows the positions of the cold carrier serum C1q, after protein staining. The reduced sample (lane 1) shows the three chains of serum C1q, A, B and C, at

apparent molecular weights 34,000, 31,000 and 27,000 (refs 13, 14), and the heavy and light chains of the rabbit immunoglobulin. The non-reduced sample (lane 2) shows the A-B and C-C dimers of C1q and the intact rabbit immunoglobulin. Figure 3b shows an autoradiograph of the whole gel. As found previously with fibroblast cultures 11, the larger-molecular-weight C1q does not enter the gel in the non-reduced form (lanes 5-7). When reduced, the radioactively labelled C1q immunoprecipitates from the three fibroblast cultures (lanes 2-4) all show the same two bands, with apparent molecular weights of 47,000 and 42,000. These same two bands were previously shown to be synthesized by fibroblasts11. Both reduced and non-reduced samples show radioactive bands near the top of the gel. The identity of these bands is not certain, but they are probably fibronectin which could bind to the collagenous part of the cold carrier Clq, present in the immune aggregates, in a similar manner to the binding of fibronectin to denatured collagen (see, for example, ref. 15).

The results clearly indicate that the C1q secreted into the medium of the fibroblast cultures, derived from the two serum C1q-deficient siblings, has haemolytic activity comparable with that of the control culture. The radiolabelled immunoprecipitates from the culture medium also show no apparent differences between the patients and the control.

If fibroblasts were a major source of serum C1q, patients with a non-functional circulating C1q might be defective in a gene

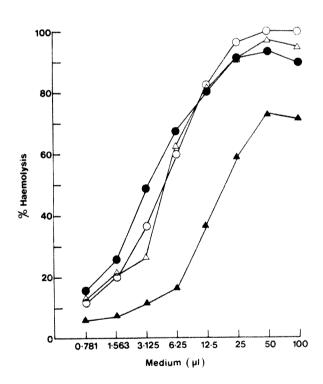


Fig. 2 Assay of component C1 haemolytic activity of day 0 control, and day 7 medium collected from fibroblast cultures of Butler (Bu), RKF and SSF. Preparation of the buffers and reagents used in the haemolytic assays was performed as described by Reid 5. Aliquots (100 µl) of doubling dilutions of the cell culture media were incubated with $100 \,\mu l$ of EAC4 cells (1×10^8) cells ml⁻¹) at 30 °C for 20 min, and then spun down at 2,000 r.p.m. for 10 min. The cell pellet was resuspended in assay buffer and incubated at 30 °C for 40 min, then component C2 (0.1 ml, 200 effective mol ml⁻¹) was added and the mixture incubated at 30 °C for 15 min. 0.1 ml of EDTA buffer, pH 7.4, was added and finally 0.2 ml of guinea pig serum, diluted 1/30 with isotonic buffer, pH 7.4, containing EDTA, was added and incubated at 37 °C for 30 min. After addition of 1.0 ml 0.15 M NaCl, the mixture was spun at 2,000 r.p.m. for 10 min and the degree of lysis determined by reading the A_{412} of the supernatant. \bigcirc , Bu; \triangle , RKF (female); ●, SSF (male); ▲, day 0 control.

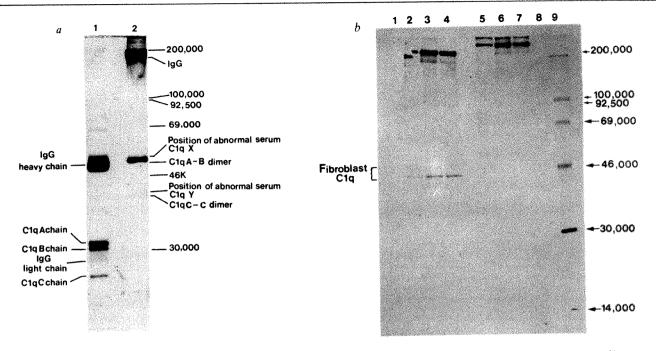


Fig. 3 a, The three fibroblast cell lines, Butler, RKF and SSF, were labelled over 72 h with 100 μ Ci ³⁵S-methionine, 16.3 μ Ci ¹⁴C-proline and 16.3 μ Ci ¹⁴C-glycine per flask of 2×10^6 cells. The radioactive amino acids were added directly to the medium containing inactivated FCS. A solution of carrier subcomponent C1q, prepared according to Reid¹⁷ (30 μ l of 3.8 mg ml⁻¹), was added to the 30 ml culture medium collected from the three fibroblast cell lines. The medium was made 10 mM with respect to EDTA, and then concentrated to ~4 ml in an Amicon Diaflo 50-ml cell using a PM10 membrane. The concentrate was mixed with enough heat-inactivated rabbit anti-human serum subcomponent C1q to precipitate all the unlabelled carrier subcomponent C1q added. The rabbit anti-C1q was prepared according to Reid et al. 13. The concentrate plus antiserum was incubated at 37 °C for 2 h, and then at 4 °C overnight. The immunoprecipitate formed was washed five times with 0.3 ml of 10 mM EDTA/150 mM NaCl, pH 7.4. After the final wash the samples were electrophoresed on a polyacrylamide gel in the presence of SDS as described by Fairbanks et al. 18. The gel was stained for 1 h and then destained overnight. Lane 1, cold reduced serum C1q, lane 2, non-reduced cold serum C1q. Molecular weight markers (14C-methylated protein mixture CFA.610, Radiochemical Centre) were myosin (MW 200,000), phosphorylase b (100,000 and 92,500). bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000) and lysozyme (14,300). Standards were run in reducing conditions. The mobility of the defective serum C1q in non-reducing conditions is indicated on this gel. This was determined in a parallel experiment (K.B.M.R., unpublished results). X and Y correspond to what are thought to be the A-B and C-C dimers of the abnormal serum C1q. b, The gel was impregnated with diphenyloxazole in dimethyl sulphoxide by the method of Bonner and Laskey¹⁹, dried and exposed to RP Royal X-Omat film at -70 °C for 2 weeks. Lane 2, reduced Bu; 3, reduced RKF; 4, reduced SSF; 5, non-reduced Bu; 6, non-reduced RKF; 7, non-reduced SSF.

coding for a processing enzyme of fibroblast C1q. In this event, one could not expect the heterozygote parents and sibling to have normal as well as abnormal circulating C1q, as codominant expression is not expected with a defect in a processing enzyme. Thus, these results strongly suggest that a cell type other than fibroblasts is responsible for the synthesis of serum Clq, and that there are distinct genes for the fibroblast Clq and serum C1q. The products of these genes would be two antigenically and physicochemically different C1q molecules.

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- 1. Lepow, I. H., Naff, E. W., Todd, E. W., Pensky, J. & Hinz, C. F. J. exp. Med. 117, 938-1008
- Gigli, I., Porter, R. R. & Sim, R. B. Biochem, J. 157, 541-548 (1976).
- Stecher, V. J., Morris, J. H. & Thorbecke, G. J. Proc. Soc. exp. Biol. Med. 124, 433-438
- Day, N. K., Gewurz, H., Pickering, R. J. & Good, R. A. J. Immun. 104, 1316-1319 (1970). Muller, W., Hanauske-Abel, H. & Loos, M. J. Immun. 121, 1578-1584 (1978)
- Muller, W., Hanauske-Abel, H. & Loos, M. J. Immun. 121, 1578-1584 (1978).
 Colten, H. R., Borsos, T. & Rapp, H. J. Proc. natn. Acad. Sci. U.S.A. 56, 1158-1163 (1966).
 Colten, H. R., Gorden, J. M., Rapp, H. J. & Borsos, T. J. Immun. 100, 788-792 (1968).
 Bing, D. H., Spurlock, S. E. & Bern, M. M. Clin. Immun. Immunopath. 4, 341-351 (1975).
 Morris, K. M., Colten, H. R. & Bing, D. H. J. exp. Med. 148, 1007-1019 (1978).
 Al-Adnani, M. S. & McGee, J. O'D. Nature 263, 145-146 (1976).
 Reid, K. B. M. & Solomon, E. Biochem. J. 167, 647-660 (1977).
 Thomson, R. A. et al. Num. Expl. J. Med. 303, 23, 244 (1980).

- Thompson, R. A. et al. New Engl. J. Med. 303, 22-24 (1980).
 Reid, K. B. M., Lowe, D. M. & Porter, R. R. Biochem. J. 130, 749-763 (1972).

- Reid, K. B. M. & Porter, R. R. Biochem. J. 155, 19-23 (1976). Ruoslahti, E., Vaento, M. & Engrall, E. Biochim. biophys. Acta 534, 210-218 (1978).
- Reid, K. B. M., Sim, R. B. & Faiers, A. P. Biochem. J. 161, 239-245 (1977) Reid, K. B. M. Biochem. J. 141, 189-203 (1974).
- Fairbanks, G., Stech, T. L. & Wallach, D. F. H. Biochemistry 10, 2606-2617 (1971).
- 19. Bonner, M. & Laskey, R. A. Eur. J. Biochem. 46, 83-88 (1974)

Dispermic origin of XY hydatidiform moles

Koso Ohama*, Tadashi Kajii†, Etsuji Okamoto*, Yasuhiko Fukuda‡, Kiyoshi Imaizumi†, Masato Tsukahara†, Kunihiko Kobayashi† & Keiji Hagiwara†

* Department of Obstetrics and Gynecology and ‡ Surgery, Hiroshima University School of Medicine, Hiroshima, Japan 734 † Department of Pediatrics, Yamaguchi University School of Medicine, Ube, Japan 755

Complete hydatidiform mole is an abnormal human pregnancy with grossly swollen chorionic villi, usually with a 46,XX karyotype, and with a propensity to malignancy1. The XX moles originate from fertilization of an 'empty egg' (resulting from either enucleation or inactivation of the female pronucleus) by a haploid sperm and its subsequent duplication^{2,3}, a process called diploid androgenesis. XY moles, although infrequent, are of special interest because their origin must differ from that of the XX moles. Two XY moles have been studied for their origin, but the results were inconclusive^{3,4}. We describe here a study of four XY moles, and provide evidence that they result from the fertilization of an empty egg by two haploid spermatozoa.

The macroscopic and microscopic findings of the four XY moles were indistinguishable from those of XX moles. The karyotypes of cells cultured from the molar tissue of the four patients were 46,XY. Q- and R-band chromosome heteromorphisms², histocompatibility leukocyte antigen (HLA) specificities, phosphoglucomutase 1 (PGM₁) and esterase D

Table 1 Mode of inheritance of heteromorphic features in XY moles

			\		C	Chromoson	al hetero	morphism	s†		Н	LA	Enzyme variants	
		Age*	Karyotype	3	4	13	14	15	21	22	A	В	PGM _i	ES-D
	(Mat	23 yr	46, XX	ab	aa	aa	ab	<u>ab</u>	aa	ab	2, 9	w51, w52	2-1	2-1
\boldsymbol{A}	{ Pat	25 yr	46, XY	aa	aa	aa	aa	bc	ab	ab	9, 11	40, w21	1-1	2-1
	Mole	67 days	46, XY	aa	aa	aa	aa	cc	ab	ab	9, 9	40,40	1-1	2-2
	(Mat	27 yr	46, XX	aa	aa	aa	aa	aa_	aa bc	ab	1,2	40, 39	2-1	2-1
В	{ Pat	27 yr	46, XY	aa	aa	ab	ab	bc	bc	aa	9, 11	7, 13	1-1	1-1
	Mole	65 days	46, XY	aa	aa	aa	aa	bc	bc	aa	11, 11	7,7	1-1	1-1
	(Mat	23 yr	46, XX	aa	aa	ab	ab	<u>ab</u>	ab	aa	2, 2	w51, 40	1-1	2-1
C	{ Pat	27 yr	46, XY	ab	aa	bc	aa	ac	ab	ab	2, 9	40, 40	2-1	2-1
	Mole	72 days	46, XY	ab	aa	bb	aa	cc	ab	ab	2, 2	40, 40	1-1	2-1
	(Mat	21 yr	46, XX	<u>ab</u>	aa	ab	ab	aa	ab	ab			1-1	1-1
D	{ Pat	22 yr	46, XY	ac	aa	cd	ac	ab	bb	bc	*****	Allenane	1-1	1-1
	Mole	122 days	46, XY	cc	aa	cc	ac	ab	bb	cc	Suction		1-1	1-1

^{*} Age at expulsion (days after last menstrual period in hydatidiform mole).

(ES-D) genotypes were studied in the four XY moles and their parents. HLA-A and -B specificities were studied using peripheral blood lymphocytes from the parents and cultured molar fibroblasts by the standard National Institutes of Health two-stage microcytotoxicity test⁵. PGM₁ and ES-D genotyping was carried out on parental red cells and on uncultured molar villi with starch gel electrophoresis^{6,7}. These genetic markers were reliable in our hands in the study of the origin of XX moles (ref. 2 and unpublished data). The chromosomal markers are situated close to the centromeres and thus are usually free from meiotic crossing-over, while HLA and enzyme gene loci are situated relatively far from the centromeres, and thus may occasionally be subject to cross-over and resulting recombination.

The results of these studies are shown in Table 1. Mole A inherited both chromosomes 15 and HLA-B specificities exclusively from the father. Similarly, mole B showed an exclusively paternal inheritance of both chromosomes 15 and 21, as well as HLA-A and -B specificities. Mole C inherited both chromosomes 15 from the father. Mole D inherited pairs 3, 13 and 22 exclusively from the father. Thus, all four moles were androgenetic in origin. Further, each of the four moles had both homozygous (a paternal marker transmitted to the mole in duplicate) and heterozygous (both paternal markers transmitted to the mole) genetic markers. The fact that both homozygous and heterozygous chromosomal heteromorphisms were observed in each of the four moles indicates that the moles resulted from dispermy, that is, entry of an X and Y sperm into an 'empty egg'. Entry of a diploid spermatozoon resulting from tetraploid spermatogonia cannot be ruled out but is less likely. The results of the HLA studies do not support the cytogenetic findings, but this could well be a chance occurrence (one in eight).

We are aware of 121 complete moles karyotyped since 1977: 80 by us and 41 in the literature 3,4,8-10. This, excluding an isolated case report4, would give five XY moles out of 120 karyotyped, a rate of 4%. Dispermy would result in XX, XY and YY sex chromosome constitution, at a 1:2:1 ratio. YY moles are lethal, while XX dispermic moles are not. Therefore, some 2% of XX moles would also be the product of dispermy, a variety yet to be identified.

Mole B in our series is worthy of comment. The human chorionic gonadotropin (HCG) level in the mother remained high after expulsion of the mole, and roentgenography 8 weeks after expulsion revealed in the right lung a 15×13 mm rounded shadow not seen before. Chemotherapy was started and the urinary HCG level fell to normal in 10 weeks, while the pulmonary shadow remained unchanged 18 months after treatment. We proposed that the propensity to malignancy of complete moles is associated with single chromosomal mutation

that is of necessity homozygous in the 46, XX variety². This explanation might only apply to the XY dispermic moles, in which each spermatozoon would contribute the same chromosomal segment carrying the causative mutation. It would be interesting to know whether such a propensity is more prevalent in one of the two classes of complete mole

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- Vassilakos, P., Riotton, G. & Kajii, T. Am. J. Ohstel. Gynec. 127, 167-170 (1977).
- Kajii, T. & Ohama, K. Nature 268, 633-634 (1977). Jacobs, P. A., Wilson, C. M., Sprenckle, J. A., Rosenshein, N. B. & Migeon, B. R. Nature 286, 714-716 (1980). Surti, U., Szulman, A. E. & O'Brien, S. Hum. Genet. 51, 153-156 (1979)
- Ray, J. G., Hare, D. B., Peterson, P. D. & Kaybee, D. E. (eds) Manual of Tissue Typing Techniques, 20-22 (NIH, Bethesda, 1974).
- Spencer, N., Hopkinson, D. A. & Harris, H. Nature 204, 742-743 (1964). Hopkinson, D. A., Mestriner, M. A. & Cortner, J. Ann. hum. Genet. 37, 119-137 (1973).
- Wake, N., Takagi, N. & Sasaki, M. J. natn. Cancer Inst. 60, 51-57 (1978). Wake, N., Shiina, Y. & Ichinoe, K. Proc. Jap. Acad. 54, 533-537 (1978).
- awler, S. D. et al., Lancet ii, 580 (1979) 11. Kajii, T. Gann Monogr. Cancer Res. 25, 189 (1980)

RNA is synthesized at the nuclear cage

D. A. Jackson, S. J. McCready* & P. R. Cook

Sir William Dunn School of Pathology and * Botany School, University of Oxford, Oxford OX1 3RE, UK

The photomicrographs of 'genes in action' taken by Miller and colleagues strikingly illustrate transcription of eukaryotic genes by RNA polymerase moving along the DNA1,2. Although completed transcripts may be associated with sub-nuclear structures3-7, the view that a mobile polymerase processes along the DNA remains unchallenged. Here we examine an alternative view-that transcription occurs as DNA passes through a transcription complex fixed to a sub-nuclear structure, and we show that transcribed sequences are closely associated with a sub-nuclear structure that we call a nuclear cage.

When nuclei are isolated by conventional procedures, their DNA is invariably broken, mainly by endonucleolytic nicking8. However, if living cells are lysed in a detergent, 2 M salt and chelating agents, structures are released which resemble nuclei 4.9-12. Such nucleoids contain naked histone-free DNA packaged within a flexible cage of RNA and protein. Some

^{-,} Neither of maternal markers transmitted to the mole, indicating its androgenetic origin. □, f a, b, c and d are symbols and do not represent specific heteromorphisms. A paternal market transmitted in duplicate to the mole [1], Both of paternal markers transmitted to the mole. A, B, C, D refer to the four moles studied.

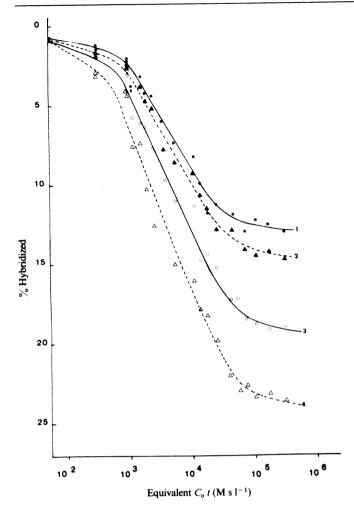


Fig. 1 Cage-associated DNA is enriched in sequences complementary to nucleoid RNA. Four different samples of cage-associated DNA containing 100% (sample 1), 35% (2), 14% (3) and 5% (4) of the DNA associated with undigested nucleoids were labelled by nick-translation, denatured and the per cent of the DNA forming a hybrid with an excess of nucleoid RNA determined. Nucleoids (prepared from cells labelled with ³H-thymidine) were incubated with various amounts of *EcoR*1 and the cages, and any associated DNA, sedimented free of unattached DNA¹⁸. The pellet was solubilized in proteinase K and SDS and the amount of DNA remaining cage associated determined by scintillation counting. The cage-associated DNA was purified, completely fragmented with EcoRI to yield populations of fragments with similar sizes¹⁸ and labelled by nick-translation³¹ using ³HdCTP (58 Ci mmol $^{-1}$) to yield products with specific activities of $50-70\times10^6$ d.p.m. per μg. After heat denaturation, these single-stranded probes had sizes between 100 and 200 nucleotides (determined by gel electrophoresis). RNA was purified from nucleoids and hybridized in excess (RNA: DNA, 50 mg ml⁻¹: 25 ng ml⁻¹) to the labelled DNA and the per cent of the DNA forming a hybrid determined essentially as described by Levitt et al.32 Self-annealing of the probe, in the presence of an excess of tRNA, never exceeded 2% and has been subtracted from the values presented. During the very long incubation required to achieve high equivalent C_0t values, <5% of the probe became acid soluble.

nucleoid proteins resemble those of various sub-nuclear structures isolated by other workers¹³⁻¹⁷. Their DNA, which is looped by attachment to the cage, is supercoiled and so must be unbroken. Therefore, these nucleoids might be expected to contain unbroken RNA.

We first studied whether DNA close to the cage was richer than total DNA in transcribed sequences. Four types of DNA were prepared by incubating HeLa nucleoids with various amounts of *Eco*RI; then cages, and any associated DNA, were sedimented free of detached fragments to yield pellets which retained various amounts of DNA¹⁸. This cage-associated DNA was purified and hybridized with total nucleoid RNA (Fig. 1). (As nucleoids contain all the nuclear RNA, this is equivalent to nuclear RNA.) The cage-associated DNA hybridized to a

greater extent than total nuclear DNA (sample 1), for example, 23% of the DNA from cages which retained 5% of the total (that is, sample 4) is complementary to nucleoid RNA. Assuming that only one strand is transcribed, about half this sample of cage-associated DNA contains transcribed sequences—a remarkable enrichment.

We next determined whether nascent RNA was attached to the cage. When HeLa cells are incubated with 3 H-uridine for 1 or 15 min, >95% of the cellular radioactivity which is insoluble in trichloroacetic acid subsequently co-sediments with nucleoids (unpublished results and ref. 4). We tested the possibility that it is entangled in the cage network or the high concentration of DNA by determining whether it could be detached with DNA from the cages. Cells were labelled with [Me^{-14} C]thymidine (0.005 μ Ci ml⁻¹; 56 mCi mmol⁻¹) for 24 h, followed by 3 H-uridine (10 μ Ci ml⁻¹) for 2.5 min. (Actinomycin D (0.08 μ g ml⁻¹) was present during, and 30 min before, the 3 H pulse to suppress ribosomal RNA synthesis ¹⁹.) Nucleoids were

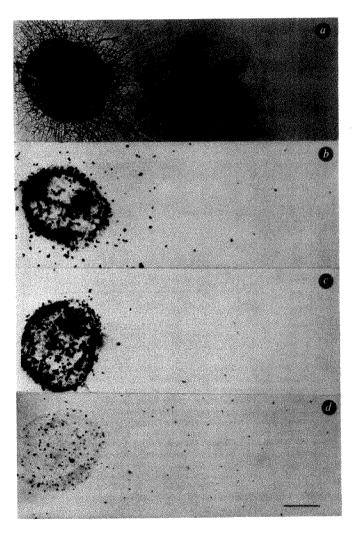


Fig. 2 RNA is attached to the cage. a, An electron micrograph of a nucleoid spread by Kleinschmidt's procedure ¹² illustrating the cage and the skirt of tangled DNA fibres. b-d, Autoradiographs of spreads²⁰ after labelling: b, HeLa cells in vivo for 24 h with [Me- 3 H]thymidine (58 Ci mmol $^{-1}$; 0.05 μ Ci ml $^{-1}$); c, cells in vivo for 2.5 min with [5,6- 3 H]uridine (46 Ci mmol $^{-1}$; 250 μ Ci ml $^{-1}$); d, nucleoids in vitro by transcribing nucleoids for 15 min using E-coil RNA polymerase (see ref. 21; 3 units per assay; [5,6- 3 H]UTP, 52 Ci mmol $^{-1}$, 20 μ Ci ml $^{-1}$). The reaction was stopped by the addition of NaCl to 2.0 M. So that silver grains can be easily seen and counted in b-d, DNA is shadowed but not stained and can be seen only at higher magnifications. Nascent RNA cannot have been torn from the DNA—and so lost—during the violent spreading because the same total aumber of grains lie over the DNA of spread and unspread nucleoids (results not shown). Scale bar, 5 μ m.

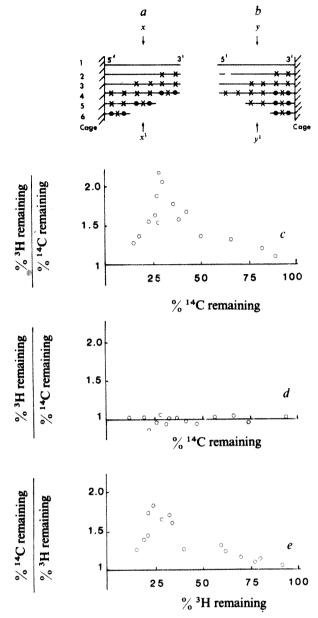


Fig. 3 RNA is attached at the 3' end. a, A simple model for labelling and cutting RNA attached at the 5'end. (1) A completed strand of RNA (attached at its 5' end to the cage. Cells are labelled for 2 min with 14C-uridine (×) followed by 1 min with ³H-uridine (•). (2, 3), Some nascent RNA molecules complete synthesis after the addition of ¹⁴C but before the addition of ³H, so becoming only ¹⁴C-labelled, whereas others (4-6), which initiate during the pulses, become labelled with both ¹⁴C and ³H. Digestion with ribonuclease (that is, cutting between x and x') detaches ¹⁴C and ³H in roughly equal proportions. Therefore, on digestion, the ratio (%3H remain-C remaining) remains at about unity. b, An array of molecules labelled as in a are attached at the 3' end. Cutting between y and y' detaches ¹⁴C but not ³H: therefore, the ratio is greater than unity. These models are gross oversimplifications and we use them only to illustrate whether or not a label becomes enriched. c-e, The 3' end of nascent RNA resists detachment by ribonuclease. HeLa cells $(5 \times 10^7 \,\mathrm{ml}^{-1})$ were incubated at 37 °C with $[U^{-14}C]$ uridine (490 mCi mmol⁻¹) and $[5,6^{-3}H]$ uridine (46 Ci mmol⁻¹) as indicated. Actinomycin D (0.08 µg ml⁻¹) was present 30 min before, and during, the labelling. Labelling was stopped by the addition of 50 volice-cold phosphate-buffered saline containing 10 mM uridine, the cells were pelleted and washed, and nucleoids isolated and diluted to 0.2 M NaCl and ~2 with 10 mM Tris (pH 8.3). They were then incubated with pancreatic ribonuclease (2-500 ngm ml-1) for 0-30 min at 37 °C and the reaction was stopped by addition of NaCl to 2 M. The per cent of label remaining associated with cages was determined by filtration²⁰. Control experiments using mixtures of labelled RNA and nucleoids showed that experiments using mixtures of labelled RNA and nucleotes 88% of the RNA could be filtered free of the cages. Labelling was for: c, 2 min with 14 C-uridine (2 μ Ci ml⁻¹) followed by 1 min with 3 H-uridine (200 μ Ci ml⁻¹); d, 3 min with both 3 H-uridine (200 μ Ci ml⁻¹) and 14 Curidine $(2 \mu \text{Ci ml}^{-1})$; e, 2 min with ³H-uridine $(100 \mu \text{Ci ml}^{-1})$ followed by 1 min with ¹⁴C-uridine $(4 \mu \text{Ci ml}^{-1})$. In these conditions sufficient label was incorporated to be counted accurately; for example, filters bearing undigested nucleoids contained about 10^4 and 2×10^3 d.p.m. of 3H and ^{14}C respectively.

isolated, incubated with EcoRI and the amount of the two labels remaining associated with cages was determined after filtering them free of detached DNA²⁰. In one typical experiment, when 90% of ¹⁴C (that is, DNA) was detached, < 5% of the ³H (RNA) was lost. (A control experiment showed that >80% of the ³H but none of the ¹⁴C could be detached by ribonuclease.)

A second experiment confirms that nascent RNA is not simply entangled in DNA. When nucleoids are spread on an air/aqueous interface their DNA, initially confined within the cage, forms a huge skirt which is attached to, and surrounds, the collapsed cage ¹² (Fig. 2a). If RNA were entangled in DNA we would expect its distribution to reflect that of spread DNA. The latter is obtained from autoradiographs of spreads prepared from cells containing uniformly labelled DNA (Fig. 2b): 55% of the DNA is outside the cage ²⁰. However, when cells are pulse-labelled with ³H-uridine for 2.5 min, >90% of the grains lie over the cage in each of 20 spreads selected at random (Fig. 2c). This is so whether or not the DNA has been lightly γ ray-irradiated (9.6 J per kg) to release supercoils ^{9.11} (results not shown).

The following controls demonstrate that RNA made in vitro can escape with the DNA from the cage and does not stick nonspecifically to the cage network. RNA was synthesized in vitro by incubating nucleoids with Escherichia coli RNA polymerase, ³H-UTP and the appropriate precursors²¹. The transcripts co-sediment with the nucleoids²¹ and are spread by Kleinschmidt's procedure; 34% of the autoradiographic grains are found over the skirt (Fig. 2d). Furthermore, digestion with EcoRI (using conditions which detached 79% of the DNA) detaches 75% of this RNA labelled in vitro so that it can then be filtered free of the cages. (The conditions for transcription and filtration are described in the legends to Figs 2 and 3.) This RNA made in vitro is not as tightly associated with the cage as that synthesized in vivo. We next determined whether this tight association was specific.

A 'cap' containing methylated bases is synthesized at the 5' end of nascent RNA as transcription begins²²⁻²⁴. Therefore, ³H-methionine can be used to label caps; however, this label is also incorporated into proteins, DNA and other methylated bases within RNA chains, especially into those within ribosomal RNA molecules. We suppress the latter using actinomycin D^{19,23}. We established what proportions were incorporated into these different fractions by labelling cells for 15 min with ³H-methionine. Of the label in nucleoids, 75% was solubilized by proteinase K and SDS, and so must be in protein, and 23% was recovered in RNA (Table 1). As others have found^{25,26}, 36% of the label in RNA was in caps, the remainder being in internal residues within the original RNA chains (control sample in Table 1).

Table 1 Distribution of incorporation of ³H label into different fractions on labelling RNA

Amount label remaining (c.p.m. × 10 ⁻³)			
Control	RNase treated		
600 (100%)	600 (100%)		
138 (23%)	68 (11%)		
90.2 (15%)	24.6 (4.1%)		
50.0 (8.3%)	48.2 (8.0%)		
	Control 600 (100%) 138 (23%) 90.2 (15%)		

Cells were labelled for 15 min with L-[Me-³H]methionine (50 μCi ml-¹; 78 Ci mmol-¹), and nucleoids isolated and diluted to 0.2 M NaCl and 2×10⁶ ml-¹ with 10 mM Tris (pH 8.0)^{4.1¹}. Actinomycin D (0.08 μg ml-¹) was present 30 min before, and during, the labelling. Nucleoids were then incubated with or without pancreatic ribonuclease (5 μg ml-¹) for 15 min at 37 °C, the reaction was stopped by the addition of NaCl to 2 M and the nucleoids were pelleted through a cushion of 7.5% sucrose containing 2 M NaCl. The pellet was digested with deoxyribonuclease (5 μg ml-¹) for 20 h and then proteinase K and SDS¹8. Next, RNA was purified³0 and the proportion of label in caps and internal residues in the RNA was determined after digestion with RNase A and T2 followed by column chromatography: mononucleotides and caps eluted in fractions containing +1 and +5 charges, respectively²³.25.26°. The amount of ³H in the different fractions is given in the table with percentages in parentheses. The amount of RNA detached (75%) by the ribonuclease from the pelleted cages was determined from an identical experiment in which cells were labelled with [5,6-³H]uridine (46 Ci mmol-¹; 10 μCi ml-¹) rather than the ³H-methionine.

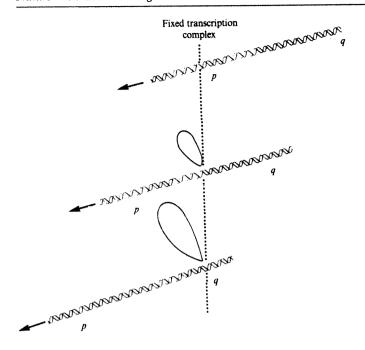


Fig. 4 A model for transcription. a, A fixed complex transcribes DNA between p and q. b,c, RNA is synthesized as DNA passes through the complex, the nascent RNA chain being attached at both ends forming a loop. Transcription of a helical template—itself attached to the cage—to yield attached transcripts poses a number of topological problems. If the polymerase rotates about the helical axis then the transcript can be separated from its template during synthesis by cutting one and passing the other through the cut (there is a precedent for cutting RNA during 'splicing'). Alternatively, a fixed topological relationship might be maintained between the cap and 3' end, perhaps by attaching both to the polymerase, so that if one rotates then so does the other. If DNA rotates, then supercoiling is inevitably induced elsewhere in the DNA and must be stored or released (for example by a nicking-closing activity).

If nascent RNA is attached at its 5' end, caps should resist detachment by ribonuclease. Therefore, we incubated ³H-methionine-labelled nucleoids with sufficient ribonuclease to detach 75% of nascent RNA. Next, cages and any associated RNA were sedimented free of detached RNA and the amounts of label in the different fractions determined (Table 1). The ribonuclease treatment did not affect the quantity of label recovered in protein or DNA (results not shown) whereas it halved that in RNA. This reduction was almost entirely due to the loss of label in internal residues with negligible loss in caps. Therefore, removal of 75% of the body of the chain detaches few, if any, caps.

We have attempted to demonstrate attachment at the 3' end as follows. Cells were incubated for 2 min with 14C-uridine and then for 1 min with ³H-uridine. The doubly labelled nucleoids were then incubated with ribonuclease, the detached RNA removed by filtration and the percentage of the labels remaining associated with the cages determined. If RNA is attached at random, 14C and 3H will be detached from the cages in equal proportions, that is, the normalized ratio (% ³H remaining): (% ¹⁴C remaining) will remain at unity independently of the amount of ¹⁴C remaining. On the other hand, if the 3' end is attached, detachment of RNA will lead to a relative enrichment of ³H: the ratio will increase above unity as the amount of 14C remaining decreases (Fig. 3a, b). (Attachment at both ends cannot be distinguished from 3' attachment using this labelling regime.) The results are consistent with attachment at the 3' end because the ³H resists detachment. For example, removal of all but 30% of 14 C leaves 54% of 3 H (thus, the ratio in Fig. 2c is 54:30 = 1.8). Experiments with simultaneous labelling and label reversal (Fig. 3d, e) rule out labelling artefacts.

Three points should be borne in mind in discussing these results. First, we are concerned mainly with heterogeneous

nuclear RNA as we have generally labelled in actinomycin D to suppress ribosomal RNA synthesis. Second, our pulse times should label many unfinished molecules. [Heterogeneous nuclear RNA—1,500–50,000 nucleotides²³—is synthesized at ~5,000 nucleotides per min (ref. 27)]. The third point concerns the anchorage by polymerases of 3' ends to DNA and so to the cage. We would expect polymerases to dissociate from the DNA in the 2 M NaCl used to make nucleoids and although nucleoids contain no polymerase activity²¹, we cannot preclude anchorage by some—perhaps undiscovered—subunit of the polymerase. However, if polymerase does hold the 3' ends in this way, it must do so close to the cage because neither transcript nor its template is readily detached by *Eco*RI.

We have demonstrated that transcribed sequences are closely associated with a sub-nuclear structure that we call the nuclear cage. Furthermore, nascent RNA is attached at the 5' cap—and perhaps at the 3'end—to the cage. The striking micrographs illustrating polymerases processing along the DNA unattached to any larger structure are obtained only after disruptive spreading or with unusually hyperactive genes which may be very special cases. (For example, lampbrush loops and amplified ribosomal genes in amphibian oocytes.)

A trivial explanation is consistent with our results. Perhaps nascent RNA, which is synthesized throughout the nucleus in vivo, sticks to the cage when nucleoids are prepared. We think this extremely unlikely: nearly all this RNA would have to stick specifically at the 5' end and remain stuck during filtration or spreading, even though the control experiments show that RNA synthesized in vitro has little if any, affinity for nucleoids. A second explanation is that a polymerase initiates at the cage and moves away to yield a transcript attached at its cap. However, this cannot readily explain why, in the presumed absence of polymerase, the 3' end resists detachment by ribonuclease. Alternatively, transcripts may be generated as DNA passes through a fixed transcription complex to yield transcripts attached at both ends (Fig. 4). Whichever proves to be correct, it seems that the cage must have an important role in transcription. As replication is initiated by attaching sequences to the cage²⁰ (see also refs 28, 29), perhaps genes are also activated during differentiation by attachment.

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    Miller, O. L. & Beatty, B. R. Science 164, 955-957 (1969).
    McKnight, S. & Miller, O. L. Cell 8, 305-319 (1976).
    Franke, W. W. Int. Rev. Cytol. Suppl. 4, 71-236 (1974).
    Cook, P. R., Brazell, I. A. & Jost, E. J. Cell Sci. 22, 303-324 (1976).
    Miller, T. E., Huang, C.-Y. & Pogo, A. O. J. Cell Biol. 76, 675-691 (1976).
    Herman, R., Weymouth, L. & Penman, S. J. Cell Biol. 78, 663-674 (1978).
    Harlan, G., Echert, W. A., Kaffenberger, W. & Wunderlich, F. Biochemistry 18, 1782-1788 (1979).
    Warren, A. C. thesis, Univ. Oxford (1977).
    Cook, P. R. & Brazell, I. A. J. Cell Sci. 19, 261-279 (1975).
    Cook, P. R. & Brazell, I. A. J. Cell Sci. 19, 261-279 (1975).
    Cook, P. R. & Brazell, I. A. J. Cell Sci. 19, 261-279 (1978).
    McCready, S. J., Akrigg, A. & Cook, P. R. J. Cell Sci. 39, 53-62 (1979).
    Berezney, R. & Coffey, D. S. Biochem. 84, 465-477 (1978).
    McCready, S. J., Akrigg, A. & Cook, P. R. J. Cell Sci. 39, 53-62 (1979).
    Berezney, R. & Coffey, D. S. Biochem. biophys. Res. Commun. 60, 1410-1417 (1974).
    Rijey, D. E., Keller, J. M. & Byers, B. Biochemistry 14, 3005-3013 (1975).
    Aaronson, R. P. & Blobell, G. Proc. natn. Acad. Sci. U.S.A. 72, 1007-1011 (1975).
    Comings, D. E. & Okada, T. A. Expl Cell Res. 103, 341-360 (1976).
    Levin, J. M. thesis, Univ. Oxford (1978).
    Cook, P. R. & Brazell, I. A. Nucleic Acids Res. 8, 2895-2906 (1980).
    Perry, R. P. Expl Cell Res. 29, 400-406 (1963).
    McCready, S. J., Godwin, J., Mason, D. W., Brazell, I. A. & Cook, P. R. J. Cell Sci. 46, 365-386 (1980).
    Furuichi, Y. Proc. natn. Acad. Sci. U.S.A. 75, 1086-1090 (1978).
    Salditt-Georgieff, M., Harpold, M., Chen-Kiang, S. & Darnell, J. E. Cell 19, 68-78 (1980).
    Perry, R. P., Kelley, D. E., Friderici, K. & Rottman, F. Cell 4, 387-394 (1975)
```

Maniatis, T., Jeffrey, A. & Kleid, D. G. Proc. natn. Acad. Sci. U.S.A. 72, 1184-1188 (1975).
 Levitt, A., Axel, R. & Cedar, H. Devl Biol. 69, 496-565 (1979).

Dijkwel, P. A., Mullenders, L. H. F. & Wanka, F. Nucleic Acids Res. 6, 219-230 (1979).
 Pardoll, D. M., Vogelstein, B. & Coffey, D. S. Cell 19, 527-536 (1980).
 Palmiter, R. D. Biochemistry 13, 3606-3615 (1974).

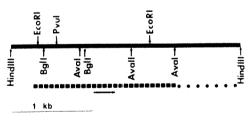
Developmental expression of a Drosophila actin gene encoding actin I

Erich Zulauf*, Federico Sánchez*, Sara L. Tobin†‡, Ursula Rdest* & Brian J. McCarthy*‡

* Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, USA
† Department of Genetics, University of California, Berkeley, California 94720, USA

The Drosophila melanogaster actin genes comprise a dispersed multigene family^{1,2} of six genes, one at each of six scattered chromosomal loci (refs 1, 2 and unpublished observations). These genes encode the Drosophila actins I, II and III3,4, which can be separated in the isoelectric focusing dimension of twodimensional gels. Actins II and III have been found in all tissues, with actin III an unstable species except in adult thorax5, while actin I, the most acidic form, appears during larval myogenesis both in vivo and in vitro 3,4. Some actins therefore seem to be developmentally regulated. If individual members of a multigene family are regulated at the transcriptional level, this implies that developmental mechanisms of gene regulation can be elucidated by comparing gene family members. We report here a pattern of transcriptional activity of the D. melanogaster actin gene at the 79B chromosomal locus. Our results suggest that the 79B actin gene encodes actin I, the larval muscle-specific actin, and that actin I is synthesized initially as a precursor. The precursor co-migrates with cytoplasmic actin at the position of actin II and is subsequently acetylated to form actin I.

Because actin is a highly conserved protein⁶, the DNA sequences encoding the protein cross-react with great efficiency, both within actin gene family members of a particular organism and between different species 1.7-10, and so the gene sequences themselves cannot be used to detect the transcript of any one individual gene. We therefore isolated a specific probe from the transcribed, but non-translated 3' portion of the 79B actin gene and used it to investigate the transcriptional activity of the 79B gene at different stages of *Drosophila* development. We also used this probe to select homologous mRNA from a heterogeneous mRNA population. The message which bound to this



Lys Cys Phe Stop
AAG TGC TTC TAA COTAGGTTGGGTGGGGTTGGTCTAGLCGGCAGAGGAGCTCCGCCGGGGGACCAG
TTC ACG AAG ATT GCATCCAAGCCAGCGGAACCAGATGGGGGTCTCCTCCAGGGCGGGGGCGGTCTCTC

Ava I clearage site

Fig. 1 Physical map of the actin gene at the 79B cytological locus. This 2.8-kb HindIII DNA fragment from phage λ insert DNA 11 containing the 79B actin gene was subcloned into the plasmid pBR322 as described previously 1. The locations of restriction endonuclease sites within the plasmid insert are indicated. The dashed line and arrow indicate the amino acid-encoding region of the plasmid and direction of transcription as determined by DNA sequence analysis 14. The AvaI-HindIII fragment at the 3' end of the gene delineated by the dotted line was used as the gene-specific probe for the 79B actin gene. The DNA sequence from the 3' end of the amino acid-coding region to the AvaI restriction endonuclease cleavage site at the beginning of the gene-specific probe is shown.

‡ To whom reprint requests should be addressed.

probe was then eluted, translated *in vitro* and the translation products electrophoresed on two-dimensional gels to distinguish between actins I, II and III.

Several clones representing the 79B actin gene were isolated from a bank of Canton S DNA cloned into phage λ by Maniatis and co-workers¹¹, using our original actin clone Kla (ref. 1) as probe. The presumptive coding region of each as identified by homology to Kla was subcloned into pBR322. The 79B cytological locus of origin¹² was established by *in situ* hybridization¹³ of a ³H-labelled cRNA probe synthesized using as template a subclone representing a region flanking the actin protein-coding

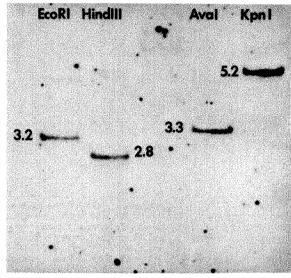


Fig. 2 Autoradiogram illustrating hybridization of the 79B gene-specific probe to genomic DNA from Canton S embryos. The 79B gene-specific probe was isolated from a preparative digest of whole plasmid DNA¹, labelled with ³²P by nick-translation ¹⁵ and hybridized to Canton S embryo DNA that had been cut with the indicated restriction endonucleases, electrophoresed in a 1% agarose gel and transferred to a nitrocellulose filter²⁵. The sizes of labelled fragments (kb) are indicated (exposure 4 days).

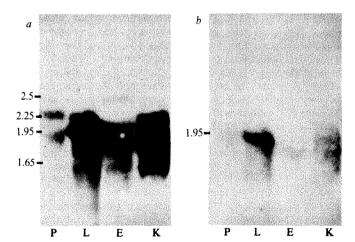


Fig. 3 Autoradiogram illustrating hybridization of coding region (a) and gene-specific (b) probes to poly(A) RNAs from embryos, larvae, pupae and Kc cells. Probes were labelled by nick-translation and hybridized to poly(A) RNAs that had been denatured, separated on a single methyl mercury-containing agarose gel and covalently bound to a diazobenzyloxymethyl paper filter and covalently bound to a diazobenzyloxymethyl paper filter and covalently from the scatting gene which contains the complete actin amino acid-coding region of that gene. This gene was one of those we have isolated from the Maniatis Canton S genome bank and is homologous to that previously described. The probe was removed by several washes with 95% formamide at 45 °C. b, Hybridization of 79B gene-specific probe to filter. The sizes of the labelled transcripts (kb) are indicated. Embryos (E); larvae (L); pupae (P); Kc cells (K).

[†] Present address: Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717, USA.

sequence¹. All 79B clones were characterized by an EcoRI restriction endonuclease cleavage site within an intervening sequence starting at amino acid 307 (data to be presented elsewhere).

Figure 1 shows a restriction endonuclease cleavage map of the 79B actin gene. The gene-specific probe is indicated and the AvaI restriction endonuclease site at which it begins has been localized by DNA sequence analysis 14 at 30 nucleotides beyond the 3' end of the amino acid coding region. We have used the fragment indicated in Fig. 1 for the experiments described here. This DNA fragment contains no amino acid-encoding nucleotides and, as shown in Fig. 2, hybridizes to only one genome DNA fragment with a molecular weight corresponding to that of the original clone fragment from which it was derived. This may be contrasted with the seven fragments labelled when a similar blot of EcoRI-digested Canton S genome DNA is hybridized to an amino acid-coding region probe 2 .

The gene-specific probe was labelled with ³²P by nick-transand hybridized to populations of poly(A)RNAs isolated from three different stages of Drosophila development and from Kc cells16 which had been fractionated on an agarose gel and transferred to diazotized paper¹⁷. Figure 3 contrasts the reaction of a coding region probe which will hybridize to all actin transcripts with the hybridization of the 79B gene-specific probe. Actin transcripts of varying lengths (1.65-2.5 kilobases (kb)) can be observed in the preparations while the 79B genespecific probe hybridizes strongly to only one RNA species (1.95 kb) from early third instar larvae and weakly to RNA from Kc cells. Because the transcripts homologous to the 79B genespecific probe constitute a significant proportion only of larval actin message, the 79B gene seems to function independently of at least some other actin genes. This experiment establishes developmental specificity of the transcriptional activity of the 79B locus.

To determine whether actins I, II or III are synthesized by the 79B actin gene, we immobilized the gene-specific probe DNA on nitrocellulose, hybridized the probe DNA to homologous poly(A) RNA from early third instar larvae, eluted the bound RNA¹⁸ and translated the RNA in vitro¹⁹. Figure 4a, b shows the translation products after separation by two-dimensional electrophoresis²⁰. Before selection of the 79B gene-specific transcripts, actins I, II and III are observed (Fig. 4a). In contrast, translation products of the RNA homologous to the 79B gene-specific probe migrate at the positions of actins I and II (Fig. 4b). These results differ from those obtained with translation

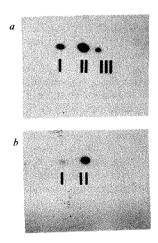


Fig. 4 Autoradiograms of two-dimensional gel electrophoresis of ³⁵S-methionine-labelled translation products of early third instar larval RNAs. a, Poly(A) RNA¹ from early third instar larvae was translated in vitro¹⁹ and the translation products electrophoresed in two dimensions²⁰. b, The same RNA was hybridized to DNA of the 79B gene-specific probe and the bound RNA eluted¹⁸. This RNA was then translated and electrophoresed simultaneously with shared buffer chambers with gel a. Actins I, II and III are indicated.

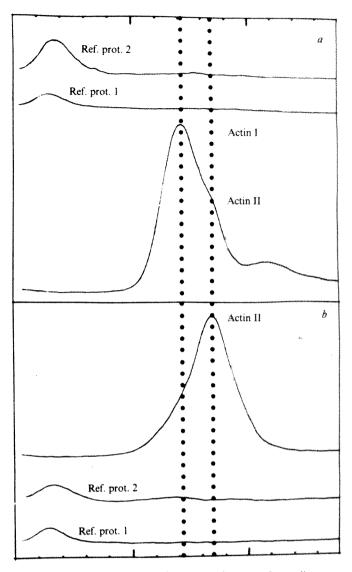


Fig. 5 Densitometer scan of an autoradiogram of two-dimensional gel electrophoresis of ³⁵S-methionine-labelled translation products with and without reagents which prevent acetylation of the *in vitro* synthesized proteins²⁴. Total RNA was isolated from early third instar larvae¹ and translated *in vitro*¹⁹. The translation products were electrophoresed simultaneously with shared buffer chambers in two dimensions²⁰ and autoradiographed. The X-ray films were scanned with a soft laser scanning densitometer (Biomed Instruments). Two reference proteins from each gel are included. a, Densitometer scan of actins and two reference proteins which were translation products of total RNA from early third instar larvae. b, Densitometer scan of actins and two reference proteins which were translated from the same RNA preparation in the presence of citrate synthase (0.88 units per 25-µlreaction solution) and oxaloacetate (1 mM) as described by Palmiter²⁴.

products (actins II and III) of RNAs selected by a cytoplasmic *Drosophila* actin gene probe². Thus, either the gene-specific probe selects more than one message or the two actins observed are related by a precursor-product relationship. We think the first hypothesis unlikely because the probe does not hybridize to other actin genes (see Fig. 2).

If the isoelectric point shift between actin II and actin I were due to post-translational processing, acetylation would seem a likely candidate for several reasons. Actin III is converted into actin II by an acetylation²¹ in Kc cells, causing a shift in isoelectric point from 5.84 to 5.77 (ref. 3). As the isoelectric points of actin I (5.70) and actin II (5.77) also differ by 0.07 pH units, acetylation of actin II could account for its conversion into actin I. The reticulocyte lysate has a ribosome-associated transacetylase²² which is capable of acetylating *Dictyostelium* actin²³.

Also, a proportion of the in vitro acetylation of proteins in the reticulocyte translation system can be suppressed by the addition of oxaloacetate and citrate synthase²⁴. Consequently, we carried out in vitro translation of total larval RNA with and without oxaloacetate and citrate synthase and separated the products by two-dimensional electrophoresis. The results of this experiment are shown in Fig. 5a, b. When acetylation is suppressed to this degree, actin I is greatly reduced, presumably because of diminished conversion of actin II into actin I. The peak of actin II is increased, presumably due to accumulation of actin II which would ordinarily have been acetylated into actin I. These results strongly suggest that actin I is synthesized as a component that initially co-migrates with acetylated cytoplasmic actin and is subsequently acetylated. The acetylation of a precursor can thus account for the characteristic isoelectric point and position on two-dimensional gels of actin I^{3,4}, and can also explain why the turnover of actin III in muscle cells is insufficient to account for the quantity of actin II observed^{3,4}. In addition, this result suggests the evolutionary conservation of mechanisms of acetylation because the rabbit acetylase is apparently able to recognize and acetylate Drosophila actins.

Thus, we believe the 79B actin gene encodes actin I, the larval muscle-specific actin, although it does not seem to be the only actin gene to do so (unpublished data). Several characteristics of the transcript of this gene are consistent with this hypothesis; for example, the stage at which the gene is active, the difference in size of the transcript from that of one of the cytoplasmic messages² and the synthesis of actin I by in vitro translation of mRNA homologous to the 79B gene-specific probe.

The 79B locus seems to be developmentally regulated independently of other *Drosophila* actin genes (Fig. 3). Differential control of actin gene expression during development may at least partly explain the multiplicity of actin genes which have been found in several eukaryotes^{7,8}. We are continuing to develop gene-specific probes for the remainder of the actin genes of *D. melanogaster* to investigate the pattern(s) of gene expression and form of actin synthesized by each. These patterns of expression will ultimately be correlated with DNA sequence information from the 5' regions of each actin gene so as to define the sequences responsible for this regulation.

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Received 10 April; accepted 23 June 1980.

- 1. Tobin, S. L., Zulauf, E., Sanchez, F., Craig, E. A. & McCarthy, B. J. Cell 19, 121-131
- Fyrberg, E. A., Kindle, K. L., Davidson, N. & Sodja, A. Cell 19, 365-378 (1980).

 Storti, R. V., Horovitch, S. J., Scott, M. P., Rich, A. & Pardue, M. L. Cell 13, 589-598 (1978).
- Fyrberg, E. A. & Donady, J. J. Devl Biol. 68, 487-502 (1979).
 Horovitch, S. J., Storti, R. V., Rich, A. & Pardue, M. L. J. Cell Biol. 82, 86-92 (1979).
 Pollard, T. D. & Weihing, R. R. CRC crit. Rev. Biochem. 2, 1-65 (1974).
 Kindle, K. L. & Firtel, R. A. Cell 15, 763-778 (1978).

- Cleveland, D. W. et al. Cell 20, 95-106 (1980).
- Gallwitz, D. & Sures, I. Proc. natn. Acad. Sci. U.S.A. 77, 2546-2550 (1980).
- Ng, E. & Abelson, J. Proc. natr. Acad. Sci. U.S.A. 77, 3912-3916 (1980).
 Maniatis, T. et al. Cell 15, 687-701 (1978).

- Bridges, C. B. J. Hered. 26, 60-64 (1935). Bonner, J. J. & Pardue, M. L. Chromosoma (Berl.) 58, 87-99 (1976).
- Maxam, A. M. & Gilbert, W. Meth. Enzym. 65, 499-560 (1980).
 Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. J. molec. Biol. 113, 237-251 (1977).
- 16. Craig, E. A., McCarthy, B. J. & Wadsworth, S. C. Cell 16, 575–588 (1979).
 17. Alwine, J. C., Kemp, D. J. & Stark, C. R. Proc. natn. Acad. Sci. U.S.A. 74, 5350–5354
- 18. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. Proc. natn. Acad. Sci. U.S.A. 76, 4927-4931
- Pelham, H. R. B. & Jackson, R. J. Eur. J. Biochem. 67, 247-256 (1976)
- O'Farrell, P. H. J. biol. Chem. 250, 4007-4021 (1975) Berger, E. et al. Biochem. Genet. 19, 321-331 (1981).

- Traugh, J. A. & Sharp, S. B. J. biol. Chem. 252, 3738–3744 (1977).
 Rubenstein, P. & Deuchler, J. J. biol. Chem. 254, 11142–11147 (1979).
- Palmiter, R. D. J. biol. Chem 252, 8781–8783 (1977) Southern, E. M. J. molec. Biol. 98, 503–518 (1975).
- Bailey, J. M. & Davidson, N. Analyt. Biochem. 72, 413-427 (1976).

Gene required in G₁ for commitment to cell cycle and in G₂ for control of mitosis in fission yeast

Paul Nurse & Yvonne Bissett

School of Biology, University of Sussex, Brighton BN1 9QG, UK Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

The control regulating commitment to the cell division cycle of eukaryotes seems to occur before the initiation of DNA replication 1-4. In the budding yeast Saccharomyces cerevisiae, this control is called start and is the earliest gene-controlled event of the cell cycle³⁻⁵. A haploid cell which has completed start is committed to cell division and unable to undergo alternative developmental fates such as conjugation. Here, we describe an analogous start control in the fission yeast Schizosaccharomyces pombe. We have tested the ability of cdc mutants blocked at various stages of the cell cycle to undergo conjugation. Only mutants of cdc 2 and cdc 10 which block during the G1 period are able to conjugate. Other mutants which block during G1, S phase, G2 or mitosis are committed to cell division and cannot conjugate. The commitment control start is located in G1, and its completion requires the gene functions of cdc 2 and 10. Completion of start occurs at the beginning of the cell cycle in rapidly growing cells, but is delayed to later in the cell cycle at slow growth rates. The cdc 2 gene function also participates in another major cell cycle control which determines the timing of mitosis⁶⁻⁸. Therefore cdc 2 is a cell cycle control gene which acts at two separate points in the cell cycle: it is required in G. for commitment to cell division and in G2 for the control of mitosis.

When dividing S. pombe cells are deprived of nitrogen, they stop proliferating, accumulate in G₁ and, if they are of appropriate mating type, undergo conjugation and sporulation^{9,10}. To determine whether conjugation is limited to a specific phase of the S. pombe cell cycle, various temperature-sensitive cdc mutants were incubated at their restrictive temperature to block cells at different places in the cell cycle, and the ability of these cells to conjugate was tested. The restrictive temperature of the cdc mutants was either 35 or 36 °C (refs 11, 12, 20), but at these temperatures conjugation in wild type was inhibited. As the highest temperature at which a reasonable level of conjugation took place was 33 °C (ref. 10), the experiment was carried out by incubating the cdc mutant cells for two generations at 36 °C to block progress through the cell cycle, followed by shifting the cells to 33 °C and mixing them with cells of opposite mating type to allow conjugation to take place. After overnight incubation the mating mix was plated out to assay the number of diploid cells formed as a consequence of conjugation (for practical details see Table 1 legend). In these conditions of temperature and medium, mutants of 11 of the 16 cdc genes known to be required for DNA replication or mitosis were blocked in their progress through the cell cycle. Mutants of the other five genes were not completely blocked and could not be used in the experiment. The levels of conjugation expressed as a percentage of a wild-type control are given in Table 1 (column 1). Only mutants of cdc 2 and 10 gave high levels of conjugation. Other mutants were <5% of the wild-type control.

Before concluding that all the cdc genes apart from cdc 2 and 10 block at cell cycle stages where conjugation is not possible, it is necessary to eliminate various other explanations for the failure of conjugation in the blocked mutants. The first possibility is that the viability of the cdc mutants is dramatically reduced after incubation at the restrictive temperature. This was not the case because the viabilities of the cdc mutants after 5 h at 36 °C and overnight at 33 °C were high, the lowest values being ~20% for cdc 24.M38 and cdc 6.23 (column 4 of Table 1). A second possibility is that the cdc mutants are partially sterile and have a reduced ability to mate even at the permissive temperature. This was not the case either, because at 25 °C all the mutants mated well, the lowest being ~30% of the wild-type control for cdc 17.K42 and cdc 25.22 (column 2 of Table 1). A third, more subtle, possibility is that the cdc mutant function is directly required in the conjugation process, and that this is the reason conjugation is prevented at the restrictive temperature (for a fuller discussion of this point see ref. 5). This possibility was tested by first accumulating the cdc mutant cells at the appropriate cell cycle stage for mating by incubation at 25 °C in nitrogen-free medium, shifting the cells to 33 °C and then mixing them with cells of opposite mating type. In these conditions, all the cdc mutants underwent conjugation, demonstrating that they were not directly involved in the conjugation process (column 3 of Table 1). Two mutants, cdc 1.7 and cdc 25.22, did have rather reduced conjugation frequencies, ~ 10 -15%, but these levels were still far greater than their conjugation frequencies at 36/33 °C.

These experiments demonstrate that mutants of cdc 17, 22, 23, 24 (involved in G_1 or S phase) and of cdc 1, 6, 13, 25, 27 (involved in G_2 or mitosis) block at stages in the cell cycle where conjugation is not possible whereas mutants of cdc 2 and 10 block at a stage where it is possible. Two different mutant alleles of cdc 2 and 10 behave similarly (Table 1), indicating that the ability to conjugate at the restrictive temperature was not due to the presence of a second mutation in the strains. The stage at which cdc 10 mutant cells become blocked is during G_1 , before the initiation of S phase^{11,12}. The situation with cdc 2 is more complex as cdc 2 mutants were previously thought to become blocked only in late G_2 just before mitosis^{8,11}. However, cdc 2

Table 1 % Conjugation for each temperature regime

		_	-			
	cdc mutant	36/33°C	25/25°C	25/33°C	% Viability	Can cells conjugate at this block?
,	cdc 1.7	0.046	76.2	10.4	47.6	No
	cdc 2.33 cdc 2.M63	30.9 19.0	106 112	105 129	97.1 32.7	Yes
	cdc 6.23	3.09	52.6	37.4	20.8	No
	cdc 10.129 cdc 10.K28	138 108	94.6 101	88.2 117	78.1 56.4	Yes
	cdc 13.117	0.205	60.3	33.5	59.4	No
	cdc 17.K42	0.295	29.0	31.9	58.7	No
	cdc 22.M45	4.44	51.2	42.8	87.3	No
	cdc 23.M36	2.93	72.0	51.0	65.8	No
	cdc 24.M38	2.83	80.3	119	19.2	No
	cdc 25.22	0.086	36.7	14.0	57.6	No
	cdc 27.K8	4.98	93.9	78.6	56.9	No

The cdc h mutant cultures were grown at 25 °C in minimal , washed and resuspended in minimal medium lacking the nitrogen source, and incubated at 25 or 36 °C. After 5 h cells were mixed with a ×10 excess of *ura* 1 *mei* 1-B102 cells¹⁰, the 25 °C culture was split and incubated at 25 °C and 33 °C, and the 36 °C culture incubated at 33 °C. After overnight incubation the mating mix was sonicated and plated onto minimal medium at 36 °C. Only complemented diploids derived from a conjugation event between the cdc h and ura 1 mei 1-B102 parents could form colonies in these conditions. All the cdc mutants were recessive to wild type. The mating type allele mei 1-B102 (also known as mat 2-Pm) was used as this allows conjugation with cells of h mating type but prevents meiosis and sporulation. Consequently a stable diploid is formed. In each batch of experiments a control ade 6 h culture was also crossed to ura 1 mei 1-B102 to determine the numbers of diploids formed from wild-type cells which were not blocked in cell cycle progress by a cdc mutation. The levels of conjugation quoted here are expressed as percentages of this wild-type control under the temperature regime used. The per cent viabilities of the cdc mutants were calculated from comparison of particle and colony counts after cultures had been incubated in minimal medium lacking the nitrogen source for 5 h at 36 °C followed by overnight incubation at 33 °C.

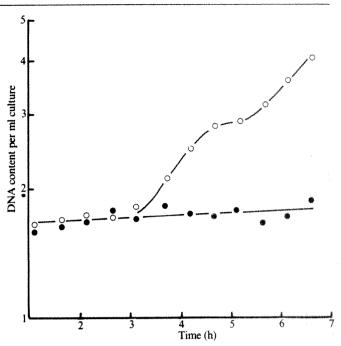


Fig. 1 cdc 2.33 h and 972 h wild-type cells were incubated in minimal medium lacking a nitrogen source at 25 °C and were re-innoculated into fresh minimal medium at 36 °C at time = 0 h. the DNA contents in the two cultures were estimated using the diphenylamine reaction Both cultures began with cells containing the G₁ content of DNA. DNA contents per ml are plotted on an arbitary log scale, and the actual DNA content per ml equivalent to 1 unit on the scale is given in parentheses following the symbol key.

○, 972 wild type (76 ng); ●, cdc 2.33 (68 ng).

mutant cells actually become blocked in G₁ as well as G₂, as is shown by the following experiment. cdc 2.33 cells were accumulated in G₁ by nitrogen starvation at 25 °C and a synchronous culture prepared by re-innoculating the cells into fresh medium at 36 °C. In these conditions wild-type cells undergo a synchronous round of DNA replication, but this does not occur in cdc 2.33 (Fig. 1). Thus, cdc is required independently for the two major events of the cell cycle, S phase and mitosis.

If cdc 2 has two cell cycle block points, it is likely that only those cells blocked at the first point in G1 are able to undergo conjugation. If this was the case it would account for the reduced conjugation of cdc 2 mutants compared with cdc 10 (Table 1, column 1), as the long G₂ period found in S. pombe would result in most cells accumulating at the second block point in late G2 where they could not conjugate. To test this, a synchronous culture of cdc 2.33 was prepared by selection of cells early in the cell cycle (mostly at the beginning of G₂), using sedimentation rate centrifugation. This culture was grown at 25 °C, and aliquots were shifted at various times during the cell cycle to 36/33 °C following the procedure described in the earlier experiments, to measure the ability of the cells to conjugate. If there is only one block point in the cell cycle, regardless of when the shift up occurred, the level of conjugation should have been the same. However, if there are two block points, one at which cells could conjugate and one at which they could not, there should have been a peak in the level of conjugation at the former block point. This second pattern was the one observed, with the mid-point of the peak occurring at the very beginning of the cell cycle coincident with cell division (Fig. 2). This is close to the timing of the G₁ period, indicating that conjugation is possible only at the G₁ block point.

We conclude that there is a start control in fission yeast which functions during the G_1 period. Before start, cells can still conjugate but after start they become committed to cell division and are unable to conjugate. Completion of start and commitment to the cell cycle requires the genes cdc 2 and 10. Rapidly growing cells complete the cdc 10 gene function very early, immediately after mitosis, but in more slowly growing cells the

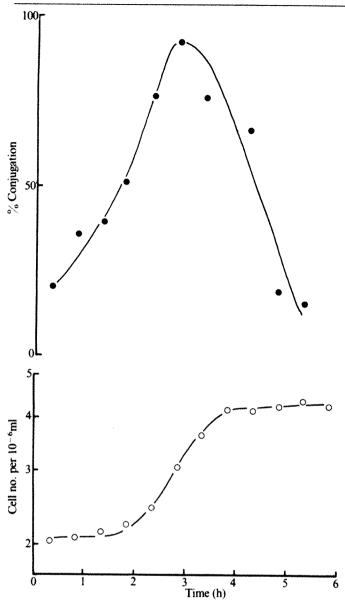


Fig. 2 A synchronous culture of cdc 2.33 h was prepared from an exponentially growing culture by sedimentation rate velocity centrifugation of the cells through a 7.5-30% lactose gradient to isolate the small cells at the beginning of the cell cycle⁶. The synchronous culture was grown in minimal medium at 25 °C and aliquots were shifted at intervals to 36 °C for 5 h in minimal medium lacking a nitrogen source, before shifting to 33 °C and mixing with ura 1 mei 1-B102. The mixing and subsequent processing were as described in Table 1 legend. Cell number was measured using a Coulter counter⁶. •, % conjugation; O, cell number per ml.

cdc 10 function is completed much later in the cell cycle¹¹⁻¹³. Thus, commitment to cell division occurs at the beginning of the cell cycle in rapidly growing cells, but is delayed to later in the cell cycle at slow growth rates. With the appropriate environmental cues these uncommitted cells can undergo an alternative developmental pathway such as conjugation. A similar situation also applies in S. cerevisiae 14

cdc 2 is unique among cell cycle genes in being required independently for two cell cycle events, start and mitosis. Together with wee 1, cdc 2 is involved in the control determining the timing of mitosis⁶⁻⁸. Therefore, cdc 2 can be considered a cell cycle control gene with regulatory functions at two control points during the cycle—start in G₁ and the timing of mitosis in G₂. Attainment of a critical cell size is required before mitosis can take place 15-17. In addition, traverse of start as marked by the completion of cdc 10 gene function¹³ or the occurrence of S phase^{6-8,18,19} also requires a critical, though different cell size.

Thus, cell size may have an important regulatory role at both cell cycle control points.

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- Prescott, D. M. Reproduction of Eukaryotic Cells (Academic, London, 1976).
- 2. Pardee, A. B., Dubrow, R., Hamlin, J. L. & Kletzien, R. F. A. Rev. Biochem. 47, 715-750
- Hartwell. L. H. Bact. Rev. 38, 164-198 (1974).
- Reed, S. I. Genetics 95, 561-577 (1980). Reid, B. J. & Hartwell, L. H. J. Cell Biol. 75, 355-365 (1977). Nurse, P. Nature 256, 547-551 (1975).
- Thuriaux, P., Nurse, P. & Carter, B. Molec. gen. Genet. 161, 215-220 (1978). Nurse, P. & Thuriaux, P. Genetics 96, 627-637 (1980).

- Nurse, P. & Thuriaux, P. Genetics 96, 627-637 (1980).
 Egel, R. & Egel-Mitani, M. Expl Cell Res. 88, 127-134 (1974).
 Crandall, M., Egel, R. & Macay, V. L. Adv. microbiol. Physiol. 15, 307-398 (1977).
 Nurse, P., Thuriaux, P. & Nasmyth, K. Molec. gen. Genet. 146, 167-178 (1976).
 Nasmyth, K., & Nurse, P. Molec. gen. Genet. (in the press).
 Nasmyth, K. J. Cell Sci. 36, 155-168 (1979).
 Jagadish, M. N. & Carter, B. L. A. Nature 269, 145-147 (1977).
 Fantes, P. & Nurse, P. Expl Cell Res. 107, 377-386 (1977).
 Fantes, P. & Nurse, P. Expl Cell Res. 107, 377-386 (1977).

- Fantes, P. & Nurse, P. Expl Cell Res. 115, 317-329 (1978).
 Nurse, P. & Thuriaux, P. Expl Cell Res. 107, 365-375 (1977)

- Nasmyth, K., Nurse, P. & Fraser, R. J. Cell Sci. 39, 215-233 (1979).
 Thuriaux, P., Sipiczki, M. & Fautes, P. J. gen. microbiol. 116, 525-528 (1980).

Hydrolysis of ATP and reversible binding to F-actin by myosin heavy chains free of all light chains

Paul D. Wagner & Edward Giniger

Cardiovascular Research Institute, School of Medicine. University of California, San Francisco, San Francisco, California 94143, USA

Myosin has two 'heads' or subfragments-1 (S-1) each containing a heavy chain, a phosphorylatable or 'regulatory' light chain and another light chain which is generally referred to as being essential¹⁻³. The regulatory light chains can be removed without loss of ATPase activity3, but until now it has not been possible to remove the other class of light chain without complete loss of ATPase activity4. However, the dissociating conditions used also denature the heavy chains, and inactivation frequently precedes light-chain dissociation⁵. We now describe the use of mild dissociating conditions combined with affinity chromatography using antibodies to the alkali light chains to produce light-chain-free S-1 heavy chains. The S-1 heavy chain binds reversibly to F-actin and has 30-80% of the ATPase activities of native S-1.

Fast skeletal muscle myosin has two different but related essential light chains, alkali-1 (molecular weight $(M_r) = 20,700$) and alkali-2 $(M_r = 16,500)^6$. Two S-1 isozymes, S-1(A1) and S-1(A2), can be separated on the basis of their alkali light-chain content. These two forms of S-1 seem to have the same heavychain compositions, that is, both contain the same two Nterminal sequences⁷. In the absence of actin, S-1(A1) and S-1(A2) have the same ATPase activities. However, at low ionic strengths, the actin-activated ATPase of S-1(A2) has a higher maximal rate of ATP hydrolysis (V_{max})⁸. Using 4.7 M NH₄Cl as a dissociant, purified alkali light chains have been exchanged into S-1 or myosin without inactivation9. Similar techniques have been used to prepare hybrids which contain heavy chains from one muscle myosin and 'essential' light chains from a different muscle myosin⁹⁻¹¹. Here we have modified the exchange conditions to allow the separation of light chains from heavy chains.

Rabbit antibodies to the alkali light chains of chicken pectoralis muscle myosin were purified and coupled to CNBractivated Sepharose 4B (3 mg of antibody per ml of Sepharose) as described elsewhere 12. The immunoadsorbent column was eluted in conditions similar to those used in the exchanges, but

Table 1 ATPase activities of S-1 and S-1 heavy-chain preparations

			Actin-activated			
	EDTA (s ⁻¹)	$Ca^{2+}(s^{-1})$	$I = 0.$ $V_{\text{max}}(\mathbf{s}^{-1})$	020 M K _{app} (μM)	$I = 0.$ $V_{\text{max}}(\mathbf{s}^{-1})$	040 M Κ _{αρο} (μΜ)
Preparation A	LDIA(8)	Ca (s)	max (5)	Tapp (part)	max (o)	realth (bette)
S-1(A2)	10.1	2.1	25	34		
NH ₄ Cl-treated S-1(A2)*	7.6	1.3	19	26		
S-1 heavy chain (10% S-1(A2))†	7.9	0.9	13.5	28		
Preparation B						
S-1(A2)	9.6	0.96	28	45	24	97
NH ₄ Cl-treated S-1(A2)*	6.5	0.58	20	53	16	110
S-1 heavy chain (15% S-1(A2))†	4.3	0.36	9.4	32	10	89
S-1(A1)	9.5	0.87	16	7.6	23	65

ATPase assays were done using a pH stat to measure the rate of H⁺ release at pH 8.0 and 25° C. Assay conditions for the EDTA-ATPase were: 2 mM EDTA, 5 mM ATP and 0.6 M KCl; for the Ca²⁺-ATPase: 10 mM Ca²⁺, 5 mM ATP and 0.6 M KCl; and for the actin-activated ATPase: 2 mM MgCl₂, 1 mM ATP and 12 or 32 mM KCl. The actin-activated ATPases were analysed using the following equation ¹⁸: $v = V_{\text{max}} \left[\frac{\text{actin}}{K_{\text{app}}} + \frac{\text{actin}}{K_{\text{app}}} \right] = \frac{V_{\text{max}}}{V_{\text{max}}} \left[\frac{V_{\text{max}}}{V_{\text{max}}} \right] = \frac{V_{\text{max}}}{V_{\text$

* S-1(A2) was diluted to 0.25 mg ml⁻¹ with 4.6 M NH₄Cl, 2 mM ATP, 2 mM DTT, 2 mM EDTA and 0.10 M imidazole, pH 7.0 at 4° C. After 1 h, MgCl₂ was added to 5 mM and the S-1(A2) dialysed as described for the light-chain-depleted samples.

† S-1 heavy-chain samples were prepared as described in Fig. 1 legend. The amount of alkali-2 light chain in the samples was determined by densitometry of Coomassie brilliant blue-stained SDS-polyacrylamide gels. The two different S-1 heavy-chain preparations shown indicate the variability in the EDTA-ATPase activities.

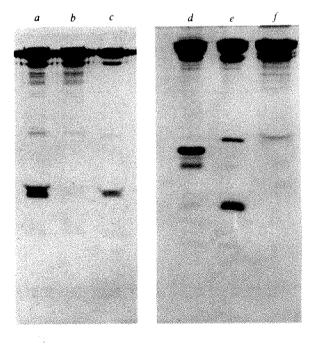
2 mM ATP was added to stabilize the heavy chains during the 1-h isolation procedure and $MgCl_2$ was added to 5 mM before the removal of the NH_4Cl .

When S-1(A2) was applied to the column, the protein eluting in NH₄Cl contained 85–90% S-1 heavy chain and 10–15% S-1(A2) (Fig. 1). (The phosphorylatable light chains were removed during the digestion of myosin to S-1.) After removing the NH₄Cl, the light-chain-depleted samples had 45–80% of the EDTA-ATPase and ~40% of the Ca²⁺-ATPase of native S-1(A2) (see Table 1). This decrease in ATPase activity may be due in part to the NH₄Cl treatment. In the exchange experiments, where the S-1 was 2.3 mg ml⁻¹, there was only a 10–15% loss in activity. However, when S-1 was left in NH₄Cl for 1 h at

0.25 mg ml⁻¹, equivalent to the S-1 heavy-chain concentration which elutes from the immunoadsorbent column, there was a 25-40% decrease in ATPase activities (Table 1).

When an equal mixture of S-1(A2) and S-1(A1) was applied to the immunoadsorbent column in the same conditions as for Fig. 1, the eluate contained no alkali-2 light chains and half the level of alkali-1 present in the applied S-1. This sample had 73% of the EDTA-ATPase and 45% of the Ca²⁺-ATPase of native S-1. There are two obvious explanations for the preferential removal of the alkali-2 light chain by the immunoadsorbent column: either these particular antibodies interact more strongly with the alkali-2 than with the alkali-1 light chains, or the latter bind more tightly to the S-1 heavy chains than do alkali-2

Fig. 1 SDS-polyacrylamide gel electrophoresis of S-1 heavychain preparations. S-1 was prepared by an α -chymotryptic digestion of chicken pectoralis myosin and fractionated into S-1(A1) and S-1(A2)⁸, S-1(A2) (5.0 mg) was incubated for 5 min at 4° C in 1 ml of 4.7 M NH₄Cl, 5 mM ATP, 2 mM EDTA, 2 mM dithiothreitol (DTT) and 0.10 M imidazole, pH 7.0, and then loaded on to a 1.6×5 cm immunoadsorbent column (containing antibodies specific for alkali light chains) equilibrated in 4.6 M NH₄Cl, 2 mM ATP, 2 mM DTT, 2 mM EDTA and 0.10 M imidazole, pH 7.0. The column was eluted with this equilibration buffer at 20 ml h and 2.5-ml fractions were collected. The two peak fractions, 0.2 to 0.3 mg ml⁻¹, were pooled and MgCl₂ added to a concentration of , were pooled and MgCl2 added to a concentration of 5 mM; 4 ml were dialysed against 0.2 M KCl, 2×10⁻⁴ M DTT and 5 mM imidazole, pH 7.0 at 4° C, for use in the ATPase assays, and 1 ml was dialysed against 20 mM NaCl, 2×10^{-4} M DTT and 5 mM imidazole, pH 7.0 at 4°C, for SDS-polyacrylamide gel electrophoresis. The immunoadsorbent column was regenerated by washing rapidly with 4 M guanidine-HCl. The capacity of the column decreased with use; after six runs only two-thirds as much S-1 could be applied. An $A_{280}^{1\%}$ of 7.5 and a molecular weight of 115,000 was used for S-1(A2) and for S-1 heavy chain these values were estimated to be 8.6 and 95,000. SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli' 50 μg of S-1(A2); b, 50 μg S-1 heavy chain, eluted from the immunoadsorbent column in NH₄Cl; c, 25 μg of S-1(A2); d, 50 μg S-1(A1); e, 50 μ g S-1(A2) and f, 50 μ g S-1 heavy chain as in b, except that it was further purified with the immunoadsorbent column equilibrated in 0.2 M KCl. S-1 heavy chain containing 20% contaminating S-1(A2), 11 ml at 0.25 mg ml⁻¹, was concentrated by ultrafiltration to 2 ml at 1.4 mg ml⁻¹ and applied to the immunoadsorbent column equilibrated in 0.2 M KCl, 2×10 DTT and 5 mM imidazole, pH 7.0 at 4° C. S-1(A2) bound to the column and pure S-1 heavy chain passed through.



light chains. The latter explanation is supported by the observation that the alkali-1 light chain exchanges into myosin more easily that alkali-2 light chain 10. As the alkali-2 light chain is more easily removed from S-1 than alkali-1 light chain, in the rest of the experiments described here, the S-1 and heavy chains were prepared from S-1(A2).

The actin-activated ATPases of both S-1 and S-1 heavy chain have hyperbolic dependences on actin concentration (Fig. 2). In preparation A of Table 1, after correcting for residual S-1(A2), the V_{max} of S-1 heavy chain was 49% that of native S-1(A2) and 67% that of NH₄Cl-treated S-1(A2). In these assay conditions, the V_{max} of S-1(A1) is about half that of S-1(A2), but when the ionic strength is increased to 0.04 M, the $V_{\rm max}$ of S-1(A1) increases to that of S-1(A2)¹³. Preparation B of Table 1 shows the effect of ionic strength on the actin-activated ATPase of the S-1 heavy chain. Although the recovery of actin-activated ATPase was lower than in preparation A, the actin-activated ATPase activities were still much higher than could be accounted for by residual S-1(A2). The $V_{\rm max}$ of the S-1 heavy chain did not increase significantly when the ionic strength was increased. At both ionic strengths, the K_{app} of the S-1 heavy chain was between those of S-1(A2) and S-1(A1).

Further purification of the heavy chains was achieved by removing residual S-1(A2) on the immunoadsorbent column in the absence of NH₄Cl. No alkali-2 light chain was detectable on SDS-polyacrylamide gels (see Fig. 1). The EDTA-, Ca²⁺- and actin-activated ATPases of pure S-1 heavy chain were all 30% of those of native S-1(A2). The decreases in activities, most noticeable in the EDTA-ATPase, probably reflect the instability of the heavy chain during the isolation procedures.

To check that the S-1 heavy chains did not contain fragments of the alkali light chains (produced by proteolysis during the isolation procedures) which were not visible on Laemmli-type SDS gels¹⁷, the lysines of an S-1 heavy-chain sample were modified in urea with ³H-formaldehyde and NaCNBH₃ (ref. 14). The sample was run on an SDS-urea gel capable of resolving small peptides¹⁵. There were no counts in the area of small peptides which could be attributed to digestion products of the alkali light chains nor were any bands detectable by Coomassie blue staining.

The binding of the S-1 heavy chain to F-actin was examined by ultracentrifugation (see Table 2). Centrifugation in the absence of actin showed a slight aggregation of the S-1 heavy chains. (Other experiments also showed that the S-1 heavy chains tend to aggregate more readily than does S-1.) After correcting for aggregation, approximately the same fractions of S-1 heavy chain and S-1(A2) bound to F-actin in the absence of

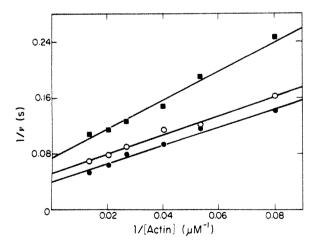


Fig. 2 Actin-activated ATPases. Assays were performed as described in Table 1 legend; v is the observed rate of ATP hydrolysis by S-1 in the presence of actin, minus that of S-1 alone. •, S-1(A2); O, NH₄Cl-treated S-1(A2); and **II**, S-1 heavy chain containing 10% S-1(A2).

Table 2 Actin binding of S-1(A2) and S-1 heavy chain preparations

	Fraction bound		
	-ATP	+ATP	
S-1(A2)	0.91 (0.02)	0.09 (0.03)	
NH ₄ Cl-treated S-1(A2)	0.82 (0.08)	0.05 (0.03)	
S-1 heavy chain (10-15% S-1(A2))	0.82 (0.06)	0.17 (0.09)	

S-1 heavy chain or S-1(A2) (1 μ M) was mixed with 36 μ M F-actin in 0.1 M KCl, 2 mM MgCl₂, 10⁻⁴ M DTT and 5 mM imidazole, pH 7.0 at 4° C. Some samples also contained 1.0 mM ATP. After centrifuging the samples at 150,000g for 30 min, the supernatants were carefully removed and aliquots electrophoresed on SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue and the intensity of the heavy-chain bands determined using a scanning gel densitometer. When centrifuged in the absence of actin, 80% of the S-1 heavy chain and 90% of the S-1(A2) and the NH₄Cl-treated S-1(A2) remained in the supernatant. These values were used as references for determining the fraction of S-1 bound to F-actin. Values are the mean (±s.d.) of three different S-1 heavy-chain preparations

ATP, but a lower fraction of S-1 heavy chain was released by ATP.

These results show that the alkali light chains are not essential for either actin binding or ATP hydrolysis, and that the ATP and actin binding sites are located on the myosin heavy chains. However, the alkali light chains may stabilize the myosin conformation, as the heavy-chain preparations seem to be unstable. It has been reported that the isolated heavy chain of the nonfilamentous Acanthamoeba myosin 1B is fully active 16. As this myosin has many unique properties, it was not possible to extrapolate this observation to other myosins. However, as fast skeletal muscle myosin is a 'typical' myosin, it is likely that the ATP and actin binding sites of other myosins will also reside on the heavy chains.

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- Lowey, S & Risby, D. Nature 234, 81-84 (1971).
- Sarkar, S., Sreter, F. A. & Gergely, J. Proc. natn. Acad. Sci. U.S.A. 68, 946-950 (1971).
 Weeds, A. G. & Lowey, S. J. molec. Biol. 61, 701-725 (1971).
 Dreizen, P. & Gershman, L. C. Biochemistry 9, 1688-1693 (1970).
 Leger, J. J. & Marotte, F. FEBS Lett. 52, 17-21 (1975).

- Frank, G. & Weeds, A. G. Eur. J. Biochem. 44, 317-334 (1974). Pope, B. J., Wagner, P. D. & Weeds, A. G. J. molec. Biol. 109, 470-473 (1977). Weeds, A. G. & Taylor, R. S. Nature 257, 54-56 (1975).
- Wagner, P. D. & Weeds, A. G. J. molec. Biol. 109, 455-473 (1977). Wagner, P. D. J. biol. Chem. 256, 2493-2498 (1981).
- 11. Hollosi, G., Srivastava, S. & Wikman-Coffelt, J. FEBS Lett. **120**, 199–204 (1980). 12. Holt, J. C. & Lowey, S. Biochemistry **16**, 4398–4402 (1977).
- Wagner, P. D., Slater, C. S., Pope, B. & Weeds, A. G. Eur. J. Biochem. 99, 385-394 (1979).
 Jenoft, N. & Dearborn, D. G. J. biol. Chem. 254, 4359-4365 (1979).
 Swank, R. T. & Munkres, K. D. Analyr. Biochem. 39, 462-477 (1971).
- Maruta, H., Gadasi, H., Collins, J. H. & Korn, E. D. J. biol. Chem. 253, 6297-6300 (1978).
 Laemmli, H. K. Nature 227, 680-685 (1970).
- 18. Eisenberg, E. & Moos, C. Biochemistry 7, 1486-1489 (1968).

Erratum

In the letter 'Lamellar-zonal bone with zones and annuli in the pelvis of a sauropod dinosaur' by R. E. H. Reid, Nature 292, 49-51 (1981), the sentence beginning on the first line of page 51 should read 'Some material in the literature cited' could also be interpreted differently, for example if bone figured by Seitz (Figs 53, 54 of ref. 14) from the theropod Allosaurus is thought to show lamellar-zonal structure'.

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BOOK REVIEWS

The case for unilateral disarmament

Hedley Bull

ROBERT Neild, a Professor of Economics at Cambridge and the first Director of the Stockholm International Peace Research Institute, has written a clear-headed and honest little book which will indeed help people to make up their minds about the bomb, even where (like myself) they are not able to accept his conclusions.

In Britain the debate about nuclear weapons policy, dormant throughout the late 1960s and 1970s, has been revived by the recent decisions of the government to purchase the *Trident* missile from the United States and to accept the deployment of United States cruise missiles in Britain. It is to this debate about British policy that Neild seeks to contribute. Does Britain need its own nuclear weapons? And is Britain's security more strengthened than it is endangered by the hosting of United States nuclear bases? To both questions his answers are firmly negative.

Neild holds that while the Soviet Union has developed a "beastly political system" (p.9) and engages in adventurism in the Third World, it does not present a significant military threat in Europe, where its policy is basically defensive. American nuclear weapons deployed in Western Europe, he thinks, would confer positive benefits on the latter only if it could be assumed that the United States is a "reluctant ally" that does not perceive its interests to lie in defending Western Europe. In fact, according to Neild, the United States can be relied upon to defend its investments and world role in Europe, whether or not it has nuclear weapons deployed there that will draw it into any conflict.

The presence of nuclear weapons on British soil, whether British or American, adds to the risk that Britain will be subject to nuclear attack, a danger which official policy is reluctant to acknowledge. As Neild says:

In any nuclear conflict between the United States and the Soviet Union, Britain runs the risk of being the victim of a pre-emptive strike or accident. This is what happens to aircraft carriers [p.18].

Britain's nuclear deterrent force, Neild thinks, expresses

the folly of a declining power which seeks influence on the one hand by ingratiating itself with the United States and becoming dependent on her and, on the other hand, by buying and maintaining nuclear symbols of independence

How to Make Up Your Mind About the Bomb. By Robert Neild. Pp.144. ISBN 0-233-9382-6. (André Deutsch: 1981.) Pbk £2.95.

from the United States, symbols for which it has no use since it does not seek to be independent [pp.99–100].

United States assistance to the British nuclear weapons programme, as Neild notes, has had two conditions: that British nuclear forces be assigned to Nato, and that Britain continues to provide the United States with nuclear bases and other military facilities. Britain's role as an American military base — a role that has been played continuously since the stationing of American bombers in connection with the Berlin Blockade in 1948 — is thus in part made necessary by the British nuclear weapons programme.

The network of American military bases in Britain is now vast (Neild cites a study that lists 103 "bases and facilities"). There has been extraordinarily great governmental secrecy, and extraordinarily little public debate, about their nature and implications. And it is clear that such control as Britain exercises over the use of them, including that of the nuclear-armed bombers now based in Britain, is purely political and informal in nature; nor will the nuclear-armed cruise missiles to be deployed in the next few years be subject to any "double key" system that will give Britain a share in their technical control, such as it has over Nato tactical nuclear weapons deployed on the Continent. This is the measure of the change that has taken place in Anglo-American relations since the Quebec Agreement of 1943, when each country undertook not to use nuclear weapons against any third party without the consent of the other.

Neild is right to insist that it is important to stand back from detailed strategic analysis and ask where the nuclear arms race is going. He is right also to see new dangers in the collapse of super-power arms control negotiations, the drift away from "simple deterrence" towards nuclear war-fighting in strategic doctrine and the tendency to substitute considerations of the balance of nuclear arms between the super powers for considerations of mutual nuclear deterrence. He has performed a service in calling for public debate about US military bases in Britain; many who do not support Neild's view that these should

be removed in toto will nevertheless feel, as I do, that given the present direction of United States foreign policy, Britain should be seeking to disengage from any close identification with it, and especially to assert positive control over the use of American nuclear weapons based in Britain.

But, like many rational men of science, Robert Neild does not recognize the part that is necessarily played in international relations by power, including military power. If it is true, as I expect it is, that Soviet leaders harbour no aggressive intentions towards Western Europe, it is also true that the balance of military power created by Nato forms part of the context in which Soviet intentions have taken shape. If the balance were replaced by a Soviet military preponderance in Europe, Soviet leaders would be likely to develop different intentions, if not to invade Western Europe, then at least to dominate it. "Finlandization", which Neild dismisses as a bogey, seems to me to be a very possible consequence of a lowering of the guard by the Western powers, even if the analogy with Finland is an inexact one. Similarly, if the Soviet military position in Eastern Europe were to weaken, pressures would be likely to develop in the Western world to adopt a "forward policy" of promoting change in those countries.

Neild, it is true, does not call for Britain's exit from Nato or for the withdrawal of British forces from Germany; rather, he wants Britain to adopt a "French" policy of national self-reliance in defence, but continued participation in the alliance, except that Britain, unlike France, would be without its own nuclear weapons. Neild, moreover, clearly thinks that Western Europe, even after it had expelled American nuclear weapons, could still look to the United States for military support. He does not consider whether it is morally justifiable for Western European nations to ask the American people to accept on their behalf the risks of having nuclear weapons on American soil, while refusing to accept these risks themselves.

While Britain and other West European countries at present need to distance themselves from an apparently reckless American foreign policy, they are still in danger of being dominated by the Soviet Union if a balance of power in Europe is not maintained. The long-run objective, for Western Europe, should be to create a military counterpoise to the Soviet Union

from its own resources, independently of the United States. But this requires a nuclear deterrent force or forces controlled by West Europeans themselves, for which the British and French nuclear forces now provide a basis, as is today increasingly recognized elsewhere in Western Europe (the West German government, for example, has officially welcomed the British decision to deploy *Trident*). And in the short run there can be no balance in Europe without the commitment of American nuclear forces, for which some price will have to be paid and some risks accepted.

Hedley Bull is Montague Burton Professor of International Relations at Oxford University, and was formerly a Director of the Arms Control and Disarmament Research Unit in the British Foreign Office.

Environmental chemistry: a mixed reaction

Peter S. Liss

The Handbook of Environmental Chemistry. Edited by O. Hutzinger. Three volumes of two parts. Vol.1A The Natural Environment and the Biogeochemical Cycles pp.258, ISBN 3-540-09688-4. Vol.2A Reactions and Processes pp.307, ISBN 3-540-09689-2. Vol. 3A Anthropogenic Compounds pp.274, ISBN 3-540-09690-6. (Springer-Verlag: 1980.) Vol.1A DM98, \$57.70; Vol.2A DM126, \$74.40; Vol.3A DM98, \$57.70.

THESE three volumes are the first part of what we are told in the preface will be a multi-part venture. Part Bs for all the present volumes are promised shortly, with further parts to be published at some later date. Readers are even asked for their suggestions as to what future books might contain! Thus we appear to be at the start of what could become a major exercise in environmental chemistry publishing. At this early stage perhaps it is timely to examine both the achievements of what is now published and see what guidance it gives for future projects of this sort.

Each of the present volumes contains between nine and thirteen chapters, each by different authors. Generally they seem to be aimed at an audience at post-graduate level. The whole is edited by O. Hutzinger who is in the Laboratory of Environmental and Toxicological Chemistry at the University of Amsterdam. He has clearly put in an enormous amount of work to get the venture started and presumably continues to labour mightily on future volumes. Although editorial supervision is obviously important for a worthwhile outcome, the editor is, as always in a project of this kind, almost totally at the mercy of the people who are writing the individual articles. In spite of the fact that the authors are generally well known in their subject areas, the final result here is a series of chapters of very uneven content and quality. Some authors have done a really excellent job in summarizing their allotted topics; conversely, several chapters appear to do considerable injustice to the sub-branch of environmental chemistry which they are supposed to cover. This patchiness seriously limits the value of the whole venture. Presumably, the whole purpose of a handbook is that it can always

be relied on to give the sort of information and understanding one requires. If this reliability is lost by some contributions being sub-standard, then one might as well go back to using a collection of textbooks and journal articles covering more restricted parts of the topic.

Since only Part As are currently available, it is not possible to comment at this stage on the comprehensiveness of the Handbook or on problems of overlap or underlap between chapters. The arrangement into three volumes is done in a rather interesting way. Volume 1 contains chapters on the hydrosphere, atmosphere. oceans and soils as well as contributions on aspects of biogeochemical cycling. In this last group the article by P. J. Craig, "Metal Cycles and Biological Methylation", deserves special mention since it is a most informative and well balanced appraisal of the topic, particularly with respect to the routes and importance of methylation

The second volume is concerned with reactions and processes which transport and modify substances in the environment.

Volume 3 contains nine chapters, each on a particular man-made chemical (or group of chemicals) or natural substance whose behaviour is being affected by anthropogenic activities. The list of chemicals covered so far is plainly far from complete. This treatment by individual substance is perhaps how one might have expected the whole *Handbook* to have been organized. The editor is surely right in adopting the approach of establishing the basic principles of the subject in Vols 1 and 2 before proceeding to a detailed examination of individual chemicals.

As to the future of the *Handbook* the message is clear – vigilance in choice of authors is paramount. Although articles must obviously be written by experts in their subject area, it is more important that they should be prepared to make the effort to do justice to their topic rather than be well known in the field.

Peter S. Liss is Reader in the School of Environmental Sciences, University of East Anglia, and Scientific Adviser in the Environmental Studies Section at CEGB Headquarters.

Immunity in fertility

W. D. Billington

Immunological Aspects of Reproduction and Fertility Control. Edited by J. P. Hearn. Pp.253. ISBN 0-85200-265-3/ 0-8391-4143-2. (MTP/University Park Press: 1980.) £19.95, \$44.50.

WHILE reproductive immunology has undoubtedly come of age as a discipline in its own right, it still bears the scars of its rather uncontrolled upbringing in the hands of enthusiastic but sometimes less than rigorous investigators of a wide range of backgrounds. From the resulting morass of scattered, uneven, occasionally confusing, frequently conflicting data, it is now time to extract the relevant and scientifically sound and to reject the remainder. Reviews in this field must therefore be selective, highly critical and, most emphatically, not merely exhaustive catalogues of information. This volume of ten reviews meets this requirement only in part, with almost the full range of possibilities from the truly excellent, through the generally acceptable, to the frankly deplorable. Readers familiar with the field as a whole will hopefully readily discern these categories but the newcomer will require some guidance.

The coverage of the book is wide, with many, but not all, of the immunological aspects of reproduction and fertility control included. It is really two books in one, with the first section largely examining the maternal-foetal relationships in pregnancy and the disorders and diseases, in particular infertility and pre-eclampsia. which may result from disturbances in the normal system, and the second section exploring the possible avenues leading to the production of contraceptive vaccines based upon reproductively unique antigens. The chapters are very variable in length and approach, some presenting reviews of a general topic and others structured around more specific interests and personal data. The philosophy that an understanding of normal reproductive mechanisms will illuminate both pathological events and the means of intervention, as well as vice versa, is here given reasonable substance, and there are many encouraging signs of advance in this fascinating field.

However, this is not to say that many of the major problems do not remain to be solved. Despite considerable endeavour it is still not known by what means the foetus, or more precisely the placenta, avoids rejection as an intra-uterine allograft, whether simply by failing to display appropriate target antigens or by complex mechanisms involving one or more of a diverse range of maternal cellular and humoral immunoregulatory factors. There is conclusive evidence for a primary immunological causation in very few clinical disorders of reproduction. An

acceptable form of contraceptive vaccine is also still awaited, since the most advanced contender, the placental protein hormone chorionic gonadotrophin (hCG), has demonstrated the fundamental difficulty of this approach: that of achieving effective levels of non-cross-reacting, highaffinity antibody in individuals of varied immune responsiveness with preparations of such low immunogenicity as to require the use of potentiating carrier agents and undesirable adjuvants, to say nothing of the possible side-effects. It is questionable whether three entire chapters should have been devoted to this specific molecule, although they certainly highlight the hopes and fears of those working in this important area, illustrated also by two further chapters giving consideration to the potential use of a hypothalamic releasing hormone and antigens of the zona

antigens of relevance in this context should have been relegated to the depths of the lengthy review by Jones on male and female infertility.

This book deserves to be read because it is a fair reflection of the current state of the art, warts and all. But if your time or interest is limited then my advice is to read only the conclusions to each chapter and then go on to digest in full those by Cooper, on immunological relationships between mother and conceptus, and by Redman, on immunological aspects of pre-eclampsia. Both show how it is possible to produce a balanced, critical analysis out of a confusion of data and thereby firmly point the way forward to the required goals. Future reviewers please note.

W. D. Billington is Reader in Reproductive

Immunology in the Department of Pathology, University of Bristol Medical School. pellucida. It is a pity that the spermatozoal

A Devonian view of vertebrate evolution

Alec Panchen

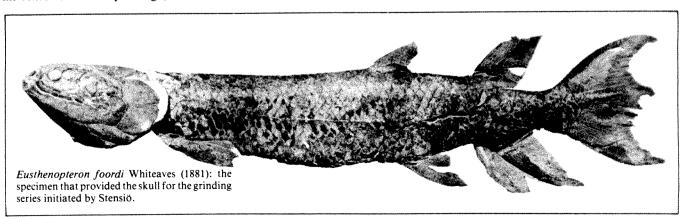
Basic Structure and Evolution of Vertebrates. By Erik Jarvik. Vol.1 pp.575, ISBN 0-12-380801-4; Vol.2 pp. 337, ISBN 0-12-380802-2. (Academic: 1981.) Vol. 1 £41, \$94.50; Vol. 2 £24.40, \$56.50.

In 1958 one of the recipients of the Linnean Society's Darwin-Wallace centenary medal was Professor E.A. Stensiö, who was honoured principally for his contribution to techniques of fossil preparation. His most important contribution was the technique of serial grinding in which specimens (particularly fossil skulls) are ground away to expose a minutely controlled series of sectional views. No specimen then remains, but a series of drawings and photographs are used to make large-scale, wax-plate reconstructions.

Stensiö may be regarded as the founder of the "Stockholm School" of vertebrate palaeontologists. Professor Jarvik, author of this two-volume work, is his former student and scientific heir, and was, until his retirement a few years ago, Stensio's successor in the Chair of Palaeozoology at the Natural History Museum in Stockholm. Thus these volumes record the meticulously detailed comparative anatomy and palaeontology, with numerous, detailed and beautiful line drawings, which characterize the Stockholm School. Unhappily, however, another characteristic feature of the work of Stensiö, Jarvik and their coworkers is very much to the fore: this is due to their isolation, in matters of principle, theory and interpretation, from colleagues elsewhere in the world and particularly from English-speaking palaeontologists. Thus anyone who expects a balanced review of important problems in vertebrate evolution, with views opposed to Jarvik's given due weight, will be disappointed. Not for him is Sir Karl Popper's dictum that the good philosopher or scientist should put his opponent's case in the most convincing manner before proceeding to criticize it.

Following an introduction Jarvik's first two chapters, amounting to 184 pages, are devoted to an uncompromisingly detailed account of the anatomy of two fish, the primitive extant form Amia and the Devonian fossil Eusthenopteron. The account of Amia is confined to the skeleton and to those features of the "soft anatomy" which can reasonably be restored in a well-preserved fossil skull. The account of Eusthenopteron is equally detailed with the "soft anatomy" restored by comparison with Amia and other vertebrates. Eusthenopteron has for many vears been accorded great importance by students of vertebrate phylogeny as the best known representative of a group of fishes standing close to the ancestry of land vertebrates. Jarvik's preoccupation with it has endured throughout his research career, largely as the result of studying ones superb skull specimen from a grinding series initiated by Stensiö.

The rest of the first volume is devoted to a review of early fossil vertebrates. Apart from details from living forms and occasional fossils used solely for comparison, none is of later than Devonian age, i.e. less than about 350 million years old. One practical and one theoretical consideration seem to have conditioned this choice: the first is that Jarvik and his present Stockholm colleagues have done little or no work on post-Devonian fossil vertebrates; the second is Jarvik's oftenrepeated view that all of the most important events in vertebrate evolution were over by the end of the Devonian, so that (with some exaggeration) Homo sapiens might be regarded as a aberrant Eusthenopteron-like fish! Specialists will find much to admire and also much to disagree with in the details of this review section: Jarvik's heterodox view that both the Dipnoi (lungfish) and the extinct acanthodians are more closely related to sharks than to bony fish will provoke many, as will the views (derived from Stensiö) on the nature of the relationship of early jawless vertebrates to extant cyclostomes. However, perhaps of more general interest is Jarvik's partial fulfilment of a frequently-expressed hope of his colleagues world-wide. Jarvik's department has on loan from the Danish Government an enormous mass of material of the world's earliest known amphibia from the Devonian of East Greenland. The original specimens, deposited in Stockholm over 50 years ago, were



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Industrial Biotechnology 5th Floor 31/33 High Holborn London WC1V 6BD 01-404 0496. Telex 912881 CW UK TXE (Micro) reviewed by the late G. Säve-Söderbergh in 1932 and since then scraps of information, mostly in the form of annotated drawings, have been released by Jarvik. This review section of his new book yields a few more crumbs in the form of figures and brief descriptions of the limbs and limb girdles, but people will still ask when the definitive monograph on these so-called ichthyostegids is to appear: 50 years is a long time, even in vertebrate palaeontology.

While Jarvik's first volume does deal to some extent with (often heterodox) theories of relationship between groups of fishes, it consists mostly of comparative anatomy and taxonomy. However, the second, shorter, volume is concerned principally with theory. It is divided into four major sections: "Basic structure and composition of vertebrate head" (sic), "The origin of the paired extremities", "The origin of tetrapods" and "Recapitulation and comment".

At first glance the opening section appears to be a very old-fashioned treatise on the embryology and anatomy of the skull and associated structures, but there is more to it than that. Jarvik attempts to demonstrate that a much larger part of the cranial anatomy of vertebrates than is normally accepted is not just segmented ("metameric") but actually formed of modified vertebrae — a reversion to the theories of the German Naturphilosophen of the early nineteenth century and particularly those of the poet Goethe. In this attempt Jarvik relies heavily on the work of his colleague H.C. Bjerring.

The following section is something of a pleasant surprise in that it opens with a brief but adequate consideration of rival theories of the origin of the paired fins of fishes. Ironically, Jarvik's views on this subject probably represent his most successful challenge to orthodoxy. However, they are followed by a treatment of the origin of the limbs of tetrapods which is strongly coloured by Jarvik's best known and most controversial theory. This is that one group of tetrapods, the Urodela (newts and salamanders) evolved from a different known group of now-fossil fish than did all other tetrapods (the remaining amphibia, reptiles, birds and mammals). Throughout both volumes this theory keeps popping up — sometimes it is assumed in the interpretation of other theories or data, at other times evidence is cited to support it, occasionally with complete circularity of argument. Within the penultimate section of the second volume it is developed in full. It arose originally from Jarvik's study of the snout of Eusthenopteron in which he documented a series of anatomical resemblances to the snout of frogs. He then claimed a second series of resemblances connecting members of another group of Devonian fish with the urodeles. Subsequently he has elaborated the comparison, seeking confirmation in other anatomical details. Many workers now

consider the theory to have been refuted by the study of amphibian snouts made by a South African, J.D. Jurgens, but Jarvik does not adequately discuss Jurgens's case.

As a bonus, Jarvik prefaces his discussion of tetrapod origin by a new theory of the mammalian ear in which he derives the three middle-ear ossicles of mammals from the hyoid arch of Eusthenopteron and, in rejecting the orthodox homologies, established over 140 years ago, virtually ignores the evidence from the rich source of fossil "mammallike reptiles".

The final section of the book opens with a chapter on general principles and methods, but does not really address the problem of the irreconcilable methodological differences between the author and his colleagues outside Stockholm. To Jarvik, the correct method of reconstructing phylogeny is the minutely detailed comparison between (inevitably) a small number of ancient fossils and an equally small number of putatively related extant forms. The enormous time gap is ignored in practice and minimized in theory by the assertion that nothing of great evolutionary importance has happened in the past 350 million years. The Anglo-American methodology on the other hand, also often gravely flawed, has been to search for ancestor-descendant sequences, studying, inevitably more superficially, large numbers of fossil species ranged over the whole span of vertebrate history. Perhaps in the future there will be a meeting of minds, also incorporating the less controversial features of cladistic analysis; but Jarvik's book, admirably scholarly in many ways but perverse in many others, will not promote it.

Alec Panchen, editor of the recent symposium volume The Terrestrial Environment and the Origin of Land Vertebrates (Academic, 1980), is Reader in Vertebrate Zoology at the University of Newcastle upon Tyne.

Nervous molluscs

Steven A. Siegelbaum

Molluscan Nerve Cells: From Biophysics to Behavior. Edited by John Koester and John H. Byrne. Pp.230. ISBN 0-87969-135-2. (Cold Spring Harbor Laboratory: 1981.) \$26, \$31.20 outside the USA.

ONE of the major goals of neurobiologists is to understand how higher processes such as learning and behaviour are related to and controlled by the electrical activity of nerve cells. Until recently, the prevailing view was that what counted most was the pattern of nerve cell connections, the nervous system's wiring diagram, and not primarily the membrane properties of individual nerve cells. However, within the



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past few years, a number of researchers have found that, at least for invertebrates, certain aspects of behaviour and "learning" can be explained surprisingly well at the level of the nerve cell membrane's jonic channels.

These recent findings depend, in large part, on our increasingly detailed understanding of the ionic basis of the electrical activity of nerve cells. In 1952, Hodgkin and Huxley demonstrated that the action potential of the squid axon could be understood in terms of two types of ion channels, one permeable to sodium, the other to potassium. While the Hodgkin-Huxley analysis still provides the framework for our present understanding of the electrical activity of nerve cells, it is now clear that no less than five distinct classes of channels exist. These channels play an important role in a variety of physiological processes, ranging from the control of transmitter release from nerve terminals to regulation of the rhythmic patterns of action potential firing produced by nerve cell bodies.

In view of this rather complex description of the nerve cell membrane, this book provides a welcome survey of recent developments in the field. The first in a new series entitled Cold Spring Harbor Reports in the Neurosciences, the book is composed of transcripts of talks from a recent symposium which concentrated on studies of molluscan nerve cells. The subjects are arranged in a logical order, starting with a discussion of the biophysical properties of ion channels and progressing to how such channel properties control spike generation patterns and behaviour. The book also includes chapters on the regulation of intracellular ions and the membrane effects of neurotransmitters and hormones.

The material has been presented clearly and at a level which should be accessible to readers with no direct experience in the field, while at the same time informative to those more familiar with the subject. The book contains two introductory chapters (by Eric Kandel and Charles F. Stevens) and should be especially useful for graduate students. One, perhaps inevitable, shortcoming of a book with such a wide scope is that some topics are treated rather briefly and uncritically. This is particularly noticeable in areas where some controversy remains, such as the control of channel opening and closing by internal calcium ions and the mechanism of neurotransmitter modulation of the action potential. Still, the book is an excellent summary and introduction to recent findings in the study of molluscan nerve cells and provides an interesting example of how membrane biophysical techniques may be successfully applied to a wide range of biological questions.

Steven A. Siegelbaum is a Research Associate at the Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris.

A question of the evolution of culture

Robert Attenborough

Man, The Promising Primate: The Conditions of Human Evolution. By Peter J. Wilson. Pp.185. ISBN 0-300-02514-9. (Yale University Press: 1980.) \$12.95, £9.10.

A NINETEENTH-century anthropologist would perhaps have found it unsurprising to read that an author's aim was to "consider human evolution from the point of view of social anthropology". That this aim, which Professor Peter Wilson declares at the outset of his book, does sound a little odd today is a measure of the gulf which has developed over the years between social and biological anthropology, making it conventional now for social anthropologists to leave problems of the evolutionary past to their biological colleagues.

Wilson, no doubt rightly in principle, will not allow such conventions to deter him from pursuing his interest in early human societies, and in doing so he meets head-on the main practical obstacle to his ambition: as he puts it, "social factors leave behind no evidence that can serve as a concrete subject of study". So readers are warned: what follows is, as the author frankly says, a purely speculative argument, and anyone who expects much in the way of facts will be disappointed.

Taken on its own terms, then, what is the nature of this argument? Wilson recognizes the value of the Darwinian perspective; he is willing to imagine that human societies must once have been more like those of apes and monkeys than they now are; and he acknowledges that the human capacity for culture must ultimately be an evolved, genetically based characteristic. But how, he asks, can we explain the emergence of this characteristic in a particular primate genus at a particular period and place? It is a good question, and, although he is by no means the first person to ask it, there is still room for fresh ideas.

The basis of Wilson's ideas is the biological proposition that primates are the most primitive and generalized of mammals, and that human beings are in turn the most primitive and generalized of primates. Lacking the evolved specializations which equip so many other animals for their particular ways of life, human beings must be flexible, they must be ready to make the best of whatever conditions they encounter, and they must do so by choosing from a range of options, rather than by giving standard responses: they must, in fact, be capable of dealing with varied day-to-day conditions of material and social life in ways that are too complicated to be genetically programmed in any specific way. From this need, which is both a constraint and a source of freedom, arose the exceptional characteristically human specialization, the large brain: and with that came the capacities for thought, consciousness and ultimately culture. Human beings are thus "promising primates", creatures of endless potential.

In all this, the focus of Wilson's attention is, naturally, on the social implications. From the generalization of sexual behaviour, he suggests, arises a longerterm mutual interest between males and females, which must be controlled by rules and bonds if it is not to lead to social chaos. The conjunction of the male-female bond with the female-infant bond forms the basis of a third bond between males and infants, and thus the cultural beginnings of kinship, the incest taboo and marriage. But it also creates a social problem, especially for the female, who plainly cannot maintain two close bonds at once. She must divide her attention and her time, and be able to convey the assurance that a neglected relationship will be returned to. Thus human beings must be "promising primates" in a second sense, committing themselves to the future of social relationships.

What, then, should one make of an argument like this? At times, Wilson's advocacy is admirable: serious but also stimulating, ingeniously argued, willing to recognize both primate capabilities and human peculiarities, and (not least of its virtues) well written and not too long. Certainly it deserves the attention of anyone interested in the field. But ultimately it is not altogether satisfying, mainly because its virtues are not maintained consistently. Sometimes Wilson labours too hard over a step in the argument, and the style suffers. And frequently he raises points of detail which tempt the reader to stop and quibble. Moreover, not all points of disagreement are mere details: let me give two illustrations.

The first concerns the use of the concept of generalization. There is, of course, more than a little truth in the notion of human beings as a biologically generalized species (though not in the idea that generalized is synonymous with primitive). But Wilson presses the concept of generalization much further than a biologist would be happy to, relying on it almost as though it were a physical law, a force which, once invoked, carries all aspects of anatomy and behaviour irresistibly along in a single current of evolutionary change. This takes Wilson away from his own correct insis-

Principles of Optics

Pergamon Press have recently published a new edition of *Principles of Optics* by Max Born and Emil Wolf. The latest edition, the sixth, incorporates revisions to the text and illustrations as well as references to the recent literature. Prices are: hbk £22, flexi £12.

tence on understanding the underlying conditions of human evolution. It also creates difficulties for his argument: most notably, it leads him to treat sexual generalization (i.e. continuous female receptivity) as a given feature of the human species, to which other features must adapt, rather than as a phenomenon to be explained in itself.

The second problematic issue is related to the first. Wilson is fairer-minded than many in the capacities that he is willing to attribute to the non-human primates: but, perhaps because his argument emphasizes their relative specialization, he does not go far enough. To take again the example of sex and bonding, he not only doubts the existence of primate sex drives, he also implies that primates do not have multiple social relationships to sustain, as early

human beings, he postulates, did. There is surely no shortage of evidence to refute this suggestion: and if one concedes this point, then Wilson's explanation of why human capacities emerged when and where they did must lose much of its force.

With flaws such as this, Wilson's argument is far from being fully persuasive, even at the frankly speculative level which he intends. But he has taken more trouble than many to present a balanced view of early human societies and, despite its less than total success, his book deserves to be read by anyone with an interest in its theme.

Robert Attenborough is a Lecturer in Biological Anthropology in the Department of Prehistory and Anthropology, Australian National University, Canberra.

Electrochemistry, principles to practice

A.J. Nozik and R.J. Gale

Electrochemical Methods: Fundamentals and Applications. By A.J. Bard and L.R. Faulkner. Pp.718. ISBN hbk 0-471-05542-5; ISBN pbk 0-471-08753-X. (Wiley: 1981.) Hbk £21, \$39.85; pbk £11.75, \$21.70.

THE field of electrochemistry is currently undergoing a major renaissance, now having a central role in such exciting research areas as photoelectrochemistry, chemically modified electrodes, novel energy conversion systems and medical electronics. These fast-moving disciplines all involve a high degree of interaction between solid state physics, physical chemistry, photochemistry, surface science, engineering and electrochemistry. The appearance of *Electrochemical Methods*, which provides a clear and thorough account of all aspects of its subject, is thus timely.

The book is encyclopaedic in scope, covering both basic principles of electrochemistry and all the modern and relevant electrochemical and electroanalytical techniques; it is truly an exceptional resource for both practising electrochemists and those eager to learn about this frequently intimidating subject. In addition to the expected material on electrochemical principles and techniques, the book also has a nice, concise introductory chapter on spectroelectrochemistry and photoelectrochemistry; this includes material on optical, electron and ion spectrometry and related phenomena.

The audience for the book will be large since it will also serve as a textbook for senior undergraduate and beginning graduate students. It is the first comprehensive text to cover electroanalytical theory and techniques since Lingane's (Electroanalytical Chemistry; Interscience, 1958) and Delahay's (New Instrumental Methods in Electrochemistry; Interscience, 1954)

books of the 1950s. Since then, the subject has grown enormously and the range of modern techniques has necessitated a reevaluation of the emphasis in applications. This has been successfully achieved in this text, and it should prove to be of considerable value in advanced courses in electroanalytical chemistry.

The omission of laboratory procedures is not especially troublesome since this area is complemented by Adams' Electrochemistry at Solid Electrodes (Dekker, 1969) and Sawyer and Roberts' Experimental Electrochemistry for Chemistry (Wiley, 1974). The absence of the more 'classical'' electrochemical topics could be a problem as most electroanalytical courses require at least a review of this material. It might also have been more appropriate to introduce concepts of the double-layer at an earlier stage than Chapter 12; Chapter 9 should be singled out as an excellent introduction to impedance concepts. The emphasis and presentation of the subject material are superlative throughout and the text could not be improved without considerable expansion in size and cost. An additional bonus is the problems, which provide useful exercises in the more important concepts and topics.

Very few errors have been noted; however, Problem 9.4 (p.368) does not tabulate the frequency as indicated, and the axes in Figure 14.8.2 (p.655) are labelled in an ambiguous (unconventional) manner.

This text has filled a definite need in present-day electroanalytical literature. We recommend it highly to students, teachers and research scientists.

A.J. Nozik is Chief of the Photoconversion Research Branch at the Solar Energy Research Institute, Golden, Colorado, and R.J. Gale is an Assistant Professor in the Department of Chemistry at Louisiana State University, Baton Rouge.

Ionic solutions

A.K. Covington

Ionic Liquids. Edited by D. Inman and D.G. Lovering. Pp.450. ISBN 0-306-40412-5. (Plenum: 1981.) \$49.50, £31.19.

Most conference volumes are conceived before the meeting itself takes place, and these days contributors are expected to arrive with camera-ready copy or place their expenses in jeopardy. The decision to publish the papers from this conference organized jointly by the Electrochemistry and Molten Salts Discussion Groups of the Chemical Society (now the Royal Society of Chemistry) apparently arose during the conference at St John's College, Oxford, in July 1978. The intention of the meeting organizers was to provide a greater exchange of ideas between aqueous solution chemists and molten salt chemists, thereby focusing attention on the somewhat neglected area of the properties of concentrated electrolyte solutions.

As with most conference volumes, the quality, approach and style of the 20 presentations are variable. Particularly valuable, however, are Enderby's account of the important neutron scattering first order difference method of determining radial distribution functions, and hence cation and anion hydration in concentrated solutions such as NiCl₂/D₂O, CaCl₂/D₂O, Duffy and Ingram's account of acid-base properties of concentrated aqueous solutions, and Adams and Hills's perceptive survey of computer simulation methods. The latter authors are somewhat scathing in their criticism of the preoccupation of solution chemists with continuum theories. In this connection, one of the contributors (Richter) quotes a 1957 paper of Fuoss and Onsager who thought that the problem of concentrated salt solutions should be approached from the fused salt side. Little progress has been made in those 24 years although recent approaches by Blander (Argonne), who was not a contributor, look promising.

Several papers highlight problems of technological interest — Dead Sea brines (Marcus), aluminium halide containing melts (Osteryoung) and sulphur and sulphide species in melts (Plichon) — and restore the balance towards practical aspects. Four papers deal with various aspects of water in melts: industrial and electrochemical (Lovering), concentration dependence of transport properties (Claes), solution chemistry (Coombes), and effect on purification (White).

The book is excellently produced with good, clear diagrams. It is to be recommended with the hope that it will, by placing the problems in perspective, encourage that progress which was the aim of the organizers.

A.K. Covington is Reader in Physical Chemistry at the University of Newcastle upon Tyne.

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Applied Biological Sciences

GEBELEIN, C. G. and KOBLITZ, F. F. Biomedical and Dental Applications of Polymers. Polymer Science and Technology, Vol.14. Based on an American Chemical Society Symposium held March 1980 at the 179th National Meeting in Houston, Texas. Pp.492, ISBN 0-306-40632-2, (Plenum: 1981.) \$59.50.

GORNALL, A. G. (ed.). Applied Biochemistry of Clinical Disorders. Pp.444.

ISBN 0-06-141010-1. (Lippincott-Harper, Philadelphia: 1981.) \$30.

HILLENKAMP, F., PRATESI, R and SACCHI, C. A. (eds). Lasers in Biology and Medicine. NATO Advanced Study Institutes Series. Series A, Life Sciences. Proceedings of the NATO Symposium on Lasers in Biology and Medicine, Camaiore, Lucca, Italy, August 1979. Pp.463. ISBN 0-306-40470-2. (Plenum: 1980.)

JENKYN, J. F. and PLUMB, R. T. (eds). Strategies for the Control of Cereal Disease. Pp.219. ISBN 0-632-00716-8. (Blackwell Scientific: 1981.) £13.

MIZRAHI, A. et al. (eds). New Developments with Human and Veterinary Vaccines. 25th OHOLO Biological conference, Zichron, Ya'acov, Israel. Pp. 444. ISBN 0-8451-0047-5. (Alan R. Liss: 1980.) \$36, Dfl.108.

PETRIE, J. C. (ed.). Cardiovascular and Respiratory Disease Therapy. Clinically Important Adverse Drug Interactions, Vol.1. Pp.,243. ISBN 0-444-80233-9. (Elsevier/North-Holland Biomedical: 1980.) \$48.25, Dfl.99.

RAINERI, A., KELLERMAN, J.J. and RULLI, V. (eds). Selected Topics in Exercise Cardiology and Rehabilitation. Ettore Majorana International Science Series. Life Sciences, Vol.4. Proceedings of the 1st Course held in Erice, Sicily, October 1979. Pp.279. ISBN 0-306-40566-0. (Plenum: 1980.) \$35.

RUBENSTEIN, I. et al. (eds). Genetic Improvement of Crops. Emergent Techniques. Pp.242. ISBN 0-8166-0966-7. (University of Minnesota Press; 1980.)

SAKAGUCHI, K. and OKANISHI, M. (eds). Molecular Breeding and Genetics of Applied Microorganisms. Pp.160. ISBN 0-12-615050-8. (Academic: 1981.) \$27.

SERROU, B. and ROSENFELD, C. (eds). Immune Complexes and Plasma Exchanges in Cancer Patients. Human Cancer Immunology, Vol. 1. Pp.344. ISBN 0-444-80237-1. (Elsevier/North-Holland Biomedical Press: 1981.) \$87.75, Dfl. 180. SKINNER, F. A., PASSMORE, S. M. and DAVENPORT, R. R. (eds). Biology and Activities of Yeasts. The Society for Applied Bacteriology Symposium Series, No. 9. Pp.310. ISBN 0-12-648080-X. (Academic: 1981.) £15.80, \$36.50.

VAINIO, H., SORSA, M. and HEMMINKI, K. (eds). Occupational Cancer and Carcinogenesis. Pp.422. ISBN 9-89116-193-7. (Hemisphere Publishing: 1981.) \$49.50.

WAGNER, J. C. (ed.). Biological Effect of Mineral Fibres, Vol.2. Proceedings of a Symposium held at the International Agency for Research on Cancer, Lyon, France, September 1979. Pp.1007. ISBN 2-85598-199-9. (International Agency for Research on Cancer, Lyon: 1980.) SwFr. 60, \$35.

WALKER, E. A. et al (eds). N-Nitroso Compounds: Analysis, Formation and Occurrence. Proceedings of the VIth International Symposium held in Budapest, October 1979. Pp.841. ISBN 92-8-321131-6. (International Agency for Research on Cancer, Lyon: 1980.) SwFr. 70, \$40.

ZWEIG, G. and SHERMA, J. (eds). Updated General Techniques and Additional Pesticides. Analytical Methods for Pesticides and Plant Growth Regulators, Vol.XI. Pp.408. ISBN 0-12-784311-6. (Academic: 1980.) \$46.

Psychology

FRANSELLA, F. (ed.). Personality. Theory, Measurement and Research Pp.256. Hbk ISBN 0-416-72770-0; pbk ISBN 0-416-72780. (Methuen: 1981.) Hbk £10.50; pbk £4.95.

FRIEDMAN, S. L. and SIGMAN, M. (eds). Preterm Birth and Psychological Development. Pp.438. ISBN 0-12-267880-X. (Academic: 1981.) \$34.

GRISSO, T. Juveniles' Waiver of Rights. Legal and Psychological Competence. Perspectives in Law & Psychology, Vol.3. Pp.295. ISBN 0-306-40526-1. (Plenum:

HERSEN, M., EISLER, R. M. and MILLER, P. M. (eds), Progress in Behavior Modification, Vol.10. Pp.243. ISBN 0-12-535610-2. (Academic: 1980.) \$27

KENDAL, P. C. and HOLLON, S. D. (eds). Assessment Strategies for Cognitive-Behavioral Interventions. Personality and Psychopathology. Pp. 425. ISBN 0-12-404460-3. (Academic: 1980.) \$29.50.

LEWIS, H. B. Freud and Modern Psychology. Vol.1, The Emotional Bases of Mental Illness. Emotions, Personality and Psychotherapy. Pp.247. ISBN 0-306-40525-3. (Plenum: 1981.) \$19.50.

LUCURTO, C. M., TERRACE, H. S. and GIBBON, J. (eds). Autoshaping and Conditioning Theory, Pp.313, ISBN 0-12-454480-0, (Academic: 1980.) \$30.

PALMER, E. L. and DORR, A. (eds). Children and the Faces of Television. Teaching, Violence, Selling. Pp.359. ISBN 0-12-544480-X. (Academic: 1980.)

PIAGET, J. Intelligence and Affectivity: Their Relationship During Child Development. Pp. 77. ISBN 0-8243-2901-5. (Annual Reviews, Palo Alto, California:

1981.) \$8 (U.S.A.) \$9 (elsewhere).
REHM, L. P. Behavior Therapy for Depression. Present Status and Future Directions. Pp.389. ISBN 0-12-585880-9. (Academic: 1980.) \$29.50.

SALES, B. D. (ed.). The Trial Process. Perspectives in Law and Psychology, Vol.2. Pp.506. ISBN 0-306-40491-5. (Plenum: 1981.) \$39.50.

TAJFEL, H. Human Groups and Social Categories. Pp.369. Hbk ISBN 0-521-22839-5; pbk ISBN 0-521-28073-7. (Cambridge University Press: 1981.) Hbk £25; pbk £7.95.

VALZELLI, L. Psychobiology of Aggression and Violence. Pp.262. ISBN 0-89004-403-1. (Raven: 1981.) \$26

WEXLER, D. B. Mental Health Law. Major Issues. Perspectives in Law and Psychology, Vol.4. Pp.270. ISBN 0-306-40538-5. (Plenum: 1981.) \$25

YENBI-KOMSHIAN, G. H., KAVANAGH, J. F. and FERGUSON, C. A. (eds). Child Phonology. Vol.2, Perception. Perspectives in Neurolinguistics, Neuropsychology and Psycholinguistics. Pp.254. ISBN 0-12-770602-X (Academic:

Sociology

BREZHNEV, L. I. Socialism, Democracy and Human Rights. Pp.247. ISBN 0-08-023605-7. (Pergamon: 1981.) £22.

COOMBS, P. H. (ed.). Meeting the Basic Needs of the Rural Poor. A Report of The International Council for Educational Development, Pp.816, ISBN 0-08-026306-2. (Pergamon: 1980.) £24.75.

History of Science

BOWERS, J. Z. When The Twain Meet. The Rise of Western Medicine in Japan. The Henry E. Sigerist Supplements to the Bulletin of the History of Medicine, New Series No. 5. Pp.173. ISBN 0-8018-2432-X. (The Johns Hopkins University Press: 1981.) \$14.

CASTILLEJO, D. The Expanding Force in Newton's Cosmos As Shown in HIs-Unpublished Papers. Pp.125. Flexi ISBN 84-85005-49-X. (Ediciones de Arte Y Bibliofilia, Madrid: 1981.) Np.

JAHNKE, H. N. and OTTE, M. (eds). Epistemological and Social Problems of the Sciences in the Early Nineteenth Century, Pp. 430, ISBN 90-277-1223-9, (Reidel: 1981.) \$31.50, Dfl.60.

KINTAKKA, J., GRUENDER, D. and AGAZZI, E. (eds). Theory Change, Ancient Axiomatics, and Galileo's Methodology. Proceedings of the 1978 Pisa Conference on the History and Philosophy of Science. Vol.1, Synthese Library. Studies in Epistemology, Logic, Methodology, and Philosophy of Science, Vol. 145. Pp.348. ISBN 90-277-1126-7. (Reidel: 1980.) \$50.

KINTAKKA, J., GRUENDER, D. and AGAZZI, E. (eds). Probabilistic Thinking, Thermodynamics and the Interaction of the History and Philosophy of Science. Proceedings of the 1978 Pisa Conference on the History and Philosophy of Science. Vol.II. Synthese Library. Studies in Epistemology, Logic, Methodology, and Philosophy of Science, Vol.146. Pp.340. ISBN 90-277-1127-5. (Reidel: 1980.) \$89.50, Dfl.95.

MARK, J. Four Anthropologists: An American Science in Its Early Years. Pp.209. ISBN 0-88202. (Science History Publications, New York: 1980.) \$20.

WALLACE, W. A. Prelude to Galileo. Essays on Medieval and Sixteenth-Century Sources of Galileo's Thought. Pp.369. Hbk 90-277-1215-8; pbk ISBN 90-277-1216-6. (Riedel: 1981.) Hbk Dfl. 95.00, \$49.95; pbk Dfl. 45.00, \$23.50.

General

BACKNELL, H. III. Energy and the National Defense. Pp.235. ISBN 0-8131-0402-5. (The University Press of Kentucky: 1981.) \$19.50.

BAILLAUD, R. Souvenirs D'un Astronome (1908-1977.) Pp.631. (Imprimerie Carrere-12-Rodez: 1980.) Flexi Np.

BALLMER, T. and BRENNENSTUHL, W. Speech ActiClassification: A Study in the Lexical Analysis of English Speech Activity Verbs. Springer Series in Language and Communication, Vol.8. Pp.274. ISBN 3-540-10294-9. (Springer-Verlag: 1981.) DM 57, \$33,60.

BARNEY, G.O. (Study Director) The Global 2000 Report to the President of the U.S. Entering the 21st Century, Vol.1, The Summary Report. A Report Prepared by the Council on Environmental Quality and the Department of State. Pp.360. Hbk ISBN 0-08-024617-6, pbk ISBN 0-08-024618-4. (Pergamon: 1981.) £25, \$50 (2 vols.).

BARRACLOUGH, N. Preology. Socieded Española de Historia Y Filosofía de la Cienca, Madrid. Pp.260. Flexi ISBN 0-08-026083-7. (Pergamon: 1981.) £5.85.

BATESON, P. P. G. and KLOPFER, P. H. (eds). Perspectives in Ethology. Vol.4, Advantages of Diversity. Pp.249. ISBN 0-306-40511-3. (Plenum: 1981.) Np. BAYNES-COPE, A. D. Caring for Books and Documents. Pp.32. ISBN 0-7141-2006-5. (British Museum, London: 1981.) £2.50.

BELLAMY, D. and MACKIE, S. The Great Seasons. Pp.153. ISBN 0-340-25720-2. (Hodder & Stoughton: 1981.) £9.95.

BÖGLI, A. Karst Hydrology and Physical Speleology. Pp.284. ISBN 3-540-10098-9. (Springer-Verlag: 1980.) DM 58, \$34.30.

BREZHNEV, L. I. Peace Detente Co-operation. Pp.197. ISBN 0-306-10971-9. Consultants Bureau, New York and London: 1981.) Np

DURAND-DROUHIN, J.-L. and SZWENGRUB, L.-M (eds). Rural

Community Studies in Europe. Trends, Selected and Annotated Bibliographies. Analyses, Vol. 1. Pp.332. ISBN 0-8-021384-7. (Pergamon: 1981.) £30, \$72. EISENBERG, L. and KLEINMAN, A. (eds). The Relevance of Social Science for Medicine. Culture, Illness and Healing, Vol. 1. Pp.398. Hbk ISBN 90-277-1176-3;

pbk ISBN 90-277-1185-2. (Reidel: 1980.) Hbk Dfl.65, \$34; pbk Dfl.30, \$14.95 OQUIST, P. Violence, Conflict, and Politics in Colombia. Pp.263. ISBN 0-12-527750-4. (Academic: 1980.) \$25.

SHAYER, M. and ADEY, P. Towards a Science of Science Teaching. Cognitive Development and Curriculum Demand, Pp.159, Pbk ISBN 435-57825-1. (Heinemann Educational: 1981.) Pbk np.

SIDERI, S. and JOHNS, S. (eds). Mining for Development in the Third World. Multinational Corporations, State Enterprises and the International Economy. Pp.325. ISBN 0-08-026308-9. (Pergamon: 1981.) £17.50, \$35.

ATTOUT CEMENTS

Awards

Dr Paul Rebut (deputy director of JET) has been awarded le Grand Prix de Physique Jean Ricard for 1981 by the Council of the Societé Français de Physique (SFP).

The annual Karl Heinrich Gyr and Heinrich Landis Commemorative Prize, which is administered by the Institution of Electrical Engineers, has been awarded to Mr Martin Weston of the BBC.

The International Union Against Cancer, with the funds provided by the American Cancer Society, will award Eleanor Roosevelt Fellowships for research on cancer only to persons on the staff of universities, teaching hospitals, research laboratories or similar institutions, and to investigators who are devoting themselves to the experimental or the clinical aspects of cancer research. The Yamagiwa-Yoshida Memorial International Cancer Study Grants are funded by the Japan National Committee for the UICC. These Study Grants are also administered by the International Union Against Cancer. They are designed to enable investigators of any nationality to gain experience in, or make comparative studies of, special techniques in both the biological and clinical aspects of cancer research. Application forms and information additional from: International Union Against Cancer, rue du Conseil-Général, 3, 1205 Geneva, Switzerland.

Twenty Harkness Fellowships tenable for between 12 and 21 months are offered each year. The award includes return fares to the United States, living and family allowances, travel in America (with car rental allowance), tuition and research expenses, a book and equipment allowance and health insurance. Candidacy is open to men and women between 21 and 30 years of any profession or field of study, who are citizens of the UK and have recieved both secondary schooling and further education (or equivalent professional experience in lieu of further education) wholly or mainly in the United Kingdom. For application forms: send a s.a.e. carrying 26p postage, and measuring not less than 10 inches by 7 inches, to The Harkness Fellowships (UK), Harkness House, 38 Upper Brook Street, London W1, UK.

Meetings

13-16 October, Indoor Air Pollution, Health and Energy Conservation, Massachusetts (Dr J.D. Spengler, Dept of Environmental Health Sciences, Harvard School of Public Health, 665 Huntington Ave, Boston, Massachusetts 02115, USA). 14 October, Geological Aspects of the Disposal of Radioactive Waste in the Sea, London (Mineralogical Society, 41 Queen's Gate, London SW7, UK).

18-23 October, Water Chlorination: Environmental Impact and Health Effects, California (Dr R.L. Jolley, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830, USA).

19-22 October, Pattern Recognition, Munich (Prof. Dr H. Marko, Lehrstuhl für Nachrichtentechnik der Technischen Universität München, 8000 München, FRG).

19-21 October, Spectroscopic Methods for Biomedical Research, Columbus (K.L. Waite, Batelle's Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201, USA).

23-24 October, The Role of Receptors in the Skin, Nice (Prof. Hans Schaeffer, CIRD, Sophia Antipolis, F-06565 Valbonne, France).

27-30 October, 10th European Symposium on Clinical Pharmacological Evaluation in Drug Control: Drugs for Infants and Children, Schlangenbad (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

25-28 October, Laboratory Health and Safety, Loughborough (Centre for Extension Studies, University of Technology, Loughborough, Leicester, UK).

25 October — 7 November, Plant Tissue Culture Methods and Applications in Agriculture, Los Banos (Dr T.A. Thorpe, Dept of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4).

27-29 October, Polynuclear Aromatic Heterocarbons, Columbus (D. Cooley, Battelle's Columbus Laboratories, 505 King Ave, Columbus, Ohio 43201, USA). 29-31 October, Rural Development and Retention of the Rural Population in the Countryside of the Developing Countries, Ottawa (J. Havet, Institute for International Cooperation, University of Ottawa, 190 Laurier Ave East, Ottawa, Ontario, Canada K1N 6N5).

29-30 October, Office Health and Safety, Loughborough (Centre for Extension Studies, University of Technology, Loughborough, Leicester, UK).

31 October — 2 November, Annual Meeting of the American Society for Cybernetics, Washington (Dr L.D. Richards, Dept of Administrative Science, Colby College, Waterville, Maine 04901, USA).

3-6 November, Working Group on Appropriate Technology for Health in Radiology, Radiotherapy and Nuclear Medicine, Rome (World Health Organization, 9, Scherfigsvej, DK-2100 Copenhagen Ø, Denmark).

3-6 November, Health and Safety at Work, Wembley (Maclaren Exhibitions Ltd, Davis House, 69-77 High St, Croydon, UK).

3-9 November, Remote Sensing of Arid and Semi-arid Lands, Cairo (E.S. Kasischke, Environmental Research Institute of Michigan, PO Box 8618, Ann Arbor, Michigan 48107, USA).

4-6 November, **EFOC** '81, Cologne (Information Gatekeepers Inc. c/o Tom Wearden Associates Ltd. 11 Chalmore Gardens, Wallingford, Oxon, UK).

9-11 November, Mechanical Behavior of Salt, Pennsylvania (Dr H. Reginald Hardy Jr, Rock Mechanics Laboratory, Room 117 Mineral Sciences Building, The Pennsylvania State University, University Park, Pennsylvania 16802, USA).

7-8 November, 32nd Annual Meeting of the American Association for the Study of Liver Diseases, Chicago (Charles B. Slack, Inc. 6900 Grove Rd, Thorofare, New Jersey 08086, USA).

9-13 November, 1981 Postgraduate Course in Clinical Pharmacology, Drug Development and Regulation, New York (Dr W.M. Wardell, Dept of Pharmacology, The University of Rochester Medical Center 601 Elmwood Ave, Rochester, New York 14642, USA). 11 November, Precambrian—Cambrian Evolutionary Explosion, Bristol (Dr R.

Evolutionary Explosion, Bristol (Dr R. Riding, Dept of Geology, University College, Cardiff, UK).

19-21 October, Working Group on Legionnaires Disease, Baden (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen O, Denmark).

12-13 November, Advisory Committee on Prophylactic, Diagnostic and Therapeutic Substances, EURO (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen O, Denmark).

14-19 November, Seminar on Appropriate Technology for Drinking Water Demineralization, Algiers (World Health Organization, 8, Scherfigsvej, DK-2100 Copenhagen O, Denmark).

12-13 November, Common Denominators in Art and Science, Edinburgh (Prof. M.R. Pollock, Marsh Farm House, Margaret Marsh, Shaftesbury, Dorset, UK).

16-17 November, HPLC of Proteins and Peptides, Washington (R. Schulthess, Varian AG, Steinhauserstrasse, 6300 Zug/Switzerland).

17-19 November, Taking Industry Offshore, London (IMechE, 1 Birdcage Walk, Westminster, London SW1, UK).

18-19 November, Business Opportunities in Genetics, Madison (C.W. Little, University of Wisconsin Extension, Dept of Engineering, 432 North Lake St, Madison, Wisconsin 53706, USA).

16-17 December, Structure of the Interfacial Region, Oxford (Dr M. Lal, Unilever Research, Port Sunlight Laboratory, Bebington, Wirral, Merseyside, UK).

17-20 December, Annual Conference of The Palaeontological Association, Exeter (Prof. J.W. Murray, Dept of Geology, University, Exeter, UK).

NEW ON THE MARKE

Colorimeter. Brinkmann Instruments Inc. have produced four new dipping probe colorimeters. These use a fibre optic light guide and detector system, and a specially designed dipping probe which is inserted into the solution to be measured. Phaseshifted, a.c. modulated light is transmitted to the probe tip and back to silicon detectors through an interference filter. This system allows measurements to be made without any effects due to extraneous or ambient light. Models PC-700 (analogue) and PC-800 (digital) have slidein interference filters which cover the visible range of the spectrum. Probe tips are available in stainless steel, glass and polycarbonate (Lexan). The diameter of the probe tip is ≤ 1 cm, thus it can be easily inserted into a test tube and readings made for a sample as small as 0.5 ml. These colorimeters have applications in the chemical and pharmaceutical industries.

Circle No. 90 on Reader Enquiry Card.



Ultrasonic system. Rinco Ultrasonics have just announced their new Bio 70, which is claimed to be the smallest ultrasonic system for biological, chemical and botanical investigations. The silent probe, which is no larger than a fountain pen, operates at 70 kHz and is powered by a portable 50 W generator, which weighs only 6 kg. Now the complete ultrasonic apparatus can be carried to the working area which decreases the possibility of cross-contamination.

Circle No. 91 on Reader Enquiry Card.

Hg vapour detector. The Data Acquisition 1500.DP5 mercury vapour detector can be easily connected to a chart recorder to provide a permanent record (and alarm) of the mercury level over a period of time. The instrument reads from $0.5 \mu g \text{ m}^{-3}$ to $1,999 \mu g \text{ m}^{-3}$ on a digital display and has its own integral charcoal filter for ease of zeroing in hazardous areas. It can be used either as a permanent or portable unit.

Circle No. 92 on Reader Enquiry Card.

Particle size analyser. The latest particle size analyser from CILAS provides extremely fast and accurate measurement of powders. It measures the diffraction of a coherent light beam by grains of the powder which are held in suspension in a suitable liquid medium. Average measurement time is 5 min and reproducibility is within $\pm 1\%$.

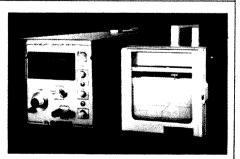
Circle No. 93 on Reader Enquiry Card.

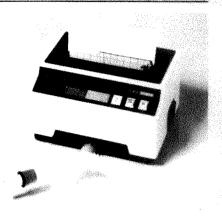
Thin film production. New from Balzers is the BAK 760, a system used for precision resistor networks and hybrid circuits where resistant and contact evaporation coatings need to be applied. As well as applying thin film to integrate circuits or liquid crystal displays, large quantities of ceramic rods coated with Ni-Cr for the production of highly stable metal-coated resistors can be processed in a tumble-cage device. Film thickness and evaporation rate are measured and adjusted during the evaporation process by the quartz crystal method and the results shown on a display. Based on these values, the microprocessor can control up to four evaporation sources and can be programmed in any desired order. The capacity of the BAK 760 ranges from 2-inch diameter to 4×4 inch substrate. The cage evaporation device has a capacity up to 31.

Circle No. 94 on Reader Enquiry Card.

Agar dilution. The agar dilution method of antibiotic susceptibility testing has long been thought to have distinct advantages over the disk diffusion method. However, there have been practical problems in the preparation of plates incorporating antibiotics. Mast Laboratories announce a major new product, ADATAB, which overcomes these problems and allows agar dilution to be used for routine susceptibility testing. Adatab consists of accurately assayed quantities of antibiotic in an inert non-interfering soluble carrier. By placing one Adatab in 100 ml of culture medium and allowing 3 min for the carrier to dissolve, six plates of a specific recommended antibiotic breakpoint concentration can be poured. This can be easily scaled up for the larger laboratory. A range of concentrations for MIC work can be prepared as required.

Circle No. 95 on Reader Enquiry Card.





Spirometer. The VS400 volume displacement spirometer can be used to perform an inexpensive and simple test for the screening of lung disorders. The VS400 is a waterless spirometer with a three-colour pen turret for simplified and accurate evaluation. The instrument has a colour-coded instruction panel, and its easy-loading card carrier and automatic or manual start make it simple to operate. The VS400 provides a volume versus time trace. Circle No. 96 on Reader Enquiry Card.

Log amplifier module. New from Bryans Southern Instruments Ltd. is a log amplifier module, an addition to the range of modules available for their high quality 50000 x-y recorder range. The new log amplifier module is designed for both A4 and A3 modular mainframes and can be easily fitted by the user. This d.c. module can be used in either the x or the y axis and if two log amplifier modules are used together, log-log plots can be recorded on a 50000 unit. Other modules for the Bryans 50000 range of high performance recorders include: low and medium sensitivity amplifiers with variable gain controls, single sensitivity amplifiers and time base/control modules.

Circle No. 97 on Reader Enquiry Card.

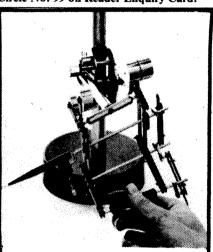
Sterilizable O₂ electrode. New Brunswick Scientific Ltd. has developed a dissolved oxygen electrode with replaceable membrane that can be rejuvenated repeatedly. The Model 900 probe rapidly responds to changes in oxygen partial pressure in biological and chemical processes. A 90% response time is reached in 60 s and linearity and reproducibility are 1% at constant temperatures. Mounted in a type 316 stainless-steel housing, the Model 900 is available in eight lengths for vessels ranging in size from 1 to > 1,000 l.

Circle No. 98 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the journal.

Coliform and streptococcal detection. Fast, accurate measurement of bacterial contamination in food, drink and pharmaceutical production is now possible using two kits from LUMAC. The streptococci detection kit allows selective measurement of streptococci after a short incubation, and the coliform detection kit selectively measures coliforms and therefore tests the hygiene of production processes. Both methods are simple, convenient and are up to 5 days quicker than conventional plate-counting techniques.

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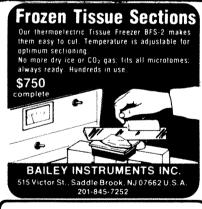


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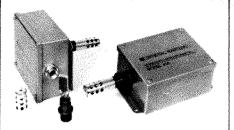
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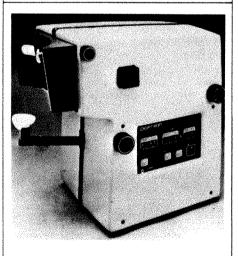
Birchover Instruments Ltd THE SPIRELLA BUILDING BRIDGE ROAD, LETCHWORTH HERTS, SGBEET ENGLAND TELEPHONE: LETCHWORTH (04626) 73097



Relative humidity transmitters. General Eastern announces a new line of per cent relative humidity and temperature transmitters — the 455 Series. These instruments are available in duct or wall-mount versions, for 115 V/230 V a.c. or ± 24 V d.c. inputs. The Model 455 provides linear 0–10 V d.c. and 4–20 mA d.c. signals for 0–100% relative humidity, and 0–12 V d.c. and 4–20 mA d.c. for 0 to +120°F. Accuracies for the measurements are $\pm 3\%$ relative humidity, between 15 and 99% relative humidity, and ± 1 °F over the entire temperature span.

Circle No. 100 on Reader Enquiry Card.

Dri-Blocks. A Techne Dri-Block can be used with Sterilin specimen containers for a more convenient method of fertility testing. A sperm sample is collected in a Sterilin 60 mm Universal clear-labelled container and heated in a special Techne Dri-Block heater for 20 min at 37°C. This ensures that the sperm is at body temperature before a sperm count is taken. The Techne Dri-Block has an advantage over a water bath in that the sperm would die if they accidentally came in contact with the water. Techne Dri-Blocks are available to cover a temperature range of 25 to 450°C. **Circle No. 101 on Reader Enquiry Card.**



Portable autorefractor. A compact, portable autorefractor, Dioptron Nova, has been developed by Coherent. It features the Coherent Auto Fog system which eliminates instrument myopia and is particularly suitable for over-refracting contact lenses. It also allows immediate vertex distance conversion from spectacles to contact lenses at the touch of a button. Circle No. 102 on Reader Enquiry Card.

Vapour adsorption cartridges. A new series of microfibre vapour adsorption cartridges has been introduced by Balston, Inc. These are designed to carry out various tasks such as removing oil vapour from compressed air, purifying breathing air, removing water vapour from a gas stream, and removing mercury vapour from gas. Designated type CI, the new cartridges are available with a choice of six standard adsorbents, including carbon, Ambersorb XE-348, silica gel, molecular sieve type 4A and 13X, and Calgon type HGR sulphurimpregnated carbon. They contain a bed of adsorbent granules in the annular space between two microfibre filter tubes, with permanently bonded end caps, and fit all standard Balston compressed gas filter housings and many cartridge filter housings of other manufacturers.

Circle No. 103 on Reader Enquiry Card.

NEW LITERATURE

Dewars. The extended range of dewars from Day-Impex Ltd. is covered in their new leaflet. Circle 150.

Affinity-isolated antibodies. Antibodies to all human immunoglobulins and mouse IgG and IgM are featured in a new catalogue from Tago, Inc. Circle 149.

Liquid handling devices. Various μ l liquid handling devices are described in a new catalogue from Drummond Scientific. Calibrated micropipettes and variable volume positive displacement digital micropipettors are included. Circle 148.

Aluminum film guide. The 'Aluminum Film Guide' from the Aluminum Association lists 30 films about aluminum from various sources. Circle 147.

Products. The range of products available for tissue culture, microbiology, serology, clinical chemistry and immunology is given in a new catalogue from Gibco. Circle 146.

Steric exclusion analysis. An applications note describing steric exclusion analysis of coatings and adhesive polymer formulations by HPLC is available from Varian. Circle 145.

Capillary columns. A new brochure from Analabs contains information on glass and metal capillary columns and column accessories for gas chromatography. Circle 144.

Freeze drying. 'Laboratory Freeze Drying Systems and Accessories' from Techmation introduces the theory and practice of freeze drying and gives full details of VirTis freeze drying equipment. Circle 143.

Air analysis. Types of industrial hygiene air analysis sampling by atomic absorption are described in a new applications note from Varian. Circle 142.

Copies of these publications can be obtained using the reader enquiry card.

NATURE

LONDON OFFICE Jean Neville 4 Little Essex Street, WC2R 3LF Telephone 01-240 1101 (Telex 262024) AMERICAN OFFICES NEW YORK Cathy Moore 15 East 26 Street, New York, NY 10010 — Telephone (212) 689 5900

PHILADELPHIA Dan Adams (215) 353 6191 ◆ SAN FRANCISCO JJHS (415) 392 6794

PASADENA JJHS (213) 796 9200 ◆ DALLAS Roy McDonald (214) 941 4461

HOUSTON Roy McDonald (713) 988 5005 ◆ FORT LAUDERDALE Brinker & Brinker (305) 771 0064

CHICAGO Didier & Broderick (312) 498 4520 ◆ BOSTON CEL Assoc (617) 848 9306

CANADIAN OFFICE TORONTO Peter Drake 32 Front Street West, Suite 201 Toronto, Ontario M5J1C5 Telephone (416) 364 1623

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CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5 p.m. ON THURSDAYS PRIOR TO ISSUE DATE.

UNIVERSITY OF ST. ANDREW'S **DEPARTMENT OF ASTRONOMY** Applications are invited for a POSTDOCTORAL RESEARCH ASSISTANT

to work on an SERC supported project investigating the properties of marginal contact and non-contact binary stars. The project involves optical and near-infrared photometry and high-dispersion spectroscopy using facilities at major international observatories. Applicants should have some experience in optical observational techniques and an interest in computational work.

The appointment will be for a period of three years from 1st October 1981 or as soon as possible thereafter. Initial salary in the range of £6,070 to £6,880 per annum on range 1A plus superannuation.

Applications together with the names of two referees should be sent by 24th August, 1981 to The Director, University Observatory, Buchanan Gardens, St Andrews, Fife KY16 9LZ, Scotland, from whom further particulars may be obtained. (9274)A

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

APPLIED PIG NUTRITION DEPARTMENT

A vacancy exists for a scientist to participate in a programme of research now being developed on nutrition and lactational physiology in the sow. The work will include studies of the energy nutrition of the sow during gestation and lactation, and on the hormonal control of mammary gland function and will involve close collaboration with the Nutrition and Physiology departments.

Candidates should have a First or Upper Second Class Honours degree in Biochemistry or Physiology.

Appointment will be as Scientific Officer (£4,809 to £6,480) or Higher Scientific Officer (£6,075 to £7,999); at least two years' relevant research experience is required for appointment to the higher grade.

Application forms are obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT.

Quote reference 81/20 (9275)A

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts AL5 2JQ Temporary Appointment **PHYSICIST**

THEORETICAL PHYSICIST

to work in the Physics Department for a period of $2\frac{1}{2}$ years on the development of physical/numerical models of the effects of drought on growth and yield of cereal crops. The modelling will be based on plant and crop physiological principles, and the work (which may include some field work) will involve collaboration with physicists, physiologists, chemists and agronomists. The post is funded by EEC and may be extended by a further few months.

Qualifications: First or upper second class honours in physics, or applied or theoretical physics, and preferably a higher degree, or equivalent post-graduate experience, in research that has included much computer analysis of experimental results and development of physical/ mathematical models.

Appointment in grade of Scientific Officer (£4,809 - £6,480) or Higher Scientific Officer (£6,075 - £7,999). (Pay Award pending). At least two years' post-qualifying experience is required for appointment as HSO. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of three referees and quoting Ref. 451 by 31st August 1981. Further details on (9252)A request

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London) DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Physicist or physical chemist required to join research group working on dynamics of bio-membranes. Experience of optical methods and laser techniques would be an advantage. Post is tenable for 3 years, supported by the SERC.

Salary on Range 1A: £7,037 to £11,542 (inclusive). Applications (cv and names and addresses of two referees) should be sent to the School Secretary, RFHSM, 8 Hunter Street, London WC1N 1BP as soon as possible. Please quote ref: SERC/B. (9284)A



INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES

Scientific Research **Position**

International Laboratory for Research on **Animal Diseases** (ILRAD)

A vacancy exists in the Pathology Division of ILRAD for a Research Scientist to continue studies on the inductive mechanisms for cellmediated immunity against the protozoan parasite Theileria parva in cattle and to analyse the antigenic composition of the parasite. Opportunities exist for collaborative research on surface markers of bovine lymphocytes and means of eliciting protective immunity against Theileriosis.

The applicant should possess a degree in Veterinary Science (or be familiar with experimentation in livestock) together with a PhD. Relevant postdoctoral experience would also be an advantage. Salary will be paid in US dollars according to experience. Perquisites include movement, housing and commutation allowances, medical and retirement benefits.

Applications with curriculum vitae and the names of three referees should be addressed to: The Director of Administration, ILRAD, PO Box 30709, Nairobi.

Closing Date: August 31st, 1981.

(W396)A

CHAIRMAN DEPARTMENT OF GENETICS

The University of Utah, School of Medicine, Salt Lake City, Utah, invites nominations and applications for the position of Professor and Chairman of the new Department of Genetics. This position requires a person with an outstanding research program in genetics, as well as, administrative and leadership qualities, who will interact with both clinical and basic science faculty. This new department will provide the opportunity and requirement to recruit strong faculty.

The university is an equal opportunity/affirmative action employer, thus applications from women and minorities are encouraged.

Inquiries including a curriculum vitae, should be sent to Dr John A Dixon, Chairman Genetics Search Committee, Department of Surgery, School of Medicine, Salt Lake City, Utah 84132. Closing date September 15, 1981. (NWX764)A

scientists

Monsanto, a large organization with European Headquarters in Brussels and Research facilities in pleasant surroundings at Louvain La Neuve, 30 km South of Brussels, is expanding its Agriculture Research Programs in the field of plant growth regulators.

The Biological Evaluation section intends to make new appointments for **scientists** with the ability to carry out and direct fundamental and applied research projects. These projects will be related to the discovery and development of plant growth regulators for use in major arable crops.

Applicants should have a PhD in plant science or agronomy a strong background of plant/crop physiology, preferably 2 years of experience in applied crop research and a proven ability to supervise research teamwork.

Candidates should apply in writing to Personnel Department Monsanto Technical Center, rue Laid Burniat, B-1348 Louvain La Neuve, Belgium.

(W395)A

Monsanto

Biophysicist/ Bioengineer

Are you the seasoned professional to pilot our new venture?

If so, you'll have full responsibility for the development and sales positioning of a new business activity and you'll be applying your knowledge of ceil culture and fermentation to the design, manufacture and marketing of diagnostic instrumentation and allied materials/equipment.

You'll need several years of solid experience in an industrial/educational environment ... with emphasis on the formulation and packaging of biological/medical commercial applications . . . as well as an advanced degree in biophysics or bioengineering or its equivalent.

The new technology is fully developed . . . all ready for your implementation. Our compensation and benefits are liberal . . . and your future will grow along with the project's success

Please send resume and salary history to:

BOX No NW804 Nature, c/o Macmillan Journals Ltd. 4 Little Essex Street, London WC2R 3LF

An Equal Opportunity Employer

(NW804)A

TUMOR IMMUNOLOGY PRE/POSTDOCTORAL

position available immediately. Monoclonal Ab, characterization for human tumor Ag.

Send CV. three ref to: Dr Daniel Eskinazi, USC, PO Box 77912, GER 323, Los Angeles, CA 90007 EOE/MFH (NW765)A

IN THE FACULTY OF

TECHNICAL AND
NATURAL SCIENCES
(TechnischNaturwissenschaftliche
Fakultät)
of the
Johannes Kepler University
Linz, Austria
the position of a full

PROFESSOR IN BIOPHYSICS is vacant

The candidate for this post will be expected to devote his research activites to experimental membrane biophysics as his main effort. His teaching should be focussed on courses, which enable graduate students in physics, chemistry, computer sciences and mathematics to acquire knowledge in biophysics. Applicants should be proficient in German.

Applications including a curriculum and a list of publications should be sent before October 30th, 1981, to the dean (Dekan) of the Faculty, Altenbergerstr. 69, A-4040 Linz, Austria. Dean Prof Dr Bruno Buchberger. (W397)A

LINCOLN COLLEGE Canterbury, New Zealand

LECTURER/SENIOR LECTURER IN ANIMAL SCIENCE

(Growth and Development)

The Council of Lincoln College, University College of Agriculture, Canterbury, New Zealand, invites applications for appointment to the position of Lecturer/Senior Lecturer in Animal Science (Growth and Development) in the Animal Science Department within the Animal Sciences Group.

Applicants should hold a degree in Agricultural Science or its equivalent and postgraduate experience in the field of growth and development in animals. The appointee will be expected to contribute to the teaching programme of animal science to students at degree and postgraduate levels, and to contribute to the research programmes of the department in the fields of animal nutrition and disease and sheep breeding.

The successful candidate will be appointed as Lecturer or Senior Lecturer at a salary commensurate with his/her qualifications and experience. Current academic salary scales are: Lecturer NZ\$19,140—\$23,520; Senior Lecturer NZ\$24,110—\$27,589 Bar—NZ\$30,035.

Travel and removal expenses will be reimbursed to specific limits. New Zealand Government Superannuation will be available.

Conditions of Appointment giving further details of this position may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on 16 October 1981. (9269)A

MASSEY UNIVERSITY Palmerston North, New Zealand

LECTURER IN BOTANY

A vacancy exists in the Department of Botany and Zoology for a lecturer in Experimental Botany. Consideration will be given to all suitably qualified applicants, but preference will be given to those with research experience and/or special interests in:- (i) plant cell biology; (ii) physiological plant ecology; and/or (iii) plant biosystematics.

The appointee will be expected to teach internal and extra-mural students at the undergraduate level, contribute to postgraduate teaching, and establish and develop research in his/her own field of interest.

Salary on the Lecturers scale:-NZ\$19,140 — \$23,520.

Further details of this position and of the University, together with the general conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF or the Registrar of the University.

Applications close on 1. September 1981. (9270)A

Molecular Biology — Virology — Immunology Syngene Products and Research — Colorado

Applications are invited from research scientists (Ph.D. or M.D.) and technicians (B.S. or M.S.) to join Syngene Products and Research, Inc. (SPAR), a TechAmerica Group Company. TechAmerica Group, Inc. is a rapidly growing company with advanced production and marketing capabilities. We are forming a diverse group of energetic, creative scientists for the purpose of developing new products for use in animal and human medicine and agriculture.

Recombinant DNA - gene splicing and expression

Virology Hybridomas - development of new vaccines - monoclonal antibodies for affinity

chromatography, diagnostics and serotherapy

Biochemistry

- isolation and sequencing of proteins

Microbiology

- physiology and fermentation technology We encourage publication and sponsor cooperative research

programs with university groups, and private research companies. SPAR laboratories are near Colorado State University in Fort Collins. located about 110 Km north of Denver, Colorado. Climate and recrea-

tional opportunities are excellent Attractive salaries and benefits, and generous bonus programs are

offered

Send curriculum vitae or resume, reprints of recent papers, and the names and addresses of three to five references to:

Dr. K.A. Larson Syngene Products and Research, Inc. 225 Commerce Drive P.O. Box 2211 Fort Collins, CO 80524 Phone: (303) 221-2050

Communications Confidential

(NW801)A

THE UNIVERSITY OF AUCKLAND New Zealand

ROTANY -LECTURERSHIPS/ SENIOR LECTURESHIP

Applicants should hold, or be nearing completion of, a PhD. Preference may be given to those who have experience in plant pathology, plant breeding, plant genetics, horticulture, or some combination of interests within these fields.

LEIGH MARINE LABORATORY -RESEARCH FELLOWSHIP

Applicants should have a PhD and research experience in some branch of Marine Science. Preference may be given to those whose research work involves diving. The Fellow will have the status and salary of a Lecturer and the appointment will be for 3 years initially.

Present salary scales are — Senior Lecturers NZ\$24,110 — \$27,589 per annum; Lecturers NZ\$19,140 -\$23,520 per annum.

Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications should be forwarded as soon as possible but not later than 12 October 1981.

EDINBURGH AND SOUTH-EAST OF SCOTLAND **Blood Transfusion Service** ROYAL INFIRMARY EDINBURGH EH3 9HB

A vacancy exists for a RESEARCH FELLOW in the Hepatitis Laboratory of the Microbiology Division. The post is for two years in the first instance and could lead to a higher degree.

The successful candidate, who should have a good honours degree (or equivalent experience) in a microbiology-related subject, will be concerned with research into Non-A, Non-B viral hepatitis. Salary on the scale of £5,346 to £6,353 pa (under review).

Telephone: 031-229 7291, ext 27, for job description. (9280)A

IMMUNOLOGIST/ IMMUNOCHEMIST

Staff research position available for experienced researcher with PhD or MD interested in applying new technologies to the study of human disease.

Experience with monoclonal antibody production and isolation of cellular antigens is desirable. Send CV and references to:

Dr. Robert Colvin, Immunopathology - Cox 5, Mass. General Hospital, Boston, MA 02114. (NW798)A

CSIRO

AUSTRALIA

Research Scientist/ Senior Research Scientist

Division of Fossil Fuels North Ryde, New South Wales

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,400 employees — 2,500 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

Field: Iron Ore Treatment

General: The Division, situated at North Ryde, Sydney, is currently undertaking a study into the pelletizing and treatment of iron ores to assess the possibility for their further treatment in Australia. Particular emphasis has been placed on the development of an understanding of the mechanism of direct reduction processes of both pellets and lumps. The application of these processes is influenced by the availability and efficient utilization of fossil fuels, especially natural gas and coal, and it will be necessary to include energy aspects of mineral processing in the research.

Duties: To carry out a theoretical and experimental investigation designed to explain the influence of process variables and ore types on the mechanism of pelletizing and reduction, and on the properties of the product.

Qualifications: PhD in metallurgy, chemical engineering, fuel technology or other appropriate fields. Alternatively, equivalent research experience together with a record of achievement relevant to mineral or fuel processing.

Salary: Research Scientist/Senior Research Scientist; \$A19,662—\$A28,564 pa.

Tenure: Indefinite with superannuation.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number A3970 should reach: The Personnel Officer, Australian Scientific Liaison Office, Australia House, Strand, London WC2B 4LA by 5 September 1981. Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue NW, Washington DC 20036. Current vacancies in CSIRO appear on PRESTEL page 252903. (9283)A

AGRICULTURAL RESEARCH COUNCIL **FOOD RESEARCH INSTITUTE NUTRITION & FOOD QUALITY DIVISION** Sensory Scientist

Higher Scientific Officer/Scientific Officer

The Institute has a vacancy for a Sensory Scientist in a new group within the Nutrition and Food Quality Division. The person appointed will be responsible for studies of the influence of sensory factors on human dietary intake of salt. The study will involve physiological and behavioural techniques and the person concerned will need knowledge of and interest in both aspects. It will involve other groups concerned with food intake measurement and sensory measurements. Collaboration with clinicians will be necessary. An aptitude for working with people is essential; travelling will be involved and a valid driving licence is essential.

Qualifications: A first or upper second class Honours degree in physiology/psychology or related subjects. Some research experienced with human subjects is desirable. At least 2 years postgraduate experience is required for grading at the higher level.

Salary: (under review)

Senior Scientific Officer - on a scale £6,075 - £7,999. Scientific Officer - on a scale £4,809 - £6,480.

Non contributory superannuation scheme.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting ref. 81/47.

Closing date: 31st August 1981.

(9258)A

HEAD OF THEORY GROUP DARESBURY LABORATORY

The position of Head of the Theory Group at Daresbury Laboratory will shortly become vacant following Dr. Pendry's appointment to a chair at Imperial College and applications are invited to fill this post. The Theory Group, which forms part of the Theory and Computational Science Division, is expected to play a major role in ensuring the effectiveness of the Laboratory's experimental programme.

There are two major research facilities at Daresbury, a dedicated synchrotron radiation source of x-ray and ultra-violet radiation, based on a 2 GeV electron storage ring which has recently started operation and a major nuclear structure facility based on a 30 MeV tandem Van de Graaff which is nearing completion. An AS 7000 and a CRAY 1 provide the basis of an extensive computing facility which supports this programme as well as supporting university research in computational science.

The Theory Group supports the experimental programme on these facilities, has close collaboration with university scientists and maintains very close links with the Computational Science Group in the Theory and Computational Science Division.

The successful applicant will head a team consisting of nuclear, solid state and atomic and molecular theoreticians. The post will be permanent. Alternatively, the Council will consider secondment from or a joint appointment with the candidate's own establishment.

The appointment will be in the grade of Senior Principal Scientific Officer at a salary within the range £13,364 – £16,250. Salaries are currently under review.

Closing date: 30th September 1981

For further information about the work of the Division please write to or telephone Professor P. G. Burke, Warrington (0925) 65000, Ext. 240.

Applications are invited from either sex and should be sent together with curriculum vitae and the names and addresses of two referees quoting reference number DL/767/ to:

The Personnel Officer

DARESBURY LABORATORY

Science & Engineering Research Council Daresbury, Warrington WA4 4AD.

(9248)A

RESEARCH INSTITUTE OF SCRIPPS CLINIC MOLECULAR GENETICS GROUP

Biochemist is required to join the Molecular Genetics Group at the Associate Member level to work on the mechanism of cell-cell interaction in Dictyostelium discoideum. Applicants should have training in both Molecular Biochemistry and Membrane Biochemistry with demonstrated exceptional productivity and creativity in research.

We offer commensurate salary and fringe benefits.

Please send curriculum vitae and statement of research interest and experience to: Nan Price, Research Institute of Scripps Clinic, Molecular Genetics Group, 10466 North Torrey Pines Road, La Jolla, California 92037.

Application deadline: September 20, 1981. (NW797)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF
ANATOMY
Applications are invited from a

GRADUATE

in biomedical sciences for the post of Research Assistant, Department of Anatomy. The successful applicant would join a small research team investigating the role of the nervous system in birth. No previous experience is necessary but a basic knowledge of nervous physiology would be beneficial. The post is supported by the Medical Research Council for three years. Starting salary £5,285 per annum.

Applications, including cv and names of two referees, should be sent to Dr A J S Summerlee, School of Veterinary Science, Park Row, Bristol BS1 5LS by 21st August, 1981. For further information please telephone Bristol (0272) 24161 ext 616, mornings only. (9286)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF HUMAN
GENETICS

POST DOCTORAL SCIENTIST

Applications are invited for a nonclinical scientist to study DNA polymorphisms in muscular dystrophy as a means of carrier detection and prenatal diagnosis. Candidates should preferably have had experience in DNA analysis, and will work in both the MRC Mammalian Genome Unit and this Department. The appointment is for 3 years from a date to be arranged, and the salary scale is that for University nonclinical academic staff.

Applications by September 14 with the names of two referees to Professor A E H Emery, University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU. (9263)A

UNIVERSITY OF ALBERTA

DEPARTMENT OF PHARMACOLOGY

FACULTY AND RESEARCH POSITIONS

The University of Alberta, Department of Pharmacology, is seeking applications for a tenure-track faculty position at the level of assistant professor. Applicants should have a PhD in pharmacology or a related discipline, proven research abilities and be enthusiastic and competent lecturers. Closing date October 1, 1981.

In addition the Alberta Heritage Foundation for Medical Research is offering 5-year scholarships (which may be renewed for a further five years) to those with excellent and well documented research skills. These positions have an academic rank (usually assistant professor) and salary similar to that of a regular faculty position, and are designed to provide support for young and very promising investigators. The Department of Pharmacology is seeking applicants for such awards. In the first instance applications should be made to the Department. It approval is given for the applicant to proceed, the appropriate forms will be submitted to the Heritage Foundation for consideration by their Scientific Advisory Committee. The closing dates for completions are September 1, 1981 and April 1, 1982.

Canadian citizens and permanent residents of Canada will be giver preference for thes positions. With this exception, the University of Alberta is an equal-opportunity employer.

Those interested should apply in writing enclosing a curriculum vitae the names of at least three referees and any other relevant information to Dr David Cook, Department of Pharmacology, University of Alberta, Edmonton, Alberta Canada, T6G 2H7. Telephone (403) 432-3575.

During Canadian mail strike University of Alberta, Box 413 Sweetgrass, MT, 59484, USA. (NW799)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF BIOCHEMISTRY
POST-DOCTORAL
RESEARCH ASSOCIATE

Applications are invited for a SERC-supported, post-doctora research associateship to work wit Dr G Turnock on the regulation or RNA synthesis during the synthesis during the synthesion of the synthesis during the synth

The appointment is available fror 1 October 1981 and is tenable for tw years at a salary within the range fc Research Associates, £6,070 - £7,290 dependent upon age an experience.

Applicants should send curriculum vitae and the names c two referees to Dr G Turnocl Department of Biochemistry University of Leicester, Leiceste LE17RH. (9247)A

TRANSGENE

STRASBOURG - FRANCE

TRANSGENE was launched by leading industrial and financial French groups.

TRANSGENE carries out high level research oriented towards applications of medical and industrial importance.

TRANSGENE is now expanding its laboratory and invites applications from:

MOLECULAR GENETICISTS

EXPERTS IN RECOMBINANT DNA TECHNIQUES AND CHEMICAL SYNTHESIS OF DNA

IMMUNOLOGISTS

from the post doctoral level to group leaders to join its first team of highly qualified scientists.

Expertise in the use of Bacillus subtilis, Yeast and Eucaryotic cells as cloning systems is of special interest.

Salaries will be competitive and commensurate with qualification.

Knowledge of French is not necessary.

STRASBOURG is a pleasant historical town, located in the heart of Europe, very close to GERMANY and SWITZERLAND.

The laboratory is located on the University campus and enjoys close contacts with well known groups in fundamental research.

For confidential consideration, send a CV, a list of publications and indication of salary requirements to:

> TRANSGENE S.A attn J-P LECOCQ B.P. 146 67028 STRASBOURG CEDEX

(W392)A

UNIVERSITY COLLEGE **CARDIFF**

DEPARTMENT OF ANATOMY BURSARY

Applications are invited from UK graduates for a Bursary in the above Department tenable in the first instance for one year but renewable up to a total of three years. While researching within the field of the Anatomical Sciences the incumbent would have the opportunity to work for a higher degree. Index-linked stipend of £2,460 includes a fee for limited teaching duties.

Applications, together with the names and addresses of three referees, should be forwarded to Professor J D Lever, Department of Anatomy, University College, PO Box 78, Cardiff CF1 1XL by 28th August 1981. Ref: 2260. (9245)A

IMPERIAL COLLEGE POSTDOCTORAL RESEARCH ASSISTANT

Plant Mitochondria

Biochemist or Plant Physiologist to study control of the NADH dehydrogenases in isolated plant mitocondria. Salary up to £7,847 pa inlcuding London Allowance. Duration 2 years from October 1st 1981.

Applications and enquiries to: Dr J M Palmer, Dept of Pure and Applied Biology, IC London SW7 2BB.

(9266)A

THE UNIVERSITY OF LEEDS DEPARTMENT OF **BIOCHEMISTRY**

Applications are invited for a post of

RESEARCH FELLOW

in the Department of Biochemistry. available from 1 October 1981 for a fixed period of up to three years, for work on the purification and characterization of androgen receptors from human and rat prostate involving the use of affinity chromatography, affinity labelling and monoclonal antibody techniques.

A good honours degree in Biological Sciences and a PhD in Biochemistry or Endocrinology are required. Experience in receptor work, affinity chromatography, affinity labelling and animal experimentation would be an advantage. Salary on the IA Range for Research and Analogous Staff (£6,070 - £10,575), according to age, qualifications and experience. Informal enquiries may be made to Professor W I P Mainwaring (Tel: Leeds (0532) 31751 ext 7437)

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LSŽ 9JT, quoting reference number 83/38/D. Closing date for applications 31st August 1981. (9241) A AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

NUTRITION & FOOD QUALITY DIVISION

Scientific Officers

The Food Research Institute has vacancies for four Scientific Officers in the Nutrition & Food Quality Division.

Nutritionist

This post is in a new group concerned with studies of the methodology of measuring food intake in man. The officer will be responsible for setting-up and operating a nutrient information and data base. The post calls for a sound background in nutrition and an interest in the application of computers. The Institute shares computer facilities with the John Innes Institute and there is an active computer group with which this officer will interact. Post No. 81/42.

Assistant Nutritionists

These posts are in the Intake Measurement Group. The officers appointed will be concerned with studies of food intake using a variety of techniques and the preliminary processing of data from these studies. The posts call for an interest and aptitude for working with people and some dietetic experience is desirable. The studies will be primarily conducted in the Norwich area; a valid driving licence is necessary. Post Nos. 81/43 and 81/44.

Assistant Sensory Scientist

The person appointed will be responsible for assisting a Sensory Scientist in studies of the influence of sensory factors on the dietary intake of salt. The work will involve both physiological and behavioural techniques and knowledge of one of these areas and an interest in acquiring an understanding of the other will be essential. A valid driving licence is necessary. Post No. 81/48.

Qualifications

Nutritionist: (81/42) - A first or upper second class Honours degree in Nutrition or Food Science with experience of, and interest in, the application of computer techniques.

Assistant Nutritionists: (81/43 and 81/44) - A second class Honours degree in Nutrition or Dietetics with some dietetic experience.

Assistant Sensory Scientist: (81/48) - A second class Honours degree in physiology or related biological sciences.

Salary: On a scale £4,809 - £6,480 (under review). Noncontributory superannuation scheme.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting the appropriate reference number.

Closing date: 31st August 1981.

(9257)A

TUFTS UNIVERSITY SCHOOLS OF MEDICINE

DEPARTMENT OF PHYSIOLOGY Tufts University has instituted a search for

CHAIRPERSON

Department of Physiology. The Department of Physiology is expected to be a center of research and scholarly excellence, as well as providing leadership in physiology instruction for the School of Medicine, School of Dentistry, School of Veterinary Medicine, and the Sackler School of Graduate Medical Science.

Individuals interested in being considered for this position should direct their letters to Dr Seymour Reichlin, Chairman, Physiology Search Committee, Box 275, New England Medical Center Hospital, 171 Harrison Avenue, Boston MA 02111. Tufts has an affirmative action program. and encourages applications from women and members of minority groups.

(NW771)A

UNIVERSITY OF KENT AT CANTERBURY RESEARCH FELLOW IN THE BIOLOGICAL LABORATORY

Applications are invited for a postdoctoral research fellowship to investigate the immobilisation of cellulases in soils and synthetic humic polymers. The project will be directed by Dr R G Burns and will initially be of two years duration. Applicants should have a first degree in biochemistry or microbiology and a PhD in some aspect of enzymology. Experience in colloid science and immobilised enzymes would be an advantage though not essential. Salary scale £6,070 - £10,575.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 8NH to whom completed applications should be returned as early as possible but not later than 24 August 1981. Please quote ref. A23/81/N. (9255)A

International Genetic Sciences, Inc. is organizing an annual conference on Introduction of Genetic Materials into Eucaryotic Cells: Present State and Future Prospects

Among the lecturers:

H. Goodman (U. Calif, S.F.), A. Graesman (Free U. W. Berlin), S. Izhar (Volcani Center, Israel), A. Loyter (Hebrew U. Jerusalem), D. Papahadjopoulos (U. Calif, S.F.), F. Ruddle (Yale U.), W. Rutter (U. Calif, S.F.), R. Rott (Inst. Virology, Gissen, W. Germany), R. Shimke (Stanford U.)

Among the topics to be included:

- 1. Transfer of Nucleic Acid into animal cells.
- 2. Introduction of genes into plant cells.
- 3. Use of Liposomes as a carrier of biological materials.
- Fusion mediated transfer of biological materials into animal cells.

Cost of \$450 includes room and meals. No registration fees, Number of participants limited to sixty. The conference to be held at the Harrison Conference Center, Glen Cove, L.I., N.Y. Arrival Nov. 22, 1981; departure Nov.25, 1981.

Interested scientists, please send as soon as possible information including name, address, phone, present position and areas of interest. Send to:

International Genetic Sciences, Inc. 155-25 Styler Rd, Jamaica, NY, USA 11433 or call (212) 526-0400

(NW802)A

THE EUROPEAN MOLECULAR BIOLOGY LABORATORY seeks an

Applications Programmer

for its nucleotide sequence database group in Heidelberg. The group is engaged in collecting and making available the published nucleic acid sequence data and will later pursue analysis and other research tasks related to the database. The laboratory's NORD-10S and VAX 11/780 computer systems currently provide computer support for the work.

The successful applicant will help to design and develop database management, data collection and analysis software for the database system. An interest in applying computer technology to scientific problems would be advantageous, although prior expertise in molecular biology is not essential.

Applicants should have at least a bachelor's degree and 3 to 5 years' experience in an industrial or research setting. Experience with modern software development methodology and block-structure languages (+ Fortran) and a demonstrated ability to develop transportable user-oriented software are essential, as is fluency in English.

The salary offered will be between DM3,300 and DM3,600 monthly, after tax. Certain allowances are payable in addition, depending on personal circumstances.

Please write briefly for an application form, quoting reference No. 81/12, to: EMBL, Personnel Section, Postfach 10.2209, D - 6900 Heidelberg, Germany.

(W398)A

UNIVERSITY COLLEGE CARDIFF

(Re-advertisement) CAREERS ADVISER CARDIFF JOINT UNIVERSITY CAREERS & APPOINTMENTS SERVICE

Applications are invited for the post of Careers Adviser in the Cardiff Joint University Careers and Appointments Service. Salary Range: Administrative Staff Grade 1A/2£5,285—£12,860 pa. Duties to commence as soon as possible. Successful careers advisers have come from many varied backgrounds. Have you the right qualities, eg relevant degree, related experience, to help students in the biological and life sciences launch their careers?

Applications (2 copies) together with the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL, from whom further particulars are available. Closing date 26th August 1981. Ref: 2255. (9251)A

UNIVERSITY OF LONDON GOLDSMITHS' COLLEGE

School of Science and Mathematics

TEMPORARY LECTURER IN PHYSICS

Applications are invited for the two year appointment as a Temporary Lecturer in Physics available from the 1st January 1982, to undertake undergraduate teaching. Candidates should have a higher degree and be actively engaged in research.

Salary on scale £7,221 × 12 increments to £12,087 per annum, inclusive of London Allowance.

Write for further particulars to the Personnel Officer, University of London Goldsmith's College, New Cross, London SE14 6NW. Closing date for applications 28th August 1981. (9272)A

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENTS OF BIOCHEMISTRY AND PATHOLOGY

Applications are invited from graduate biochemists for a temporary post of

JUNIOR RESEARCH ASSOCIATE

to work on the isolation and characterisation of human monocyte antigens. The post is tenable for a period of one year.

Salary will be at an appropriate point on the Range 1B scale: £5,285 to £7,700 pa according to age, qualifications and experience.

Applications, with full curriculum vitae and the names of three referees, should be lodged with Professor R H Pain, Department of Biochemistry, The University, Newcastle upon Tyne NEI 7RU, not later than 28th August 1981. (9279)A

UNIVERSITY COLLEGE, BOTSWANA

Applications are invited for the post of

LECTURER IN GEOMORPHOLOGY

Applicants should hold at least a Masters degree with specialisation in Geomorphology. Those with a PhD will be given preference. An ability to teach Hydrology would also be an advantage. The appointee will be required to teach an introductory course in Physical Geography, and a practical course in map and air photo interpretation at Part I level, and an advanced course in Geomorphology at Part II level. The Department would welcome an appointee with a strong interest in the use of remote sensing techniques in Geomorphological and Land Evaluation. There are excellent opportunities for research in Botswana, and the Department has continuing programmes in Quaternary studies in the Kgalagadi and in land use survey and evaluation related to resource development.

Salary scale: Lecturer P7,212—11,604 pa (£1 sterling = P1.64). The University College has a small number of positions within its establishment for which the British Government provides salary supplementation. The present posts carry no such benefits and are offered on ordinary expatriate terms only. Two-year contract (renewable); contract addition; family passages; baggage allowance; gratuity; assistance with accommodation, hard furniture provided.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent to the Registrar, University College Botswana, Private Bag 0022, Gaborone, Botswana, to arrive no later than 11 September 1981. Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London W1P ODT. Further details are available from either address. (9267)A

UNIVERSITY OF BIRMINGHAM Faculty of Medicine and Dentistry DEPARTMENT OF CANCER STUDIES

A RESEARCH FELLOW

with several years' post doctoral experience is required to join a team examining the interactions of virus transforming proteins with cell proteins. Applicants should have experience in protein biochemistry and have a good knowledge of mammalian cell biology. The post is supported by the Cancer Research Campaign. For three years.

Salary research range 1A (£6,070 to £10,575) with superannuation.

Applications (three copies) with full curriculum vitae and the names of two referees to Assistant Registrar, Medical School, Birmingham B15 2TJ by 30 September 1981. Quote reference RF/CS/381. (9282)A

YOU HAVE GOT A **BRIGHT IDEA**

TO FIND OUT BIOLOGICAL OR **CHEMICAL COMPOUNDS**

WE HAVE THE CAPITAL TO DEVELOP THESE NEW DRUGS

We are not a pharmaceutical firm, but a swiss based independant financial group, and we are prepared to let you participate in a capital venture for the exploitation of your

Our object: the development of new biological or chemical products (which have previously had a pharmacological screening) up to the stage of clinical tolerance and effectiveness required to permit licensing negotiations.

To obtain all the information required for initial selection, please write in English, French or German to: DEBIOPHARM S.A. Petit-Chêne 38 - 1001 LAUSANNE (Switzerland).

(W368)A

POST DOCTORAL -**RESEARCH POSITION**

for Biochemist-Cell Biologist available September 1, for two to three years, to identify, isolate and characterize insulin-like growth factor and α -thrombin receptors. (J. Biol. Chem. Vol 256: 2767, 1981) from cultured cells and tissues.

Experience in membrane protein chemistry, monoclonal antibody production, and/or ligand receptor characterization is desirable but not essential. \$15,000 to \$20,000 yearly depending on qualifications.

Send curriculum vitae and names of three references to Dr James F Perdue, Lady Davis Institute for Medical Research — Sir Mortimer B Davis — Jewish General Hospital, 3755 Cote St Catherine Road, Montreal, Que Canada H3T 1E2. (NW760)A

UNIVERSITY OF GLASGOW DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant-ship, supported by the Science Research Council, for work on the biosynthesis of pyrrolizidine alkaloids. The appointment will be available for two years from 1st October, 1981, at a salary within the range £6,070 — £6,880 (Range 1A, Research & Analogous Staff scales) with USS benefits.

Applications including a full curriculum vitae and the names of two referees should be sent to Dr Robins, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ.

In reply, please quote Ref. No. 4839. (9260)A



Medical Research Council Centre

Neurochemical Pharmacology Unit

NEUROPHYSIOLOGIST

Applications are invited for this NON-CLINICAL SCIENTIFIC post in an active multidisiciplinary research team investigating the actions of neurotransmitters, neuropeptides and psychoactive drugs in mammalian CNS. The successful candidate will head our neurophysiology laboratories, with modern and well equipped facilities. Applicants should preferably have several years of post-doctoral experience and familiarity with modern neurophysiological techniques, and be available for appointment from around October 1981. The post may be filled by either a SHORT-TERM appointment of 3 (to 5) years or a CAREER (TENURED) appointment according to the age and experience of the successful candidate. A career appointment may subsequently entail changes in a scientist's place of work or field of work.

Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Applications in writing within the next four weeks, with CV and names of two professional referees, and quoting reference number NP/3 to: The Administrator, MRC Centre, University Medical School, Hills Road, Cambridge CB2 2QH.

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

NUTRITION & FOOD QUALITY DIVISION

Nutritionist

Senior Scientific Officer/Higher Scientific Officer

The Institute has a vacancy for a Nutritionist to take charge of a new group working on the methodology of measuring food and nutrient intake in man. The officer appointed will be responsible to the Head of Division (Dr. D. A. T. Southgate) and will be expected to develop the research programme of the group.

The post calls for an interest in quantitative human nutrition and the problems of assessing food intake of individuals and groups of the population over long periods. An interest in food composition and the application of computer techniques in the assessment of nutrient intakes is essential.

Qualifications: A first or upper second class Honours degree in Nutrition or a related subject with research experienced that has involved studies of measuring food intake in man. At least 2 years relevant post-graduate experience required for HSO; 4 years for SSO grading.

Salary: (under review)

quoting ref. 81/41.

Senior Scientific Officer — on a scale £7,644 — £9,619. Higher Scientific Officer — on a scale £6,075 — £7,999.

Further particulars and application form from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA

Closing date: 31st August 1981.

(9259)A

LIVERPOOL POLYTECHNIC

Department of Chemistry and Biochemistry

RESEARCH ASSISTANT

£3,918 — £4,590 p.a.

Required to work on sweat physiology/biochemistry in relation to cystic fibrosis. The work will mainly involve radioimmunoassay and electrolyte determinations. The project is funded by the Cystic Fibrosis Research Trust and initially is for one year commencing October, 1981.

Applicants should have a first degree in Biochemistry or Physiology. It may be possible for a suitable candidate to register for a higher degree (CNAA).

Further details and applications from Dr. F. McEvoy, Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool L3 BAF.

Closing date is 21 days from the appearance of this advertisement. The Council is an Equal Opportunity Employer and welcomes applications irrespective of race, sex or marital status.

THE FLINDERS UNIVERSITY OF SOUTH AUSTRALIA SCHOOL OF PHYSICAL SCIENCES

Graduates in chemical engineering, chemistry or physics are invited to apply for the position of

Research Assistant (High Temperature Electrochemist)

The appointment will be initially for one year. Under a grant from ALCOA of Australia Limited a feasibility study will be carried out to measure oxygen concentration in molten fluoride electrolytes using the principles of the Flinders Oxygen Probe. Candidates with a high-temperature-electrochemical background will be preferred.

Salary will be up to A\$15,664 a year, depending on qualifications and experience.

Enquiries regarding this position should be directed to Associate

Professor H J de Bruin, telephone (08) 275 2192 or 275 2111.
Written applications, including curriculum vitae and the names and addresses of three referees, should be lodged with The Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042 not later than Friday 28th August 1981

NMR Spectroscopist

A vacancy exists for an NMR Spectroscopist in the Analytical Department of our Pharmaceutical Research Laboratories. The Department is involved in the development and application of methods of measuring drugs in biological fluids as well as the provision of mass spectrometry and NMR services to other departments, notably Synthetic Chemistry and Drug

The ideal candidate will be a young graduate or equivalent with 2-3 years experience of the operation and maintenance of Fourier transform NMR spectrometers and who have some knowledge of the interpretation of data. We expect shortly to take delivery of a Brucker 250 MHz Fourier transform spectrometer and the successful applicant will take charge of this and provide a service to the other departments as outlined above.

The Company is situated at Milton Keynes where housing is available for rent or purchase.

Salary and conditions are of the order to be expected from a world leader in the pharmaceutical field and include contributory pension with free life insurance, membership of PPP and a generous relocation allowance, if appropriate.

Please write or telephone for an application form to: Adrian Forrest, Personnel Department, Hoechst UK Ltd., Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

(9264)A

Hoechst



UNIVERSITY OF **CALIFORNIA** Los Angeles Tenure Faculty Position ASSOCIATE OR FULL PROFESSOR IN THE FIELD OF PLANETARY **ATMOSPHERES**

A position has become available for a joint appointment in the Department of Atmospheric Sciences and the Institute of Geophysics and Planetary Physics.

Preference will be given to Senior Scientists with recognized accomplishments, whose interests are in global atmospheric composition evolution of planetary atmospheres.

The position will require the successful candidate to develop a strong research program. A moderate teaching load will be expected in the Department of Atmospheric Sciences.

Applicant deadline: 30 November 1981. Please send résumés to Hans Pruppacher, Chairman, Department of Atmospheric Sciences, University of California, Los Angeles, California 90024. An Equal Opportunity/Affirmative Action Employer. (NWX796)A

FELLOWSHIPS

IMPERIAL CANCER **RESEARCH FUND** POSTDOCTORAL RESEARCH **FELLOWSHIP**

Applications are invited for a three year postdoctoral appointment in the joint laboratories of Tumour Virology and Viral Leukaemo-genesis at Lincoln's Inn Fields to apply recombinant DNA techniques to the study of virus-induced oncogenesis. Molecular cloning of RNA tumour viruses is being undertaken to investigate, by a combination of restriction enzyme analysis, DNA sequencing and DNA transfection, the structure and function of mutant and wild type viral transforming genes and their interaction with host cell sequences in which they integrate. Salary range £7,700 - £9,750 plus London Allowance £967 a year, with entry point according to qualifications and experience. Wyke (tel. 01-242 0200 ext 270).

Further information from Dr. John Applications with curriculum vitae and names and addresses of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 by 31st August, 1981 quoting reference (9243)E 124/81

UNIVERSITY OF LEICESTER SCHOOL OF BIOLOGICAL

SCIENCES

ICI/UNIVERSITY JOINT LABORATORY Applications are invited for a POST-DOCTORAL **FELLOWSHIP**

in a group studying the molecular genetics of methylotrophic bacteria.

The post provides an opportunity to use modern bacterial genetics and recombinant DNA techniques to study chromosomal organisation in an industrial micro-organism. Applicants should have postgraduate experience in a relevant discipline, but experience in molecular cloning is not essential.

The salary will be in the research and analogous scale £6,070 — £10,575 and the appointment will initially be for two years.

Applicants should send a curriculum vitae and the names and addresses of two referees to Professor W J Brammar, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, from whom further details can be obtained. (9192)E

UNIVERSITY OF KENT AT CANTERBURY RESEARCH FELLOWSHIPS IN PHYSICS

Applications are invited for two posts of Research Fellow associated with projects involving structural studies of molecular liquids and amorphous solids by diffraction methods. One appointment is for three years for work concerned primarily with the use of pulsed neutrons from the Harwell Electron Linac but some work on the high flux reactor at ILL Grenoble is also envisaged. The second post is available for two years and involves the use of X-rays from the Synchrotron Radiation Source at the Daresbury Laboratory, Cheshire. Both projects, under the supervision of Dr John Dore, will exploit the short wavelength nature of these new sources to obtain detailed information on molecular conformation and orientational correlations. Prior experience with diffraction and computing techniques will be an advantage but is not a prerequisite. Salary range £6,070 — £7,700 on the Grade IA scale.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Chemical Canterbury, Kent CT2 7NH to whom completed applications should be submitted by 28 August 1981, Please quote ref. A24/81/N. (9256)E

UNIVERSITY OF **BRITISH COLUMBIA**

Vancouver

POSTDOCTORAL RESEARCH FELLOWSHIP IN IMMUNOLOGY

An Immunologist is being sought to join a research group investigating network aspects of the immune system. The salary will be in the range 14,000 dollars to 15,500 dollars (Canadian).

The position is for one year initially, and can be extended up to two years.

Applicants should write to Dr G W Hoffmann, Depts of Microbiology and Physics, UBC, 2075 Westbrook Mall, Vancouver, BC Canada V6T 1W5, and ask two referees to write to (NW803)E him on their behalf.

UNIVERSITY OF DUNDEE **DEPARTMENT OF BIOLOGICAL SCIENCES** POSTDOCTORAL RESEARCH FELLOWSHIP EST/48/81J

Applications are invited for the above post to work with Professor J.A. Raven and Dr. J. Beardall on an investigation of the significance of 'slippage' and protein turnover in the energetics of shade-adapted phototrophs. Appointment from 1 January 1982 for up to three years within the salary range £6,070 — £6,475.

Further particulars from Personnel Office, The University, Dundee Office. DD1 4HN, to whom applications (2 copies) should be sent by Wednesday 30 September 1981 (9262)E

The Izaak Walton Killam **POSTDOCTORAL FELLOWSHIPS**



1982-1983 DALHOUSIE UNIVERSITY

Fellowships valued at \$16,000 (1981-82), plus travel allowances, are tenable for one year at Dalhousie University in most fields of study. Application for extensions may be considered.

Qualification

Applicants should have recently completed a Ph.D. degree at a recognized university, or expect to obtain a degree before taking up an award

Application

Application forms may be obtained by writing

The Office of the Dean Faculty of Graduate Studies Dalhousie University Halifax, Nova Scotia B3H 4H6

The closing date for receipt of applications is 15 December, 1981.

(NW794)E

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY **POSTDOCTORAL**

RESEARCH FELLOW

Applications are invited for a postdoctoral research fellowship in the above Department for work involving the cloning of developmentally regulated MRNA sequences in Xenopus. The appointment available from 1st October 1981 will be for three years on an SERC funded research grant. Salary starting at point 3 (£6,880) on the 1A Range for Research and Analogous Staff. Candidates with directly relevant experience would be preferred, but inquiries are also invited from people who have recently completed PhD work in related fields and would like to, acquire methods of nucleic acid and cloning technology. Informal enquiries may be made to Dr P Ford (031) 667 1081 ext 2873/8.

Applications should be made in writing, quoting at least two referees, to Dr P J Ford, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 Please quote Reference 5042 (9273)E

UNIVERSITY OF KENT AT **CANTERBURY** RESEARCH FELLOWSHIPS IN PHYSICS

Applications are invited for two posts of Post-Doctoral Research Fellow for a new research programme directed by Dr D A Jackson to develop optical sensors, using lasers and fibre optics, for industrial, surveying and research applications. The programme is funded by the SERC and the Royal Society and the appointment will be for three years. Experience in optics and electronics would be an advantage though not essential and the successful applicants should be prepared to collaborate with potential users of the sensors. Salary will be in the range £6,070 - £8,515 on the Grade 1A

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Chemical Canterbury, Kent CT2 7NH to whom completed applications should be submitted by 28 August 1981. Please quote ref. A25/81/N. (9254)E

IMPERIAL CANCER RESEARCH FUND POSTDOCTORAL RESEARCH **FELLOWSHIP**

Applications are invited for a three year, postdoctoral appointment in the Viral Leukaemogenesis Laboratory at Lincoln's Inn Fields to work on control of expression of membrane proteins during differentiation of erythroblasts transformed by avian retrovirus, in particular avian erythrobalstosis

Salary range: £7,700 to £9,750, plus London Allowance of £967 a year, with entry point according to

qualifications and experience. Further information from Dr. M. Hayman (telephone 01-242 0200 extension 448). Applications including full curriculum vitae and names and addresses of two referees should be sent to the Research Fund, Lincoln's Inn Fields, London WC2, by 31st August, 1981, quoting reference 121/81. (9244)E

GRANTS

UNIVERSITY OF LONDON GRANTS FOR RESEARCH

Applications are invited from members of the University and teachers in Schools of the University for grants from the Central Research Fund to assist specific projects of research and for the provision of special materials and apparatus. Grants are not made for normal maintenance.

Applications must next be received not later than 8 September 1981. Forms of application and further particulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, Malet Street WC1E 7HU.

(9268)H

CONFERENCES

THE INDUSTRIAL LIAISON PROGRAM OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY PRESENTS A COLLOQUIUM ENTITLED

"Microcapsules and Microcarriers in Biotechnology"

October 15, 1981 Date:

Professor Robert S. Langer, Jr., MIT Chairman:

Professors Marcus Karel, Alexander Klibanov, Co-Chairmen:

William Thilly, MIT

Professor E. Katchalski-Katzir Other Speakers:

Weizmann Institute of Science Professor D. Papahadjopoulos University of California, San Francisco Professor T. Chang, McGill University

J. Salk, M.D., Salk Institute

MIT Kresge Auditorium, Cambridge, MA 02139 Location:

Maria Clara V. Suva Martin Contact:

Industrial Liaison Program Tel: (617) 253-2691. Telex: 921473

Industrial Liaison Program (ILP) Members — free Non-ILP Members — \$500 Attendance Fee:

Immobilized Enzymes Topics:

Enzyme Stabilization

Controlled Drug Delivery Systems Liposomes — Drug Targeting

Microcapsules

Diffusion Control in Food Systems Microcarriers for Mammalian Cell Culture Microcarriers in Vaccine Production

Major advances have been witnessed in the past few years in the area of biotechnology, which can be defined as the application of engineering principles to biological processes. In this coloquium, the use of novel concept - microcapsules and microcarriers - in biotechnology will be discussed. These particles possess significant potential in both basic and applied research. The applications in several important areas will also be discussed including their use as (i) supports for cells which make useful products, (ii) matrices for enzymes to be used as bioproducers or bioconverters, and (iii) the central components in drug and food delivery systems. (NWX793)C

Please mention **nature** when replying to these advertisements

ASSISTANTSHIPS

UNIVERSITY OF EXETER DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral Research Assistantship (supported by the SERC) to work on the metabolism of the branched chain amino acids. The project will include the stereospecific synthesis in isoptopically labelled form of biological intermediates and studies of their conversions into both primary and secondary metabolites.

The appointment will be for two years, commencing 1 October 1981. Salary within the range £6,070 to £6,880 per annum, placement dependent on age and experience. Applications with curriculum vitae, and the names and addresses of two referees, should be sent as soon as possible to Dr D H G Crout, Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4OD. (9278)P

UNIVERSITY OF GLASGOW DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited from Organic chemists for a 3 year postdoctoral research assistantship supported by the SRC to study stereochemical aspects of the biosynthesis of some fungal lactones. The initial salary will be within the range £6,070 — £6,880 (Range 1A, Research and Analogous Staff scales).

Applications including curriculum vitae and the names of two referees should be sent to Dr N J McCorkindale, Department of Chemistry, The University, Glasgow G12 8QQ.

In reply please quote Ref. No. 4840. (9261)P

UNIVERSITY OF EXETER DEPARTMENT OF

CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a post-doctoral Research Assistantship (supported by the SERC) to work on the biosynthesis of riboflavin. Previous experience in the isolation and purification of plant or microbial metabolites in the application of isotopic techniques to the investi-

gation of biological pathways and in

the handling of fungal systems would be an advantage.

The appointment is for two years, commencing 1 October 1981. Salary within the range £6,070 to £6,880 per annum, placement dependent on age and experience. Applications, together with a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Dr D H G Crout, Department of Chemistry, University of Exeter, Exeter EX44QD. (9277)P

UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

GRADUATE TEACHING ASSISTANTSHIPS

1981/82 Academic Year

Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the MSC and PhD Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Bio-Inorganic Chemistry to Chemical Physics. Attractive and competitive teaching stipends and summer research assistantships are available for all students in the programme. Numerous graduate scholarships are available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned: James Trotter, Professor and Acting Head, Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada V6T 1Y6.

(NW795)P

UNIVERSITY OF GLASGOW

DEPARTMENT OF ORGANIC CHEMISTRY POSTDOCTORAL

RESEARCH ASSISTANTSHIP IN BIO-ORGANIC CHEMISTRY

Applications are invited for an SERC-sponsored Research Assistantship to study terpenoid metabolism in plant-tissue cultures and micro-organisms. Applicants should have a good background in organic synthesis. Experience with radio-isotopes and micro-organisms would be an advantage. The post is available from 1.10.81 and is tenable in the first instance for one year at a salary in the range £6,070 to £6,880 (Range 1A, Research & Analogous scales).

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to Professor K H Overton, Department of Chemistry, The University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4838 (9246)P

Please mention

nature

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ASSOCIATESHIPS

National Research Council Canada

Conseil national de recherches Canada

1982 RESEARCH ASSOCIATESHIPS

for research in science and engineering in the following laboratories of the NATIONAL RESEARCH COUNCIL OF CANADA:

Atlantic Research Laboratory
Division of Biological Sciences
Prairie Regional Laboratory
Division of Building Research
Division of Electrical Engineering
Industrial Materials Research Institute
Division of Mechanical Engineering
Division of Chemistry
Herzberg Institute of Astrophysics
Division of Physics

Applicants should have recently acquired a PhD in natural science or a Master degree in an engineering field or expect to obtain the degree before taking up the Associateship.

Associateships are open to nationals of all countries although preference will be given to Canadians.

Research Associates will be offered appointments to the staff of the National Research Council on a term basis and will be offered salaries and benefits currently available to members of the continuing staff.

The initial appointment will normally be for a twoyear term and may be renewed, subject to the Associate's performance and subject to the requirements of the Division. Renewals are considered annually. The term as a Research Associate will vary by Division but will not exceed five years.

An allowance will be provided towards the cost of travel.

Applications may be obtained from the Research Associates Office, National Research Council of Canada, Ottawa, Ontario, Canada, K1A OR6.

Closing date: 15 December 1981. (9253)P.

Canada

STUDENTSHIPS

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF BIOLOGY SERC — CASE STUDENTSHIP IN PLANT PATHOLOGY

A research student is required to work on the effectiveness of the growth of cereals in mixtures in reducing disease. The work will be carried out in collaboration with the National Institute of Agricultural Botany, Cambridge, where some of the experimental work will be done.

The person appointed will be able to register for the Degree of PhD at Southampton. Further particular available from Dr J G Manners, Department of Biology, Building 44, The University, Southampton SO9 5NH. (9249)F

UNIVERSITY OF DURHAM

DEPARTMENT OF BOTANY

Applications are invited for a STUDENTSHIP

from science graduates with a first or upper second class degree to read for a higher degree in the field of recombinant DNA technology. Starting date 1 October 1981 or as soon as possible thereafter.

Applications, naming two referees, should be sent to Professor D Boulter, Department of Botany Science Laboratories, South Road Durham DH13LE. (9250)F

Solar flares

HUMAN TOXICOLOGY

An International Journal

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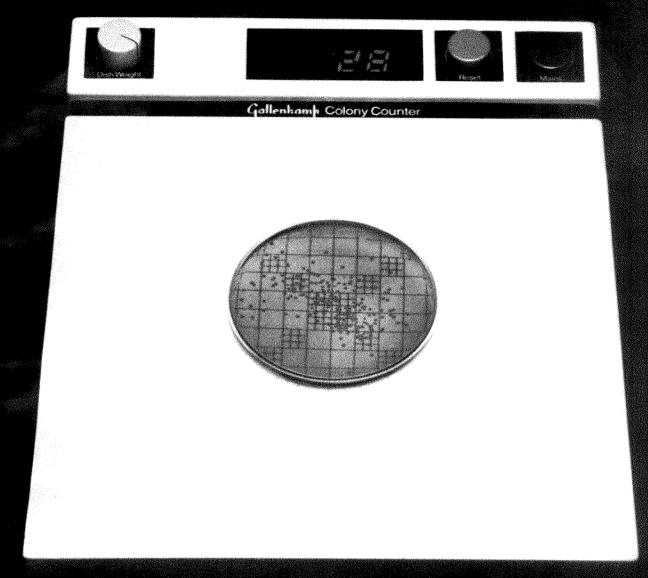
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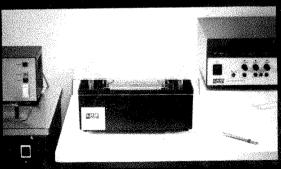
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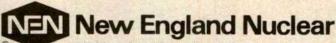
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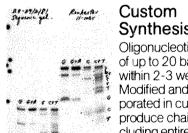
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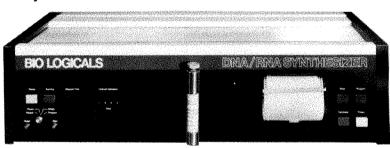
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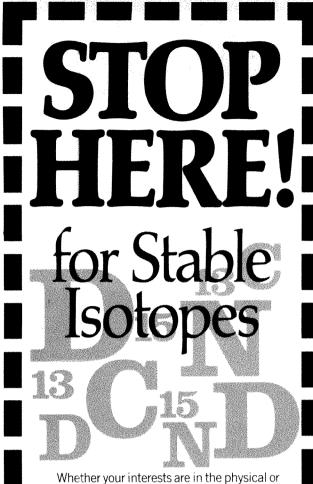
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13 August 1981

Odd ways with neutron bombs

That President Ronald Reagan has taken the decision on neutron bombs from which his predecessor shrank in 1979 is no great surprise. But the National Security Council's advice last week - with Secretary of State Alexander Haig apparently dissenting — will reverberate for several months to come. Although the Administration's plan is that the neutron weapons, when manufactured, should be stockpiled in the United States and not shipped to Europe, several democratic European governments will find that circumstance of very little help in dealing with their own electors. The only potential battlefield in which neutron weapons might have a place is in Central Europe but there they could be a powerful reinforcement of the tactical nuclear weapons, euphemistically called "theatre weapons", now or soon to be deployed. It is understandable that Mr Reagan should resist the notion that European governments should hold some kind of veto over the munitions policy of the United States, but it is not merely insensitive of him but dangerous to the cohesion of the chief alliance in which the United States is engaged, to behave as if European opinions are irrelevant.

Scepticism about the feasibility and effectiveness of neutron weapons has by now been largely dissipated. If it is possible to make a high-yield thermonuclear weapon by using a fission bomb to detonate a fusion mixture in such a way that the neutrons produced cause fast fission in a casing made of, say, uranium-238, why not play the same trick within a casing of beryllium, so multiplying the yield of neutrons? The only wonder is that something like this can be done within the diameter of eight-inch howitzer shells. And given the penetrating power of energetic neutrons, there is probably something in the claim that neutron weapons would be relatively more damaging than fission weapons to people rather than property - which is not to say that tanks or other battlefield equipment would remain in working order, ready to be turned against their original owners once the dead (and dying) bodies had been removed. So why is there so much opposition in Europe to what seems to be a sensible improvement of military technology?

Part of the reaction against neutron weapons springs from the widespread conviction that, because all nuclear weapons are abominations, the addition of a new design to existing armouries must be a calamity. The argument is mistaken. European defence is for the time being based on the assumption that nuclear weapons would have to be used in a serious conflict, even if the consequence might be the widespread destruction of European life and property. Neutron weapons, less damaging than "ordinary" nuclear weapons and more easily directed against military personnel, should therefore be more acceptable to European opinion. That is the simple counter-argument. It does not fully meet the subtle European complaint that the availability of relatively usable nuclear weapons will encourage their earlier use in any future European conflict. But that argument cuts both ways. The earlier use of comparatively safe nuclear weapons might just as well serve to prevent a more damaging exchange of nuclear weapons at a later stage as to invite early nuclear retaliation. In the bizarre logic of the nuclear battlefield, in which strategic nuclear weapons are intended to stay forever in wonderland, neutron weapons are a blessing and not a curse.

Unfortunately, especially for President Reagan and for those politicians in Western Europe who must now learn to live with last week's decision, this is not the end of the argument. Protests against the manufacture of neutron weapons may be inconsistent with the assumptions on which the defence of Western Europe is

based, but this does not mean that they count for nothing especially at the European ballot box. For the past three years, since Chancellor Helmut Schmidt courageously put his political head on the block by saying that he would allow neutron weapons in West Germany (without foreseeing that President Carter would find it politic to stab him in the back), it has been plain that the wayward trend of the past few years in European opinion will be countered only by a resumption of negotiations between the United States and the Soviet Union on nuclear armaments. These negotiations are, in any case, necessary for other and more compelling reasons, the need in Western Europe to modify strategic stand-off by flexibility in East and West European relations chief among them. In the past few weeks, the United States Secretary of State, followed with a needless show of diffidence by the Administration of which he is a part, has been talking hopefully of a resumption of the negotiations on the Salt II treaty (still in limbo) later in the year. (The diffidence is needless because the Administration has no choice.) Has this brave prospect now disappeared?

The most cynical reading of last week's decision on neutron weapons is that it is not (as Pravda and the Soviet news agency TASS have been saying) a proof that the United States is not serious about negotiations on strategic weapons but, rather, that it is the opposite — a preparation for Mr Haig's promised talks. For what could be more astute than to stake a claim to the right to deploy neutron weapons in Europe so as to increase the range of possible concessions during a negotiation? The trouble is that last week's meeting of the National Security Council — perhaps the most fully reported on record - seems to have been more iconoclast than that. And even if the underlying calculation is machiavellian, it is wrong. While the Netherlands (reluctantly a potential host for United States cruise missiles and Pershing II missiles in 1983 or thereabouts) still lacks a government, with the danger that the democratic and electoral rot might spread (Belgium?) and with the West German defence budget rising less quickly than expected, the United States Administration may find that it cannot hold the ring in Western Europe, even until Mr Haig's target date later in the year, if it fails to reckon with its allies' ballot boxes, not just with its own. That is a penalty of being a superpower, as the Soviet Union has found in Poland.

Stanford's patent prize

Is Stanford University just lucky? Or unlucky? Or do the problems stemming from its exploitation of the Boyer-Cohen patent, which goes to the root of many present attempts to make a commercial success of genetic manipulation, derive simply from its good sense, over several decades, in recruiting faculty members with skill, imagination and flair? The answer is yes on all three counts.

When universities of all kinds are under serious financial pressures, one that finds itself blessed with what may be a substantial source of revenue in the 1980s (until the patent runs out) will be the envy of both private and public universities in the United States and elsewhere. Even so, it is unlikely that Stanford would have sought the invidious position of being the first university in the United States to have patented a scientific discovery that is already of outstanding importance in research laboratories (where patent protection entails no restriction) and may yet be the basis of a new industry.

Stanford's patent has been made possible by the changes of policy nearly a decade ago at the National Institutes of Health, which made it possible for universities to apply for their own patents even when financial support had come from federal funds. (One irony in the case of the Boyer-Cohen patent is that the University of California never succeeded in negotiating what is called an Institutional Patent Agreement, whence its partnership with Stanford in this venture.) Last year, the doctrine that recipients of federal research grants may nevertheless patent discoveries that arise, and not leave them in the public domain. was extended by the new patent legislation to most agencies of the federal government. The arrangement has some virtues. It provides universities with an independent source of income, and there is a chance that some universities will be more efficient merchants of innovation than the government has been. Given its traditions, Stanford could hardly have stood aside from these developments. In that sense, its bad luck has been thrust upon it.

The university and its members have in the circumstances conducted themselves with the utmost propriety. First, the authors of the discovery have assigned all their personal rights to their respective universities, and will make no financial gain from whatever happens in the next few years. Second, Stanford has apparently consulted widely with the companies likely to be the first in the field with practical applications of the new techniques. Third, patent protection has apparently not been sought outside the United States (but less liberal regulations on prior publication elsewhere might have been an obstacle). Fourth, the terms to would-be licensees are modest, as these things go; neither university seems out to make a quick buck, or an unseemly number of bucks. Even the imminence of the deadline (15 December) before the terms are made stiffer is forgivable, given the advance consultation there has been. And licensees will have to comply with National Institutes of Health guidelines. Finally, Stanford stands out among universities in the United States in having formulated an explicit policy for the seemly division of the spoils from patent exploitation between the university, the inventors and the departments to which they belong (see Nature 18 June, p.524); the Stanford faculty will be debating these proposals early in the new academic year.

So why should there be a fuss, if fuss there is? Most probably the commercial companies, especially those which Stanford has consulted in advance, will take out licences promptly and without complaining that they are being held to ransom. They will know that it will cost them more to buy exclusive (as distinct from nonexclusive) licences for particular applications of genetic manipulation from the genetic engineering companies that have mushroomed in the past few years. Even so, a legal challenge to the patent is more than possible if less than probable, either on the grounds that Boyer and Cohen were not the only authors of the research or that, in the early 1970s, the techniques now patented were not as novel as they may have seemed. Stanford seems prepared for such a challenge (see page 573) and may well be right in thinking that it would win its case. What will concern its friends, those who admire and owe an intellectual debt to Boyer and Cohen as well as those concerned for the reputation of scientific scholarship, is that a wrangle about scientific priority in the courts would be damaging to institutions and people.

Another difficulty goes to the root of current policy in the United States on the exploitation of federally sponsored inventions. There is now a danger that, in the years ahead, drafts of papers for publication will be read with an eye not merely for scientific merit but for exploitability. Moreover, Stanford and the University of California at San Francisco apart, it remains to be seen whether the federal government's new liberality on patents will turn out to be a golden goose or merely another addition to the cost of university administration. A more serious problem is that most universities, within and outside the United States, still lack policies for deciding how the commercial benefits of invention should be divided internally among the interested parties; one obstacle is that the financial interests of the faculty are in many cases already too deeply entrenched. The danger now is that the commercialization of academic work will be reinforced

- and that the benefits will be spread throughout the university system more or less at random, according to the chance that some discoveries will be patentable and others not.

Although in the short run some universities may benefit, all must know that precarious income from patents is no basis for running a university system. Worse, they must also fear that the further they go along the money-making path, the harder it will be for all of them to stick to their proper tasks of teaching and scholarship. And while it may be pleasing to be able to demonstrate to governments that universities can turn their hands to business when they are pushed, that proposition conceals a trap. For the universities have always contributed to socially and commercially valuable innovations. If they now accept the notion that they can do so effectively only when they are rewarded, their place in public esteem will be diminished.

Polytechnics in passing

Once upon a time, when there seemed no limit to the growing demand for higher education in the United Kingdom, the late Mr Antony Crosland, in his capacity of Secretary of State for Education and Science, wove a marvellous spell. In a speech at Woolwich fifteen years ago, Mr Crosland brought into being an entirely novel system of higher education, which he called the "binary" system. One half consisted of the traditional universities. The spearhead of the other half was to be 26 technical colleges, which were redesignated as "polytechnics", urged to provide degree courses for their students (which many already did) but to concentrate on practical things such as engineering, technology, business - the "world of work" as it has since been called. In a curious way, Mr Crosland's magic wand marked the beginning of the universities' fall from grace. If the "other" half was to be the provider of practical higher education, how could the universities fail to be thought effete? But now it is the polytechnics' turn to take a tumble.

After two years of blustering, the British government has now issued a discussion document that will spread gloom and despondency in the polytechnics — and among the local authorities which are their titular sponsors (Higher education in England outside the universities: policy, funding and management: Department of Education and Science). The government's supporters, if they have the time to read such a specialized document, may also take fright. For the government now seeks to set up some kind of central body to supervise working of "higher education outside the universities". The local authorities know they have no ground on which to stand — the cheques they write each month to pay the salaries of teachers in polytechnics are immediately discounted by the central government. Worse still, there is a suspicion that the local authorities which happen to be the channels of the central government's cash flow towards the polytechnics (now grown in number to 29) would put higher education high among their priorities. There are cheaper ways of catching votes and, in any case, local authorities are probably as disappointed as the rest of the United Kingdom that the performance of the polytechnics has been so disappointing. Those which have chosen not to ape the universities have all too trendily destroyed their claims to intellectual respectability. There are some exceptions.

The government's solution, modestly called "Model B" in the discussion document, is that the polytechnics (and the rest of nonuniversity higher education) should be controlled by a body analogous to the University Grants Committee. The local authorities have lamely countered with what is called "Model A" a scheme in which their representatives will call the shots. Everybody knows how the consultation will be resolved. The government's supporters (if they had the time) would be horrified to learn that the crucial argument is the case (spelled out) that central planning of higher education is not merely necessary but possible. For some time yet, it will be forgotten that the most immediate need is to bring the two halves of the binary system into

some kind of coordination.

Stanford ready to fight for patent

No challenge as yet in sight

Washington

Officials at Stanford University's Office of Technology Licensing are bracing themselves to meet possible challenges to the patent on the set of basic genetic engineering techniques, used to replicate foreign genes in microorganisms, which was offered for licensing last week.

The patent was awarded last December covering the results of research by Dr Stanley Cohen, of Stanford Medical School's departments of medicine and genetics, and Dr Herbert Boyer of the University of California at San Francisco. Stanford is confident that it can successfully resist any challenge, if necessary in court; but patent attorneys in several large companies are already poring closely over the scientific literature in case they decide to question the basis on which the patent was awarded.

The key question is whether these companies are prepared to accept the terms of the Stanford licence. The university is asking for an initial non-exclusive licence fee of \$10,000 and an annual fee of the same amount for the use of the techniques in research. (University scientists will be exempt.) Royalties on product sales will vary from 1 per cent for the first \$5 million, to 0.5 per cent for sales over \$10 million.

Before deciding on these terms, which are relatively low for patent agreements although these usually offer exclusive licensing rights, the university had consulted several companies — including Eli Lilly, Upjohn, Smith-Kline, Schering-Pough, Du Pont and Monsanto — to gauge their reaction to early drafts.

Several have already indicated that they are prepared to accept Stanford's terms. Dr Peter Farley, for example, president of the Berkeley-based Cetus Corporation, said last week he thought the terms were "very reasonable" and that he expected his company to accept them. Similarly Genentech, of which Dr Boyer is a cofounder, has also indicated that it is likely to agree to the terms.

Other companies, however, are refusing to commit themselves — at least in public — until they have studied the implications of the licensing agreement more closely. Patent attorneys for several large pharmaceutical companies said last week that it was too soon to comment on the actions they may take.

Privately there are rumours that at least one company may decide to challenge the patent in court. But company officials list a number of factors they will have to take into account.

One is the public impact of a major court case that could rekindle debate on the ethics and safety of genetic engineering. There is also uncertainty over how much sympathy there would be for a company which appeared to be preventing the two universities profiting from discoveries made in their laboratories. (Both Drs Cohen and Boyer have assigned any personal interest in the royalties to their support teaching and research.)

Against this there is the argument put forward by the president of one small company that, since royalty payments will push up product costs — even if only marginally — the taxpayer wil be paying twice for the same research.

The principal determinant of whether a challenge is filed, however, is likely to be the cost. Each company must calculate whether the amount of money it could save by not paying Stanford the licence fee and royalties would exceed the amount required to challenge the patent successfully.

According to patent attorneys, any such challenge could take one of two principal forms. A company (or group of companies) could seek a court ruling that the patent had been improperly granted. Or — perhaps more likely — a company could continue to use the recombinant DNA techniques without a licence from

Stanford, waiting for the university to sue for the infringement of patent rights.

In either case there are two likely ways in which the Patent Office's decision to issue the patent might be challenged. The first would be to claim that the research results described in the patent were not "novel" since they were already being used by other scientists, and should therefore be considered part of the so-called "prior art". Alternatively, they could argue that the applicants had failed to show that the particular combination of techniques which they describe for the replication and expression of exogenous genes in microorganisms met the legal criterion of being "non-obvious".

Having granted the patent, the patent examiner responsible must have been convinced that the process described met both of these criteria. However, such a judgement is based largely on the material presented to the Patent Office to support the claim, which was filed by the two universities on behalf of Drs Cohen and Boyer.

A challenge on these grounds would not be a surprise. But officials at Stanford are confident that, although many scientists were involved in the discoveries and developments that laid the basic groundwork, they can demonstrate that scientific collaboration between Cohen and Boyer was sufficiently crucial to justify the claims to the patent.

David Dickson

Antecedents of Cohen-Boyer patent

The text of the Boyer-Cohen patent is a nice bibliography of the early history of genetic manipulation between 1972 and 1974. The patent, granted on 2 December 1980, was filed on 4 January 1980 and partly replaces three previous patent applications filed in 1974, 1976 and 1978.

The abstract of the patent says that "method and composition are provided for the replication of exogenous genes in microorganisms", and refers to the use of either plasmid or virus DNA as replicating elements into which foreign genes can be deliberately inserted for later replication and expression. "The method provides a convenient and efficient way of introducing genetic capability into microorganisms for the production of nucleic acids and proteins, such as medically or commercially important enzymes", and the abstract goes on to list possible applications in the production of drugs, the fixation of nitrogen, fermentation, the "utilisation of specific feedstocks, and the like".

The patent says that the process now protected includes the three steps in the preparation of recombinant plasmids, their use for the transformation of bacteria and the replication and transcription of the recombinant plasmids in transformed

bacteria. In its description of the "prior art" the patent lists a series of papers in *Proc. natn. Acad. Sci. U.S.A.* including those by Cohen et al. (69, 2110; 1972), Cohen et al. (70, 1293; 1973), Cohen et al. (71, 1030; 1974), Morrow et al. (71, 1743; 1974) and Jackson et al. (69, 2904; 1972). In addition, the patent cites the paper by Novick in *Bact. Rev.* (33, 210; 1969).

On the preparation of recombinant plasmids, the patent points to the need for preserving the site of replication and to the convenience of some "phenotypical property" that will aid the selection of transformants. Bacteria are mentioned simply as the most convenient of unicellular organisms with which to work, while usable plasmids are said to range in molecular weight from 1 to 50 million daltons. The plasmid pSC101 (developed in 1972 by Cohen et al.) is mentioned as a convenient plasmid for cloning purposes, while the techniques of cleaving plasmid DNA and its subsequent ligation to other pieces of DNA are also described.

Acknowledging that some recombinant plasmids can arise naturally, the patent says that the techniques covered make possible the construction of artificial recombinants, including those between bacteria "which do not exchange information naturally" and between bacteria and eukaryotic organisms "which cannot naturally occur".

The patent says that the techniques can be used with genes to form other bacterial cells, mammalian cells, plant cells and so on. The cloning of multiple genes is mentioned and the production of multiple copies of peptide hormones said to be of especial importance in the cases of parathyroid hormone, growth hormone, gonadotropin, insulin, ACTH, somatostatin and prolactin. Other useful products of genetic manipulation listed

Europe waits to see

European pharmaceutical companies have reacted cautiously to the announcement by Stanford University that licences on the Boyer-Cohen patent are now for sale. But many of them are huddled with their patent lawyers.

Under the terms of US patent law, companies outside the United States will be obliged to apply for licences only if they intend to market within the United States products made with techniques covered by the patent. Because most European companies are anxious not to foreclose future markets, however, they face the same dilemma as US companies in deciding whether to pay the licence fee and subsequent royalties.

Most companies this week were unwilling to guess what they may in the end decide to do, although it seems that some of them have already been consulted (through their American subsidiaries) by Stanford. Some point out that Stanford's advertised terms are reasonable, but also say that the university has been well advised in fixing a low fee and royalty rate for a non-exclusive licence to exploit a patent as general as Boyer-Cohen. Larger proprietary costs will accrue, they say, when the time comes to buy an exclusive licence to use a more specialized technique from one of the companies set up in the past few years to carry out research and development in genetic engineering.

Some legal anomalies stand out. Will, for example, a European manufacturer that seeks to market in the United States a product made by a process developed by a non-American genetic engineering company itself be required to pay a royalty, or will the legal obligation fall on the company that carried out the research and development?

In the circumstances, three options are being considered: to ignore the licence and face Stanford when products are placed on the US market; to challenge the patent; or simply to pay up. European companies, however, are unlikely to initiate direct challenges, preferring to wait and see what happens in the United States.

Judy Redfearn

include serum proteins, ferritin, myoglobin, interferin (sic), kinins and transcortin.

While illustrating the claims made with specific examples, the patent says that "it is obvious that certain changes and modifications may be practised" within its scope. Among the specific claims are for the ligation both of blunt-ended and "staggered" DNA molecules, to form recombinant plasmids.

On the face of things, the patent would seem not to cover the use of vehicles other than plasmids for the cloning of DNA, so that techniques of genetic manipulation involving the use of animal virus grown on susceptible cells in tissue culture would seem not to be protected. Nor is the development of cloning vectors able to switch between eukaryotic and prokaryotic cells. Yeasts are, however, mentioned.

The patent makes no claim to protection for organisms or plasmids with particular properties, presumably because it was not known whether organisms could be patented until the Supreme Court decided earlier this year in favour of a patent application on behalf of Dr A.M. Chakrabarty by the General Electric Company for a strain of *Pseudomonas* designed for digesting oil spills. Stanford is apparently waiting to hear whether its applications for protection for specific bacterial cloning strains will be successful.

Soviet biotechnology

Further resolve

Soviet progress in biotechnology is being hampered by shortages of reagents and apparatus, and delays in the construction of new facilities. Experiments are limited in scale, and too little is being done to implement theoretical results.

This is the tenor of last month's resolution of the Central Committee of the Party and the Council of Ministers on the further development of biotechnology. The official Soviet commitment to biotechnology began suddenly in May 1974 with a similar resolution, which in effect gave biotechnology priority status and funding from then rather than from the start of the succeeding five-year plan.

Reviewing progress in the past seven years, the Central Committee and Council of Ministers last month noted that the 1974 resolution had introduced "radical changes" in the biological sciences, especially in molecular biology, bioorganic chemistry, molecular genetics and immunology. Research cadres had been trained within a very short time, and in several fields Soviet scientists had attained a "leading position in world science". Despite high hopes, a flourishing biotechnology industry had not materialized.

The resolution suggests, however, that this is not simply because of the difficulty of turning research results into practice. The resolution charges the Academy of Sciences, the State Committee for Science and Technology, the State Planning Committee (Gosplan) and the governments of the union republics with the task of ensuring the conditions for these results to be introduced in agriculture, medicine and industry. But it also calls for intensified fundamental research both directly within the framework of the Academy of Sciences, and the ministries.

The State Committee for Science and Technology, the Academy of Sciences and Gosplan are to adopt a special target-orientated research programme in biotechnology, which will be coordinated by the State Committee's special council on biotechnology and the academy.

The supply problem remains. The resolution merely says that means of ensuring supplies of equipment, reagents, "biochemical preparations", computer technology and an information base in biology and biotechnology have been "noted", but makes no suggestion of what form these measures are to take. Vera Rich

US air pollution

Reagan retreats

Washington

Unexpectedly strong opposition has forced the Reagan Administration to retreat from plans to seek sweeping changes in US air pollution legislation. Initially Mr Reagan had announced his intention to demand a substantial weakening of the Clean Air Act when it comes up for renewal by Congress next month; but following objections not only from environmentalist groups and Democratic congressmen, but also from several leading members of the Republicandominated Senate, the Administration said last week that it was looking for more modest amendments.

One of the most significant shifts in the Administration's position has been its decision to continue to use health standards and "sound scientific data", rather than economic costs, as the basic principle for determining how air pollution should be regulated. Mr Reagan's budget director, Mr David Stockman, as well as members of his Council of Economic Advisors and outside industrial groups, had strongly urged that cost-benefit techniques be added to the statutory requirements of the Clean Air Act, first passed by Congress in 1970.

However, Mrs Anne M. Gorsuch, the new administrator of the Environmental Agency, announced last week that the Administration did not intend to go far down this particular path. Listing the basic principles to be followed in developing legislation to extend the act, she said that "cost-benefit analysis should not be included as statutory criteria in setting these standards", but that standards "should be based on sound scientific data demonstrating where air quality represents

real health risks".

Both supporters and critics of the Administration agree that the concept of "real health risks" will now become the central focus of debate over new regulations — and that the decision may reflect recent rulings by the US Supreme Court which, while denying that costbenefit calculations need be used in setting standards, have also emphasized the legal importance of scientific or epidemiological evidence of existing levels of risks.

Several of the principals announced by Mrs Gorsuch have pleased industry. For example, emission restrictions for nitrous oxides on new cars wil be eased. Under present legislation, all cars produced from this year onwards are allowed to produce only one gramme of nitrous oxide for every mile travelled. The Environmental Protection Agency intends to raise this limit to two grammes, the level at which it stood between 1977 and 1980 and a move which it is claimed could save \$100 on the price of a family car.

There will also be a relaxation in a particularly controversial section of the present legislation aimed at preventing the "significant deterioration" of air quality, even in areas where present levels of pollution are relatively low. This provision stems from a legal interpretation of the 1970 Act following court cases brought up by several large environmental groups; it hass been bitterly contested by industry on the grounds that it restricts the growth of new industries outside existing urban areas.

Mrs Gorsuch said last week that the present programme for the prevention of significant air quality deterioration "should be maintained for the protection of park and wilderness areas". She said that protection in other areas should be based on uniform technology requirements for pollution control.

The Environmental Protection Agency has also committed itself to asking for more funds from Congress for acid rain research. This has recently become a major source of conflict between the United States and Canada, which has accused Washington of failing to comply with an agreement signed last August to reduce air pollution from automobiles and large coalburning facilities. The Administration, with the utilities industry, is now arguing that considerably more research is required before the need for clear action is demonstrated, a position which is unlikely to be warmly greeted in Ottawa.

Critics of the Administration's earlier proposals, contained in draft legislation leaked to the press by congressional Democrats in May, have been given other concessions. Senator Robert T. Stafford of Vermont, for example, insisted that no change should be made to the principle that the young, the elderly and other high-risk groups should be given an extra margin of protection from the effects of polluted air. Furthermore, pollution standards will continue to be set at the federal level and

not, as industrial groups hoping for a more sympathetic hearing had asked, by individual states.

The Reagan Administration's decision to limit its proposed changes seems largely based on a realization that, unlike the cuts in public spending and taxation, there is no national consensus on the need for a major retreat on environmental protection. White House officials are said to have been concerned about the impact of recent aggressive anti-environmentalist statements by Interior Secretary James Watt, and have asked him to adopt a more conciliatory attitude.

But if the battle over the Clean Air Act extension is not going to be as fierce as once promised, it will still be heated. The National Clean Air Coalition claim that even the Administration's more limited proposals are a "blueprint for destruction" of the present laws. Mrs Gorsuch says that, even with the suggested changes, the quality of the nation's air will improve, but "at a more reasoned pace". Congressional hearings will begin next month.

David Dickson

Science jobs famine

Bangalore

Nearly three million scientists in India are unemployed and an equal number are working in posts requiring much lower qualifications, for lower pay than a government clerk receives. These were among the worrying facts which came to light at a recent meeting of the Science Advisory Committee of the Union Cabinet, headed by Planning Commission member and agricultural scientist Dr M. S. Swaminathan, and held to discuss ways of making the best use of the country's science and technology personnel, particularly those unemployed.

For instance, more than 50 per cent of scientists and engineers employed at the prestigious Bhabha Atomic Research Centre draw a monthly salary of less than \$75. And nearly 25 per cent of the personnel working in government-sponsored research and development institutions have a monthly pay packet of less than \$120. However, people working in science and technology in the private sector are better off, seven per cent of them earning \$200 a month.

According to a note circulated to members of the committee, unless the annual rate of industrial growth rises to four per cent there is little prospect of speeding up the absorption of unemployed science and technology personnel into jobs.

The note further recommends regulation of intake of such personnel for training, more informal education, facilities for improving qualifications while on the job and greater interaction between industrial and academic scientists.

B. Radhakrishna Rao

Industrial noise

Let them hear

Britain's Health and Safety Commission is tackling the difficult business of regulating noise at work. Last week it published draft regulations in a consultative document intended to pave the way for legislation which will place a statutory obligation on employers to protect workers from noise. A voluntary code of practice, drawn up in 1972, is being widely ignored, according to the commission.

Devising effective legislation is, however, proving controversial — noise regulations are notoriously difficult to enforce and statutory levels of noise are fiercely disputed. The consultative document proposes limits of no more than 90 decibels(A) — a weighted decibel unit discounting inaudible frequencies — averaged over an eight-hour day and no more than 600 pascals of peak sound pressure. But the trades unions, in particular, argue that as hearing damage can occur at lower levels, the daily average limit should be set initially at 85 dB(A) and later lowered to 80 dB(A).

The number of British workers exposed to high noise levels is considerable. The commission estimates that 50 per cent of production workers (about 2.5 million people) are exposed to more than 80 dB(A) and 10 per cent (500,000 people) to more than 90 dB(A). Nobody seems to have disputed the effect of noise on hearing since the late 1960s. Using the work of Burns and Robinson, the commission estimates that roughly 32 per cent of people exposed to 100 dB(A) over a working lifetime will suffer a 50 dB hearing loss by the age of 65.

The commission has settled for the 90 dB(A) limit on the basis that the hearing of 21 per cent of workers previously exposed to more than 100 dB(A) will be protected. Reducing the limit to 80 dB(A) would save the hearing of only a further 8 per cent. Clearly acknowledging the problems of reducing noise, it argues that a 90 dB(A) limit will give greater benefit for cost.

Under the commission's proposals, however, employers would not be freed from obligations even at lower noise levels. The draft regulations require that they reduce any noise likely to damage hearing to the "lowest level reasonably practicable", which they would have to work out for themselves. Employers fear that this general but unspecific obligation may expose them to civil suits from all those who suffer from hearing loss.

The draft regulations require that at noise levels above 90 dB(A) employers must survey noise, train staff, keep exposure records and appoint a qualified noise adviser. Workers exposed to 105 dB(A) would have to receive regular hearing checks. The proposed regulations also oblige employees to make full use of noise control measures and manufacturers to reduce noise levels of industrial

machinery to below 84 dB(A) if possible. The commission has no estimate of the cost of its proposal.

On this occasion, Britain's Health and Safety Commission seems to be a step ahead of the European Commission, whose directive is still some way off. But the signs are that Europe will go for a limit of 85 dB(A).

Judy Redfearn

Bulgarian astrophysics

Cosmic celebrations

The "Interkosmos-Bulgaria-1300" satellite, Bulgaria's cosmic celebration of 1,300 years of statehood, was launched last Friday (7 August) to the surprise of the Bulgarian public, who had expected it would form the climax of the celebrations in October.

The designation of the satellite was equally unexpected — although the original plan was for a single celebratory satellite, analogous to the Polish Interkosmos-Kopernik-500 in 1972, it was announced earlier this year that there would be two satellites, one containing Bulgarian experiments only and the other a mixed Soviet and Bulgarian payload. However, only in the Moscow radio coverage of last Friday's launch was it made clear that the mixed payload was already in orbit — aboard the Priroda-Meteor satellite launched on 10 July.

In the Bulgarian celebrations, the timing has proved unfortunate, since the head of the jubilee committee, Lyudmila Zhivkova (the daughter of First Secretary Todor Zhivkov) died suddenly on 20 July.

Scientifically, however, the satellite is operating well, and has done much to offset Bulgarian disappointment that the Interkosmos manned programme did not allow them a second chance of a cosmonaut aboard a Salyut craft. By way of consolation, Georgi Ivanov, whose Soyuz transporter failed to achieve a linkup with Salyut, was invited to attend the launch and spoke warmly of Soviet-Bulgarian cooperation.

Although the experiments on the satellite (a modified "Meteor" meteorological probe) are said to have been produced "with the assistance of Soviet scientists", the twelve experiments comprising the 350-kg payload are of Bulgarian design, and concentrate on those branches of space physics of particular interest to the Bulgarian space team, including plasma, high energy charged particle studies, atmospheric luminescence and magnetosphere studies. According to Dr Kiril Serafimov, chairman of the Bulgarian Space Research Council, a special study will be made of ionosphere troughs and equatorial anomalies in the magnetosphere, which it is hoped will provide valuable data for such applied fields as radio-wave propagation, the mechanism of rare meteorological anomalies and the radiation balance of the Earth. Vera Rich

Carcinogenicity testing

Well, yes and no

Washington

Semantic juggling has allowed US health officials to escape the embarrassment of discovering two reports on the widely-used chemical dimethyl terephthalate (DMT) which are virtually identical but contain directly conflicting conclusions about its carcinogenicity.

Re-examination of the test data has raised a dilemma for scientists with the Department of Health and Human Services' National Toxicology Program (NTP) that is frequently faced by regulatory agencies: how to present policymakers with the results of ambiguous animal tests.

The solution proposed by NTP's peer review committee is to describe data on increased cancer rates in male mice as being "statistically significant" but "biologically equivocal".

DMT is widely used to provide flexibility in plastic products from food wrapping to bottles. The source of the confusion is a technical report on its potential carcinogenicity prepared in the 1970s for the now-defunct Clearinghouse on Environmental Carcinogens of the National Cancer Institute (NCI).

The tests were carried out by a private contractor, Hazleton Laboratories. They revealed no evidence of increased cancer rates among male and female rats, or among female mice exposed to low or high doses of DMT. However, a 27 per cent incidence of lung tumours was found among male mice receiving the high doses, considerably higher than the 4 per cent incidence in a group of matched controls. On the basis of these data, which were approved by an NCI peer review group, a report was published describing DMT as a carcinogen in male mice.

Soon afterwards, however, the report was challenged by scientists working with Hercules Incorporated, a major manufacturer of DMT. They claimed that the relatively low incidence of lung tumours among the matched controls was out of line with results from other control groups. NCI scientists reassessed the Hazleton data, this time using for comparison not the matched control group, but pooled results from three other control groups associated with different experiments which had spent overlapping periods of time in the same room. The latter groups revealed a lung tumour incidence of between 10 and 18 per cent over a two-year period, much closer to the 27 per cent of the exposed group.

A revised version of the technical report was subsequently issued through the National Technical Information Service (this time without peer review) which stated in the summary that the new data indicated that DMT was not a carcinogen.

Several research libraries, however, still

have copies of the first version on their shelves. And the apparent discrepancy between the two reports was brought to the attention of NTP, which has taken on the responsibilities of the NCI clearinghouse, three months ago.

Re-examination of the data revealed that the tests appeared to have been properly conducted, even though the incidence of tumours in the matched controls seemed inordinately low. "We view the incidences of total lung tumours in the matched male controls with some suspicion" reported Dr John Moore, deputy director of NTP.

A report on the NTP staff review was brought before the peer review panel of the programme's board of scientific counsellors last month. Discussion soon focused on whether, in revising the original report, the NCI staff had been correct in ignoring the matched control data and

One more journal

A new scientific journal is to be launched at the beginning of 1982 by the European Molecular Biology Organisation (EMBO). One of the objectives of *The EMBO Journal* is to redress the balance between Europe and the United States where, it is argued, the predominant and preeminent position won by American journals in the publication of molecular biology has put European molecular biologists at a disadvantage.

The new journal, which has been in the air for the past three years, was finally agreed after a ballot of the 500 members of the organization held in January. The journal will resemble Proceedings of the National Academy of Sciences of the United States of America in that it will publish papers communicated by any of its members, each of whom will be rationed to a maximum of five papers a year by non-members of the organization.

A statement by the publishers of the new journal, IRL Press Limited of Oxford, England, says that EMBO members will be expected to take responsibility for the "scientific standard" of the papers they communicate. One of the obvious difficulties will stem from the heterogeneity of the membership, which is elected, and the differing interpretation of what molecular biology consists of in various European countries.

The editor of *The EMBO Journal* will be Dr John Tooze, executive secretary of EMBO and also, at present, the editor of *Trends in Biochemical Sciences*. One feature of the new journal is said to be speed of publication — the publishers have undertaken to print manuscripts accepted by the editor within twelve weeks of their receipt. A pilot issue of the journal is to be made available in September.

basing their reassessment on the three other control groups; or whether all four groups should have been pooled into a single set of controls.

Various members of the committee expressed unease with the statistician's technique of ignoring so-called "outsider" data points which did not appear consistent with the other data being evaluated — but which, according to the biologists, there might be no particularly good scientific reason to disregard.

Members of the NTP peer review committee agreed to recommend the aggregate data as the basis of the evaluation about carcinogenicity. But it left the problem of deciding how to describe evidence that was in Dr Moore's words, "something between equivocal and positive".

The proposed solution was an appendix to the second version of the technical report, which will give details of the new analysis and point out that, statistically, the raised incidence of lung tumours in mice exposed to DMT is "highly significant". But the appendix will add that the results are also biologically equivocal — in other words the data do not provide clear evidence on whether DMT is a carcinogen.

The next step rests with the Food and Drug Administration's Bureau of Foods. Previously the bureau's cancer assessment committee had decided, on the strength of the NCI report, that no action was needed against DMT. With the new interpretation from NTP, the committee will have to reassess its position.

Meanwhile the NTP's board of scientific counsellors is meeting soon to discuss whether a more rigorous system of classification, possibly based on procedures developed by the International Agency for Research on Cancer in France, should be adopted in the United States. The DMT episode will encourage their efforts in this direction.

David Dickson

Alternative energy

Enter the Nibe

Brussels

By 1995, about 15 per cent of Denmark's electricity requirements will be met by wind power — if the Danish Ministry of Environment goes through with its plan to plant 3,000 windmills across the country. The plan is being seriously considered and has now been passed to the relevant regional and local authorities for their views.

Denmark is more dependent on imported energy than most other industrialized countries, having to provide 95 per cent of primary energy from outside sources. The only domestic form of primary energy is natural gas under the North Sea, and this will not start flowing until 1984. So research into renewable energy sources has been given high priority.

Even before the last government plan, a 1979 law provided government subsidies of up to 30 per cent of the cost of small

domestic windmills. These have to be of an approved type, and the electricity utility companies are prepared to buy any surplus electricity from the owner.

The new plan is altogether more ambitious. The 630 kW three-blade rotor mills, called Nibe wind turbines, were developed under the 1977–80 wind energy research programme which cost Dkr 39 million (\$7.2 million). The Nibe windmills are raised on a 41-metre concrete tower and the span across the turbine blades is about 40 metres. The mills will operate in wind speeds of between 21 and 90 kilometres per hour. The research programme has evidently ironed out most of the major technical problems, leaving the economic and environmental issues to be debated.

Denmark is a small, densely populated country, so the choice of suitable sites is severely limited. The windmills cannot be placed in areas where they would interfere with recreational, agricultural or other economic interests. If it were possible to find coastal sites for all 3,000 mills, electricity production would be 25 per cent greater than if they were all inland.

Somewhat surprisingly, there is evidence to show a relationship between electricity demand and high winds in Denmark. Apparently heating requirements are greater the stronger the wind blows and, conveniently, this would be countered by more power from the windmills.

But before the windmill ideas take off, it will be necessary to bring down the unit cost of construction — or the other sources of electricity will have become prohibitively expensive. Present cost of windgenerated electricity is \$2,000 per kW of installed power.

As with nuclear power stations, however, there may be objections from local residents. Anyone living near one of these Nibe wind turbines would have to put up with noise, interference with radio and television, and landscape eyesores. But Danish public opinion is likely to favour wind power and Denmark seems likely to be the first country to launch a major commercial wind power programme.

Jasper Becker

India in space

Apple limps on

New Delhi

Although still operating on power from only one if its two solar panels, India's experimental 'Apple'' communications satellite has now been successfully placed into geostationary orbit 36,000 kilometres above Sumatra in Indonesia. Apple's solid-fuelled apogee boost motor was fired on 22 June to achieve near-geosynchronous orbit but one of the solar panels failed to respond to radio commands and did not open. Despite this the satellite was stabilized and two other major manoeuwres were performed to correct an eastward drift and increase the orbital height. On 16 July Apple reached its intended orbit, with its antenna pointing towards Nagpur in central India.

The experiments in telecommunications techniques began on 22 July, and the bulk of the programme should be completed in spite of the reduction in the satellite's useful life from two years to one and the 160 watts of power available instead of the planned 280 watts. After the earlier anxiety, scientists at Apple Mission Control Centre in Sriharikota are buoyant at having manoeuvred the spacecraft successfully.

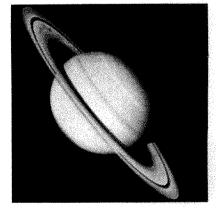
Good news was long overdue for the Indian space programme, for only 9 days after its launch aboard India's own SLV-3 launcher on 31 May, the remote sensing satellite Rohini II had burned up in the atmosphere — far short of its expected lifetime of 90 days. Rohini I was also ill-fated, crashing into the Bay of Bengal in August 1980 only a few days after launch.

Meanwhile, provided it is still functioning, Apple is to be used to broadcast nationwide an address from Prime Minister Indira Gandhi on Independence Day, 15 August. Only a few metropolitan centres will be able to pick up signals, but it marks a beginning to the plan to bring communications up to date throughout the Indian subcontinent.

Sunil Saraf

Saturn seen by Voyager 2 as the spacecraft approaches its encounter with the planet on 26 August, en route to Uranus (1986) and Neptune (1989). In the light of the results of the Voyager 1 encounter last November, the experimental programme of Voyager 2 has been considerably revised. Next week's *Nature* is a special Saturn issue containing new results from Voyager 1 and a description of the Voyager 2 mission by Dr Edward Stone, the Voyager mission's director.

The issue will also contain the usual features, including a report on the total synthesis of an interferon gene.



CORRESPONDENCE

Scientists in the Soviet Union

Sir — News of oppression crosses my desk daily. It is so commonplace that after a while its impact is almost lost. Like physicians who must become hardened to deal daily with death, human rights workers become somewhat inured to a daily fare of suffering. Nothing breaks down this shell more quickly than a visit to the victims of oppression.

As executive director of the Committee of Concerned Scientists. I went to the Soviet Union recently to investigate at first hand the trying circumstances confronting refusnik and dissident scientists. I found that the familiar litany of persecutions continues unabated. Most scientists who apply for emigration or speak out in defence of human rights are dismissed from their jobs. The regime then does its utmost to ensure their intellectual death by isolating them from their colleagues. In their personal lives too these individuals are plagued by countless harassments. Their homes are searched, their telephones disconnected, their mail intercepted. Scientists who apply to emigrate are routinely refused exit visas on spurious grounds. Anti-semitism is rife in all phases of academic life from admission to universities, to conferral of degrees, to obtaining employment. Those who have fallen from grace are frequently threatened with prosecution on criminal charges. Some are tried and sentenced to prison, labour camp and internal exile. These violations of human rights and scientific freedom are not new developments. Regrettably we have been hearing about them for some time.

I had learned much about this bleak situation during my four years with the Committee of Concerned Scientists. But I was not prepared for reports of the latest acts of persecution. Harassment has assumed new forms. Even prisoners are subjected to additional vengeful punishment. The health and very lives of prisoners of conscience Sergei Kovalev, Yuri Orlov and Anatoly Shcharansky are in grave jeopardy. Lengthy periods of solitary confinement, reduced rations and inadequate medical attention have taken their toll.

Other ominous trends in the treatment of refusniks and dissidents emerged from my discussions with leaders of these communities. First and most distressing is the wave of trials of scientists. Never before have so many activist scientists been threatened with lengthy sentences at one time. In Kiev Vladimir Kislik was sentenced to three years in a labour camp. Several years ago authorities had sought to imprison him for "compromising secrets" in an article published in an international journal. Yet this research had been officially cleared for publication some five years earlier. When confronted with a flood of protests from Kislik's Western colleagues pointing to the absurdity of this charge, the authorities backed off. In June, however, they achieved their goal through a contrived criminal charge. Computer scientist Viktor Brailovsky, a leader of the Moscow Sunday Scientific Seminar since its inception nine years ago, has been sentenced to five years of internal exile for advocating the right to emigrate, deemed defamatory to the Soviet state. In Leningrad,

refusnik mathematician Yevgeny Lein will also soon face trial. Interestingly, Kislik, Brailovsky and Lein all played key roles in organizing seminars designed to maintain the scientific skills of refusnik scientists.

The attempt by the Soviet authorities to close down these unofficial seminars is another cause for alarm. While seminars in provincial cities, such as Kiev and Vilnius, were squelched, those in Moscow and Leningrad had operated over a period of years with relatively little interference until recently. The Moscow Sunday Seminar, which had been blocked in the wake of Viktor Brailovsky's arrest in November, was able to resume regular Sunday sessions unmolested in early February. But this respite was short-lived; the authorities have shut it down three times since the end of April. On one Sunday during my visit at the end of May, the KGB barred the way to three different apartments making it impossible for a session to take place. Also in Moscow Alexander Lerner's seminar on mathematical biology, which recently held its 200th session, was blocked twice in April and May. In mid-May the seminars went into official recess for the summer. Our Soviet colleagues anxiously await the official reaction when they attempt to resume their

meetings in the fall.

Still another cause for concern is the use of academics as an instrument for persecution. Many scientists are now refused exit permits not because of allegations (usually unfounded) that they know state secrets, but simply because they are well educated. In fact the head of the administration department of the Communist Party's Central Committee bluntly told scientists that they are being detained "because you have degrees". At the same time the authorities are punishing scientists by abrogating their advanced degrees. Indeed three refusnik scientists told me their degrees had been revoked for "unpatriotic behaviour," that is, exercising their legal right in applying to emigrate.

While the situation of refusnik and dissident scientists has deteriorated alarmingly over the past year, this course is reversible. We can have an impact. Witness the return of Benjamin Levich, Mark Azbel, Valentin Turchin and others to productive scientific life due to the untiring efforts of Western scholars. We cannot afford to become hardened to the plight of oppressed colleagues who have much to contribute to a vital international science.

DOROTHY HIRSCH

Executive Director, Committee of Concerned Scientists, New York, USA

An Irish question

SIR - In your editorial of 25 June 1981 (p.601), you quite rightly in my view criticized the Science and Engineering Research Council (SERC) for turning down the proposal "that there should in future be an exchange of graduate students between the the United Kingdom and the Irish Republic on the modest scale of a dozen or so a year". You may not be aware that the Department of Education (N. Ireland) awards postgraduate studentships to good graduates who have normally been resident in N. Ireland for at least 3 years immediately preceding the start of the proposed period of study. One type of research studentship, the so-called XNI award, is tenable at institutions in Great Britain or the Republic of Ireland or exceptionally, outside the British Isles. This arrangement, which I believe has existed for many years, meets the points you make about student exchange in respect of graduates from N. Ireland. Unfortunately, there is no reciprocal arrangment in the Irish Republic.

I have had several enquiries from final-year honours students reading biochemistry in the Republic who would like to have carried out postgraduate research in this department. The only suggestion I have been able to make is that the prospective research students should apply to Queen's University for a Visiting Studentship of which there are about two per annum for the whole world including Great Britain. Competition is very keen for these studentships and the prospects of success are small. Perhaps you should give the Ministry of Education in the Irish Republic a nudge in addition to the black mark which you awarded to SERC.

D.T. ELMORE

Queen's University, Belfast, UK

University cuts

SiR — Union members have expressed particular concern over two aspects of the leading article "Change wanted" (*Nature* 11 June, p.442).

(1) It seems to accept the principle of cutting back on funding of universities, an extraordinary position for a journal concerned with science and research.

(2) It does not answer the questions and propositions posed by Swinnerton-Dyer but suggests the necessary financial savings can be made by college managements acting in a managerial capacity and shedding staffs from all sections except academic.

With regard to this last point, there is no suggestion as to how teaching laboratories and research groups could maintain their standards or volume of work when numbers of technicians and other ancillary staff, already at a bare minimum in many institutes, are reduced even further.

You cannot fail to be aware that the whole question of Swinnerton-Dyer is currently the subject of intense discussion not only in the university but also between the University of London and the trade unions that represent all sections of staff. We feel that these discussions should continue in a serious way on the basis of the evidence submitted in the discussion documents and that they should not be prejudiced by statements such as yours that the "armies of ancillary staff" should be chopped by the administrative action of the management of the schools.

M. OSMUNDSON C. SCOTT L. WALDOCK

Queen Elizabeth College branches of ASTMS, NALGO and NUPE, London, UK

NEWS AND VIEWS

The location of nucleosomes in chromatin: specific or statistical?

from Roger Kornberg

THE finding that histones are arrayed as nucleosomes along a DNA molecule immediately raised the question of whether the array occurs in a specific relationship, or 'phase', with respect to the DNA sequence1. Were this the case, then regulatory molecules might act in a novel way. For example, a protein responsible for setting a specific phase would determine which DNA sequences are protected by nucleosomes and which sequences are exposed in the regions between them. An alteration in this protein could shift the array of nucleosomes, exposing one set of sequences and covering up another. A single protein could thus control the function of a series of regulatory signals on DNA from a distance.

This intriguing possibility was investigated by comparing the distribution of nucleosomes on rat liver chromatin containing single-copy DNA with the distribution of nucleosomes formed essentially at random by reconstitution of DNA from the same source². The two distributions proved to be indistinguishable: no greater specificity with respect to sequence was apparent in the location of nucleosomes on the rat liver chromatin than in their location on the reconstituted chromatin. This ruled out a specific phase relationship of nucleosomes with rat liver DNA sequences.

An important assumption behind the notion of nucleosome phasing was that the spacing of nucleosomes was constant along a chromatin fibre and an array of nucleosomes thus periodic, akin to a linear crystal. If, however, there were no regular spacing, no long-range order, then a specific phase relationship between nucleosomes and DNA sequences could not persist for any distance along the DNA. This assumption was tested by two separate experiments which established that the spacing of nucleosomes is not constant, but variable within a cell^{2,3}.

Nonetheless, there have been several reports indicating that even if nucleosome spacing is irregular, it is not random; and

some indicating that it is both regular and non-random. Most of these experiments depend on the use of micrococcal nuclease, which cleaves DNA preferentially between nucleosomes, and they fall into three groups according to the extent of digestion. In studies of genes for heat-shock proteins and histones in Drosophila melanogaster4,5, very brief digestion of chromatin was followed by extraction of the DNA, cleavage with a restriction enzyme, gel electrophoresis and Southern hybridization with a restriction fragment produced by the same enzyme. This procedure reveals the length of DNA from the restriction site to the various points of cleavage in chromatin by micrococcal nuclease. Well defined locations of nucleosomes will result in a discrete set of DNA lengths and thus a pattern of bands, whereas random locations are expected to give a continuous distribution. Invariably a series of bands extending for thousands of base pairs was observed. The bands were irregularly spaced and those approximately 130-230 base pairs (bp) apart were attributed to defined locations of nucleosomes, although this attribution is a little problematic because the nucleosome core DNA is generally not less than 146 bp

In experiments with genes for tRNA in chicken embryos6, micrococcal nuclease digestion was more extensive, sufficient to generate a mixture of nucleosome monomers, dimers, trimers, and so on. The mixture was fractionated according to numbers of nucleosomes by sedimentation in a sucrose gradient, and the location of tRNA genes within individual oligomers determined by extraction of the DNA and cleavage with restriction enzymes, as well as by other procedures. Once again, the locations of nucleosomes with respect to DNA sequence were found to be nonrandom, though only for a distance of about five nucleosomes from the tRNA gene after which they became indistinct. In

Roger Kornberg is in the Department of Structural Biology at Stanford University.

this case, the defined locations were regularly spaced, with a periodicity the same as that found by micrococcal nuclease digestion of bulk chicken embryo chromatin.

In a third set of experiments, very extensive digestion of chromatin with micrococcal nuclease was used to degrade all the DNA to 146-bp core fragments, which were then mapped with respect to specific sequences. A unique location of nucleosomes in African green monkey satellite chromatin and a set of alternative locations of nucleosomes on genes for 5S rRNA in Xenopus laevis⁸ were found in this way.

Additional evidence of both random⁹⁻¹⁶ and non-random¹⁷⁻¹⁹ locations of nucleosomes has come from restriction enzyme digestion of chromatin and from other approaches. We are thus left with a serious conflict that needs to be resolved. One view, widely shared²⁰, is that 'phasing' of nucleosomes is a general phenomenon of considerable biological interest, despite any evidence to the contrary. An alternative view is that the two sets of apparently conflicting data may be reconciled, for example by the following simple picture.

Nucleosomes are deposited essentially at random on DNA, subject to two constraints. First, they are not formed on DNA associated with other proteins, for example sequence-specific regulatory molecules such as repressors and activators, but are restricted to the regions of DNA between such molecules. Second, nucleosomes occupy 166 bp of DNA²¹⁻²³ and they may not overlap one another. The average locations of nucleosomes subject to these constraints have been determined by computation24. It is only average properties that are of interest, because all experiments are done on millions of genomes. Remarkably, although nucleosomes are deposited at random, the computations show a distribution with a periodicity and degree of order similar to that revealed by micrococcal nuclease digestion of bulk chromatin. Furthermore, near the

boundary of a region of nucleosomes. specific locations are strongly favoured, with regular spacings identical to the periodicity of bulk chromatin and with probabilities that gradually diminish until there is less than a 10 per cent preference over neighbouring locations at a distance beyond about five nucleosomes from the boundary.

The simple statistical picture can account for the evidence for non-random locations of nucleosomes on chicken tRNA genes, provided that there is a sequencespecific protein or structure in the vicinity of these genes to form a boundary for a region of nucleosomes. The presence of regulatory proteins near the 5' end of the genes would satisfy this requirement. The similarity between the distribution of nucleosomes computed on statistical grounds and that found in the experiments on tRNA genes is striking. Both distributions show a regular spacing of nucleosomes identical to the periodicity of bulk chromatin, and both show defined locations over a range of about five nucleosomes but not far beyond. Whereas these two distributions appear so very alike, both differ markedly from that reported for heat-shock and histone genes in Drosophila, where a pattern of irregularly spaced bands extending for thousands of base pairs was produced by very brief digestion with micrococcal nuclease and interpreted in terms of specific locations of nucleosomes. This interpretation can be questioned on several grounds, the most serious of which concerns the preference of micrococcal nuclease for cleaving certain DNA sequences²⁵. It has recently been shown that striking patterns of bands are produced by digestion of naked DNA and furthermore, that the patterns resulting from digestion of naked DNA and chromatin are the same 16,26-29. This specificity of digestion poses the greatest problem when the period of digestion is very brief. It creates a need for additional evidence to support the assignment of a band pattern to an array of nucleosomes, such as the physical demonstration of nucleosomes by sedimentation in a sucrose

A further difficulty is that the interpretation in terms of an irregular spacing but well defined location of nucleosomes leads to a contradiction. Irregular spacing can only be explained by sequence-specific binding of histones to DNA. This implies that the location of nucleosomes is the same in all tissues of an organism since the DNA sequence remains the same. However, the spacing of nucleosomes has been shown^{30,31} to vary between different tissues, so the locations of the nucleosomes cannot remain constant but must vary as well.

Although the formation of nucleosomes is unlikely to be strongly sequence specific, neither is it likely to be completely independent of sequence, and the

assumption of random deposition of nucleosomes in the statistical picture can only be regarded as an approximation. Other proteins that associate with nucleic acids in a uniform way, such as the coat protein of tobacco mosaic virus, show some preference, perhaps by an order of magnitude or more, for binding to some sequences over others. It would be surprising if a similar preference were not shown by histones. Indeed, there is clear evidence for such sequence preference in the distribution of nucleosomes on polyoma DNA³², and the non-random locations of nucleosomes on satellite DNA and 5S rRNA genes mentioned above may be explained in this way (or by this effect combined with that of proteins bound at specific sites, which will be frequent in these DNA repeat regions).

Perhaps the most striking example of specificity in the location of nucleosomes so far discovered is the specific absence of nucleosomes from a region of about 400 bp encompassing the replication origin and

Kornberg, R.D. Science 184, 868 (1974).

- Prunell, A. & Kornberg, R.D. Cold Spring Harb, Symp. quant. Biol. 42, 103 (1977).
- Lohr, D., Corden, J., Tatchell, K., Kovacic, R.T. & VanHolde, K.E. Proc. natn. Acad. Sci. U.S.A. 74, 79 (1977).

Wu, C. Nature 286, 854 (1980).

Samal, B., Worcel, A., Louis, C. & Schedl, P. Cell 23, 401 (1981).

Wittig, B. & Wittig, S. Cell 18, 1173 (1979).

- Musich, P.R., Brown, F.L. & Maio, J.I. Cold Spring Harb. Symp. quant. Biol. 42, 1147 (1977).
- Gottesfeld, J.M. & Bloomer, L.S. Cell 21, 751 (1980). Cremisi, C., Pignatti, P.F. & Yaniv, M. Biochem. biophys. Res. Commun. 73, 548 (1976).

- Res. Commun. 13, 396 (1976).
 Lipchitz, L. & Asel, R. Cell 9, 355 (1976).
 Birnboim, H.C., Holford, R.M. & Seligy, V.L. Cold Spring Harb. Symp. quant. Biol. 42, 1161 (1977).
 Garel, A. & Axel, R. Cold Spring Harb. Symp. quant. Biol. 42, 701 (1977).
- 13. Gottesfeld, J. & Melton, D. Nature 273, 317 (1978).
- Singer, D.S. J. biol. Chem. 254, 5506 (1979)
- 15. Baer, B.W. & Kornberg, R.D. J. biol. Chem. 254, 9678 (1979)
- 16. Young, D., Humphries, S.E. & Carroll, D. Cell (in the press). 17. Pfeiffer, W. & Zachau, H.G. Nucleic Acids Res. 8,
- 4621 (1980). lgo-Kemenes, T., Omori, A. & Zachau, H.S. Nucleic
- Acids Res. 8, 5377 (1980).

 19. Levy, A. & Noll, M. Nucleic Acids Res. 8, 6059 (1980).
- Zachau, H.G. & Igo-Kemenes, T. Cell 24, 597 (1981).
 Noll, M. & Kornberg, R.D. J. molec. Biol. 109, 393
- (1977).
- 22. Simpson, R.T. Biochemistry 17, 5524 (1978).
- Thoma, F., Koller, T. & Klug, A. J. Cell Biol. 83, 403 (1979).
- Stryer, L. & Kornberg, R.D. (in preparation).
- Von Hippel, P.H. & Felsenfeld, G. Biochemistry 3, 27 (1964)
- 26. Fittler, F. & Zachau, H.G. Nucleic Acids Res. 7, 1 (1979).
- 27. Nedospasov, S.A. & Georgiev, G.P. Biochem. biophys. Res. Commun. 92, 532 (1980).

 28. Horz, W. & Altenburger, W. Nucleic Acids Res. 9,
- 2643 (1981) Lomonossoff, G.P. & Laskey, R.A. 29. Dingwall, D.,
- Nucleic Acids Res. 9, 2659 (1981). 30. Compton, J.L., Bellard, M. & Chambon, P. Proc. natn.
- Acad. Sci. U.S.A. 73, 4382 (1976). Thomas, J.O. & Thompson, R.D. Cell 10, 633 (1977).
- Ponder, B.A.J. & Crawford, L.V. Cell 11, 35 (1977). Varshavsky, A.J., Sundin, O. & Bohn, M. Nucleic Acids
- Res. 5, 3469 (1978). Scott, W.A. & Wigmore, D.J. Cell 15, 1511 (1978). Waldeck, W., Fohring, B., Chowdhury, K., Gruss, P. & Sauer, G. Proc. natn. Acad. Sci. U.S.A. 75, 5964
- (1978) Varshavsky, A.J., Sundin, O. & Bohn, M. Cell 16,
- 453 (1979). Saragosti, S., Moyne, G. & Yaniv, M. Cell 20, 65 (1980).
- Yu, C., Bingham, P.M., Livak, K.J., Homgren, R. & Elgin, S.L.R. Cell 16, 797 (1979).
- Stalder, J. et al. Cell 20, 451 (1980). Keene, M.A., Corces, V., Lower
- S.C.R. Proc. natn. Acad. Sci. U.S.A. 78, 143 (1981).

promoters of the SV40 chromosome. The DNA in this region appears naked, as judged from accessibility to restriction enzymes and electron microscopy³³⁻³⁷. The 'DNase I hypersensitive site' adjacent to the 5' end of many genes undergoing transcription^{4,38-40} may represent a similar region of naked DNA. The exclusion of nucleosomes from these regions is most simply explained by the sorts of constraint on the location of nucleosomes mentioned above. Thus the binding of non-histone proteins to any two sequences less than 166 bp apart will prevent the formation of a nucleosome on the entire region between them. T antigen is known to bind in the vicinity of the replication origin of SV40, and other regulatory proteins are doubtless bound near the 5' end of active genes as well.

In all the cases of non-random locations of nucleosomes discussed here, the key question concerning biological significance is the degree of preference over adjacent locations. All known regulatory proteins bind their cognate DNA sequences with affinity many orders of magnitude higher than unrelated DNA. The processes that would be regulated by mechanisms involving nucleosomes surely require at least as great precision. The degree of specificity that can arise in the statistical picture described above is at most an order of magnitude and so would seem unlikely to have any biological role. It remains to be determined whether any greater precision is achieved in other examples of nonrandom locations of nucleosomes. Unfortunately the methods in use are incapable of giving this information. The experiments are carried out, for the most part, using enzymes that cleave anywhere in the region between nucleosomes, and the data that result are mostly qualitative, in the form of bands in gels, which are visually striking even when intensity of only a factor of two separates them from the background.

The biological significance of specific locations of nucleosomes, if they are found, is apparent, but the advantages of a statistical distribution of nucleosomes. while not as obvious, are also great. A statistical distribution allows the packaging of DNA without regard to the length or the sequence involved. Were there genuine 'phasing' of nucleosomes, the loss or addition of a single base pair could have deleterious effects. A statistical distribution, on the other hand, would accommodate the extraordinary variations of genomes observed in nature.

Thus there is no convincing evidence for ordered arrays of nucleosomes in a fixed relation to the DNA sequence over a distance of many thousands of base pairs. Constraints on a random distribution of nucleosomes resulting from the presence of other proteins at specific DNA sites may impose some short-range order, but the degree of this order is unlikely to be sufficient for biological specificity.

Stripped for action

from Peter D. Chantler

STRIPPING proteins down in order to assess the individual functions of their peptide subunits has long been a preoccupation of biochemists. Hexameric myosin molecules are an ideal choice for this practice and an investigation of the role of the essential light chain in vertebrate skeletal muscle myosin was recently reported in *Nature* by Wagner and Giniger (Vol. 292, p.560).

All muscle myosin molecules contain two heavy chains (molecular weight ~ 2×105) and two pairs of low-molecularweight light chains (molecular weight ~ 2×10^4 each). One of these pairs comprises the DTNB light chains, so called because they were first partially dissociated from rabbit skeletal myosin using DTNB (5,5'-dithiobis [2-nitrobenzoic acid]). However, because their removal (in amounts greater than 1.5 moles per mole myosin) was found to have no effect on the $(Ca^{2+} + K^{+} - EDTA)$ - or actin-activated ATPase activities of myosin, they have also been described as 'non-essential'. In contrast, the other pair of light chains became known as 'essential' because their removal by various treatments always seemed to result in an irreversible loss of these ATPase activities; although as heavy chain denaturation appeared to precede essential light chain removal, the precise role of the essential light chain remained ambiguous.

The problem is further complicated in skeletal muscle by the presence of myosin isozymes. Thus there are two forms of essential light chain, A1 and A2; both exist within a single muscle cell¹ and the presence of myosin homodimers and heterodimers of A1 and A2 light chains has been confirmed²⁻⁴. In addition, at least two forms of heavy chain exist within fast skeletal myosin⁵.

Using the results of earlier workers as a guide, Wagner and Weeds⁶ found that in 4.7 M NH₄Cl, 2 mM EDTA and 2 mM dithiothreitol, pH 7.0, an equilibrium existed between dissociated essential light chain and heavy chain⁶. A 10-min incubation at 4°C did not adversely affect the ATPase activities. Exchange experiments were easily feasible in the presence of excess light chain, using isolated chymotryptic subfragments of myosin, S-1 (A1) and S-1 (A2), containing Al and A2 essential light chains respectively. These two types of myosin 'head' are easily separated on a DEAEcellulose column and lack the non-essential DTNB light chain, which is digested away by the chymotrypsin leaving the essential light chain intact. The results of such exchange experiments, now well known,

Peter D. Chantler is in the Department of Biology, Brandeis University, Massachusetts.

were that the kinetic parameters of the two subfragments appeared to be determined by the type of essential light chain, provided the assays were performed at low ionic strength (6mM)⁶; at higher ionic strength the parameters are virtually indistinguishable for the two isozymes⁷.

To study the separate roles of the heavy chain and essential light chain, Wagner and Giniger have now combined two powerful approaches: the above exchange procedure and the antibody column techniques of Holt and Lowey2, with the additional trick of including nucleotide in all these procedures to stabilize the nude heavychain fragment (so simple that one wonders why nucleotides were not included in dissociation experiments years ago). Thus, S-1 (A2) was incubated for 5 min at 4°C in 4.7 M NH₄C1, 5 mM ATP, 2 mM EDTA, 2 mM dithiothreitol, 0.1 M imidazole, pH 7.0, and then applied to an anti-essential light-chain antibody column equilibrated in the same buffer. The antibody is fully operational in these conditions and removes the dissociated A2 light chain. The subfragment eluting from the column still possesses about 10 per cent of A2 light chains but retains 50-100 per cent of the ATPase activities and 100 per cent of the actin-binding capabilities of S-1(A2) that has been similarly treated but not passed down the antibody column. Contaminating S-1 (A2) can be removed by second passage of the material through the antibody column in the absence of NH4 C1, to give a pure, active heavy-chain fragment. These results have been substantiated by Sivaramakrishnan and Burke (submitted for publication) who have used a different approach to produce light chain-free heavy chains of rabbit S1; the heavy chains retain full ATPase and actin-binding capabilities.

Taken together, these results show that the essential light chain is, in fact, inessential! They suggest that the active site and the actin-binding site are exclusive to the heavy chain in the head region of mvosin. The essential light chain (now a misnomer) can modulate this activity at low ionic strength but the activity is primarily determined by the heavy chain. The combined work of Wagner and Giniger and of Sivaramakrishnan and Burke represents the first clear demonstration of an active myosin heavy chain, free of light chains (notwithstanding the active minimyosin heavy chain from Acanthamoeba8 which is regarded as peculiar among myosins).

The corollary of pulling peptides apart is to reconstitute the original protein by adding together the constituents. Wagner and Giniger have not reported such an experiment, although it is possible and will be reported shortly by Sivaramakrishnan



100 years ago

PROF. Raoul Pictet of Geneva, who has been giving his attention of late to marine architecture, announces, according to the Times correspondent, a discovery which, if his anticipations be realised, will effect a revolution in the art of shipbuilding and greatly augment the speed of sea-going and other ships. The discovery consists in a new method of construction and such an arrangement of the keel as will diminish the resistance of the water to the lowest possible point. Vessels built in the fashion devised by Prof. Pictet, instead of sinking their prows in the water as their speed increases, will rise out of the water the faster they go, in such a way that the only parts exposed to the friction of the water will be the sides of the hull and the neighbourhood of the wheel. In other words, ships thus constructed, instead of pushing their way through the water, will glide over it. According to the professor's calculations, steamers built after his design will attain a speed of 50 to 60 kilometres the hour. A model steamer on the principle he has discovered is being constructed at Geneva From Nature 24, 18 August, 362, 1881.

and Burke. In a sense, however, the thunder of such a reconstitution experiment has been stolen by direct exchange experiments producing hybrid myosins9 using essential light chains of skeletal myosin and heavy chains of cardiac myosin, and vice versa. The results of these hybrid experiments indicate that the ATPase properties of myosin are largely determined by the heavy chain, in agreement with the results above on S-1.

Thus relatively minor roles have been assigned to the essential light chain and the DTNB light chain of skeletal myosin. Furthermore, the role of phosphorylation of the DTNB light chain in skeletal myosin remains an enigma. This is in contrast with the role of the regulatory light chain in scallop myosin (chemically related to the DTNB light chain), also deduced from stripping experiments, of governing

- Weeds, A.G., Hall, R. & Spurway, N.C.S. FEBS Lett. 49, 320 (1975).
- 2. Holt, J.C. & Lowey, S. Biochemistry 16, 4398 (1977).
- d'Albis, A., Pantaloni, C. & Béchet, J.J. Eur J. Biochem. 99, 261 (1979).
 Lower S. Berffeld, P.A. Silbertoin J. & Lower J. M.
- Lowey, S., Benfield, P.A., Silberstein, L. & Lang, L.M. Nature 282, 522 (1980).
- John, H.A. Biochem. biophys. Res. Commun. 92, 1223 (1980).
 Wagner, P.D. & Weeds, A.G. J. molec. Biol. 109,
- Wagner, P.D. & Weeds, A.G. J. molec. Biol. 109, 455 (1977).
 Wagner, P.D., Slater, C.S., Pope, B. & Weeds, A.G.
- Eur. J. Biochem. 99, 385 (1979).
 Maruta, H., Gadasi, H., Collins, J.H. & Korn, E.D. J. biol. Chem. 253, 6297 (1978).
- 9. Wagner, P.D. J. biol. Chem. 256, 2493 (1981).
- Kendrick-Jones, J., Szentkiralyi, E.M. & Szent-Györgyi, A.G. J. molec. Biol. 104, 747 (1976).
- Chantler, P.D. & Szent-Györgyi, A.G. J. molec. Biol. 138, 473 (1980).
- Wallimann, T. & Szent-Gwörgyi, A.G. Biochemistry 20, 1188 (1981).
 Hardwicke, P.M.D., Wallimann, T. & Szent-Györgyi,
- A.G. Biophys. J. 33, 2 (1981).

 14. Margossian, S.S., Lowey, S. & Barshop, B. Nature

actomyosin interactions by determining the structure of the calcium-specific switch situated on the scallop myosin molecule 10,11. There is also increasing evidence for involvement of the essential light chain of scallop myosin in this regulatory mechanism 12,13. In addition, many recent papers point to the importance of regulatory light chain phosphorylation in smooth muscle and non-muscle myosins.

Is it possible then that the DTNB and

essential light chains of vertebrate skeletal myosin represent vestigial peptides — protein subunits whose principal role of regulation has long since been usurped by thin-filament control and the evolution of an architecture devoted entirely to efficient, repetitive contraction? In this context the increased stability conferred on the heavy chain by the presence of the essential light chain and the small modulation of actin binding by DTNB

light-chain phosphorylation¹⁴ may be seen as evolutionary vestiges of a once important regulatory role; the loss of the calcium-specific site could be seen as an example of 'silent' mutations. Whatever the merits of such a depressing (for some) speculation, it is clear that Wagner and Giniger, together with Sivaramakrishnan and Burke, have performed a long awaited and much needed experiment and thereby given us ample food for thought.

A glimpse of new results: the preliminary Solar Maximum Mission data

from Robert Rosner

THE first extensive reports of results from the (partially defunct) Solar Maximum Mission (SMM) satellite have recently appeared in the Astrophysical Journal Letters (244; L133, 1981). Following the precedent set by the Einstein Observatory consortium two years ago, the SMM experimental teams joined in submitting a series of papers which has now been published as a single issue of the Letters. Most teams contributed two papers: the first describing the particular experiment and its in-flight observational capabilities, and summarizing some of the principal preliminary results; the second focusing on a particular event or observation. The SMM observational programme also had a strong ground-based component, which is in evidence both in the accompanying article by Rust et al. 1 and in the individual papers (in which simultaneous radio and other ground-based data figure quite strongly in guiding the interpretations). The result is a useful overview of solar physics research conducted from SMM (as well as of the ancillary ground-based work), concentrating on the solar flare problem, and a good indicator of the quality of the data base generated by SMM. Astronomers wishing to acquaint themselves with the current status of the solar flare problem can do little better than to combine the reading of this volume of Astrophysical Journal Letters with a perusal of the Skylab Solar Flare monograph2. SMM will clearly provide significant observational constraints for flare theorists.

The SMM satellite carried on board seven instruments (see refs 3,4 and refs therein), six of which were spectroscopic, specifically optimized to observe dynamic events in the solar chromosphere and corona; the seventh instrument was a very broad-band (active cavity) radiometer intended primarily for temporal studies of the total solar irradiance. The energy range of the spectrometers spanned from the visible (6,583 Å) to 160 MeV, with the only significant gap in the extreme UV (in which, unfortunately, fall a number of

strong resonance lines from plasma species at transition region temperatures, such as O v_I [1,032 Å]). I will attempt here to describe what I believe are the main features of the results reported by the seven experimental teams.

Coronagraph/polarimeter (C/P). The C/P was a coronagraph capable of highspeed multicolour photometry and polarimetry. Built by the High Altitude Observatory (Boulder; L.L. House, Principal Investigator [PI]), it was specifically designed to observe transient coronal mass ejection with high time resolution and short response time (to flare onset alarms) as close to the solar limb as possible⁵. The instrument had a spatial resolution of 100 arc s and was capable of observations as close as $\sim 0.51 R_0$ from the solar limb; the spectral range covered was 4,435-6,583 Å, with particular emphasis in these reports on the classic coronal green line of Fe xiv (5,303 Å) and the red line of H α (6,563 Å). The preliminary data presented by Wagner et al.6 of the large coronal transient of 7 April 1980 are largely morphological, as the detailed calibration required to retrieve plasma diagnostic information had apparently not been completed. The images presented are, however, spectacular; successive images show rapid motions in both radial and nonradial directions, with peak velocities near 650 km s-1 at the outermost edges of the transient. Simultaneous radio observations made with the Culgoora three-frequency radioheliograph established the presence of a moving type IV source very near the outward-moving loop (surprisingly the 80-MHz source moved differently both in time and space from the 43-MHz source). Analysis of the radio data shows that the magnetic energy density in the transient region exceeded the thermal energy density, a conclusion which is essentially independent of the emission mechanism hypothesized as responsible for the observed radiation; and, if the instrument

Robert Rosner is at the Harvard-Smithsonian Center for Astrophysics.

calibration bears out the estimates for excess mass in the transient made by Wagner et al., one finds that in this event. the kinetic energy density is comparable with the magnetic energy density, and so far exceeds (by at least an order of magnitude) the internal (thermal) energy density. The conclusion - that estimates of total flare energies released based solely on the observed radiative fluxes can be mistaken by up to several orders of magnitude - is a caution well known from previous flare studies7. Furthermore, these observations confirm the conclusions of the Skylab Solar Flare workshop that loop transients must be driven largely by lorentzian forces, rather than by gaspressure gradients8.

Ultraviolet spectrometer and polarimeter (UVSP). The UVSP team (E. Tandberg-Hanssen, PI) comprised members of many collaborating institutions, including the Marshall and Goddard Space Flight Centers, the High Altitude Observatory (Boulder) and the Lockheed Palo Alto Research Laboratory. Spanning the spectral range 1,170-3,600 Å, the telescope/spectrograph laid claim to both the highest spatial $(3 \times 3 \text{ arc s})$ and spectral ($\lambda/\Delta\lambda \sim 150,000$) resolution on SMM; in addition, the instrument could carry out polarimetric observations, using a MgF, waveplate (and thus was, in principle, capable of the first measurements of the four Stokes parameters in the solar UV spectrum below 2,000 Å). One of the most surprising results of SMM in fact derives from the UVSP polarimetry: using observations of circular polarization of the 1,548 Å line of C Iv above a large sunspot, Tandberg-Hanssen et al.9 derive a line-of-sight magnetic field strength of $\sim 1,100\pm300$ G. This remarkably high field strength (considering the height of formation of the C IV line) is based on an assumed Doppler width, and so it is crucial that the measurement be confirmed in further detailed studies. If it were confirmed, standard models for the extrapolation of magnetic fields above sunspots might need

substantial revision. Another observational feature reported by Tandberg-Hanssen et al. is the detection of significant oscillatory mass motions above sunspots. Using the C_{IV} line once again, the UVSP found oscillations with periods of ~ 130 and ~ 170 s over two different spots; the fluctuations were in phase over four adjacent pixels (which were repeatedly rastered) but out of phase by ~ 180 deg in the blue and red sides of the line for any given pixel. It is not certain to what process such oscillatory motions are attributable, although calculations of the wave motions of flux tubes by H. Spruit (Max Planck Institute) and B. Roberts (St Andrews University) and collaborators appear very promising. Studies of the spatial and temporal behaviour of the density in the transition region of solar active regions, as determined from the intensity ratio of the Sizy (1,402.8 Å) and Ozy (1,401.2 Å) lines, show further evidence for a substantial level of dynamical behaviour in 'quiescent' regions. Thus, the UVSP has observed very large (~5-10 times) spatial variations in transition region densities on scales of ~ 4 arcs, as well as temporal variations on time scales of minutes or less. From the point of view of modelling, it is relevant to ask whether the observed fluctuations take place within magnetically 'confined' plasma structures (for example, whether they occur at the re-entrant footpoints of hot coronal 'loops'). If so, then these data suggest that the transition regions of coronal 'loops' are far more complex in their behaviour than allowed for in standard models for quiescent loop structures. Turning to yet more dynamic phenomena, Woodgate et al. 10 discuss thoroughly the morphological aspects of the limb flares of 30 April 1980, as seen in the C_{IV} line discussed above; several of the other teams have in fact found it useful to take advantage of the high spatial resolution imaging capability of the UVSP to place their (lower-resolution) observations in the proper geometric 'context'. The main conclusion of Woodgate et al. seems to be that, although the pre-flare state resembles the scenario of models for flare initiation of, for example, Canfield et al. 11 and Heyvaerts et al. 12 — a small loop of emerging flux rising into an overlying complex of magnetic loops — the detailed evolution of loop brightening and plasma motions does not. This conclusion must be tempered by the realization that it may not be reasonable to confront a theory which is designed to explain the 'primary' flare features with morphological details it may be, in principle, incapable of accounting for (see ref.2 for a particularly lucid account of this difficulty in flare research).

Soft X-ray polychromator (XRP). The XRP actually consisted of two instruments, the flat crystal spectrometer (FCS), with spatial resolution of 12×12 arc s and spectral resolution of $\sim 1,000$ at 20 Å, and the bent crystal spectrometer

(BCS), with a substantially lower spatial resolution of 6×6 arc min, but far higher spectral resolution ($\lambda/\Delta\lambda \sim 25.000$). The experimental team reflects a collaborative effort between Lockheed Palo Alto (L.W. Acton, PI), Mullard Space Sciences Laboratory (J.L. Culhane, PI) and the Rutherford and Appleton Laboratories (A.H. Gabriel, PI). One of the most interesting results reported of the SMM mission is the XRP observations of strong line broadening in both quiescent active regions and flaring regions. For example, Acton et al. 13 discuss the observations of quiescent active regions with the FCS; the spectra show substantial broadening beyond the thermal line width (which is estimated on the basis of electron temperatures inferred from the measured Ne IX/Mg XI line flux ratio). Assuming an isothermal model. Acton collaborators derive r.m.s. speeds of ~100±28 km s⁻¹ and argue convincingly that the excess broadening is probably due to random motions on spatial scales far smaller than the 'loop' structures themselves. How these observations relate to specific proposed coronal heating mechanisms is not yet clear because virtually all the current contenders predict some level of flow 'turbulence'. Turning to higher spectral resolution, a nice example of the power of spectroscopy is provided by the analysis of BCS flare data by Culhane et al. 14. These authors show in a succinct and convincing way that: (1) electron temperatures via the satellite-to-resonance line flux ratio for Ca $x_i x k$ and Fe $x_i x y j$ lines can be obtained, and the Fe lines are formed in different regions than the Ca lines (suggested by differences in derived temperatures, temperature evolution and intensity light curves; see also previous observations of the Caxix and Fexxvlines by Doschek et al. 15 and Feldman et al. 16); (2) line widths are strongly variable during the course of a flare, starting with rather broad profiles and narrowing significantly after the hard X-ray impulsive event; the broadening may be associated with turbulent flow speeds of $\sim 60-100 \text{ km s}^{-1}$; (3) fluorescence, rather than ionization by fast electrons, is the most plausible excitation mechanism for Fe Ka emission in at least some cases. In one case, however, the excellent temporal coincidence between a hard X-ray burst and an early Fe Ka 'spike' suggests (following calculations) that the energetic electrons responsible for the hard X-ray burst also excite the Ka transitions (in fact, calculation of the expected Ka flux from fluorescence yields far too little emission in this case).

Hard X-ray imaging spectrometer (HXIS). The HXIS was an imaging spectrometer with a field of view of 2 min $40 \text{ s} \times 2 \text{ min } 40 \text{ s}$ of arc at a resolution of $8 \times 8 \text{ arc s}$, and a field of view of 6 min 24 s $\times 6 \text{ min } 24 \text{ s}$ of arc at a resolution of $32 \times 32 \text{ arc s}$. It had some energy resolution covering the spectral range 3.5-30 keV in six channels and variable time resolution in

the range 0.5-7.0 s. The HXIS team (C. de Jager, PI) represents a collaboration between the Astronomical Institute at Utrecht and the University of Birmingham (UK). Its two contributions focused on providing a complete evolutionary description of the morphology and plasma characteristics of three flares observed in April 1980 (7 and 10 April: Hoyng et al. 17; 30 April: van Beek et al. 18). Although the gross morphological characteristics of flares seen in X rays were well established by the Skylab observations, these HXIS data illustrate the value of extending timeresolved imaging to higher photon energies. The flare observations in early April showed emission regions characterized by a hard spectrum to be spatially associated with, and to overlie, the brightest Ha emission patches, and furthermore that associated, softer emission components tended to lie between the harder components. If the emission source geometry is assumed to be that of a coronal 'loop', then a natural and immediate interpretation of these data is that the hard X rays are emitted at the loop footpoints (where the $H\alpha$ emission would be expected to take place), whereas the softer X rays are emitted at the top portion of a loop. If one views the footpoint excitation as a result of particle (electron) beams impinging on the dense footpoint boundary, then the 'thick target' model for the hard X-ray emission is clearly favoured. Further support for 'streaming' of fast electrons comes from the late-April flare data. Van Beek et al. show that the onset of flare emission could be associated with a very compact source and that the subsequent development of an extended 'tongue'-like component, whose temperature is higher than that of the compact component, is then consistent with production of energetic electrons within the compact component and subsequent streaming or diffusion into the larger (but magnetically confined) volume. In particular, this sequence of events is consistent with the time scale for the development of the extended source and the lack of clear evidence for large-scale mass motions within this source, and accounts well for the simultaneous appearance of an Ha 'tongue' spatially coincident with the extended X-ray tongue. Note that the above argument depends crucially on the assumed source geometry and that at X-ray wavelengths, SMM observers invoke the Skylab observations to justify their choice of loop geometry.

Hard X-ray burst spectrometer (HXRBS). The HXRBS (built by the Goddard Space Flight Center; K.J. Frost, PI) was not an imaging instrument and so provided no spatial resolution (the field of view encompassed the entire Sun). However, the lack of spatial resolution was compensated by high sensitivity and extremely high (1 ms) temporal resolution (because the probability of more than one flare occurring is low and because the

quiescent corona contributes a negligible fraction of the observed emission, source confusion for the HXRBS is of little concern); the spectrometer covered the ~25-400 keV energy range in 15 channels. The HXRBS team chose to discuss observations of three flares (29 March, 30 April and 7 June 1980) for which the timeresolved data allowed access to a previously inaccessible observational domain (Dennis et al. 19; Orwig et al. 20). The combination of large effective area with high potential time resolution allowed this instrument temporally to resolve several hard X-ray bursts into subfeatures: a combination of short (~1 s), quasi-periodic 'sub'-bursts, separated by 1-2s and characterized by a fairly hard spectrum, and a much softer, slowly varying component. In addition, HXRBS detected persistent intensity fluctuations significant down to the 100 ms level and spectral variability down to a time scale of seconds. By taking advantage of the capability for simultaneous observations with the other SMM (as well as ground-based) instruments. Orwig et al. were also able to compare the details of their light curves of the 30 April flare with those obtained with the UVSP, HXIS and BCS. The onset of Ca xix resonance line broadening discussed above seems to be well correlated in time with the onset of the impulsive burst and essentially all the flux observed by the HXRBS probably derives from a small loop (seen by the UVSP) coincident with one of HXIS's 8×8 arc s pixels.

y-Ray spectrometer (GRS). The short wavelength limit of solar radiative emission was explored by the GRS, an instrument whose experimental team (E.L. Chupp, PI) reflects a collaboration between the University of New Hampshire, the Max Planck Institute for Physics and Astrophysics (Munich) and the Naval Research Laboratory (Washington, DC). At these energies, the Sun emits both a continuum and a number of y-ray lines; the latter are thought to arise from the interaction of fast ions with the denser portions of the solar outer atmosphere. y-Ray emission is thus a direct diagnostic for the presence of, for example, accelerated ions and examination of the spectral and temporal behaviour of the emission provides a clue to the characteristic of the incident fast particles (compare with ref. 21, p.117). The GRS explored the energy range ~ 0.3-9 MeV with an energy resolution of ~7 per cent (FWHM at 662 keV) and a time resolution of ~ 16 s (or, using the 'burst window' 64 ms time resolution in the very limited singlechannel energy range 330±25 keV). The two contributions of the GRS team (Chupp et al. 22; Ryan et al. 23) focused on analysis of continuum and line emission from two flares; only in one were both the continuum and lines detected. Of particular interest are the data for the flare of 7 June 1980, for which the first detailed observations of the full light curve of the 2.223 MeV line from

the reaction $n+p\rightarrow^2H+y$ were obtained. The power of nuclear physics to 'recreate' the sequence of events which must have led to the emission of this y-ray line (as well as the continuum) is nicely laid out: not only must both electrons and nucleons have been accelerated, but the time constraint on neutron production (imposed by the requirement that the fast neutrons be thermalized before ¹H capture and the 2.223 MeV photon emission occur) is such that the fast ions responsible for their production must have been accelerated in virtual time coincidence with the fast electrons. This result imposes a non-trivial constraint on particle acceleration mechanisms anticipated (as an SMM result) at the time of the Skylab Solar Flare workshop (see ref. 21, p.162).

Active cavity radiometer irradiance monitor (ACRIM). ACRIM was the only SMM instrument not specifically designed for the study of flare transients. The culmination of an evolutionary sequence of successively more refined radiometers built by R.C. Willson (Jet Propulsion Laboratory), ACRIM was the first instrument put into orbit which had some assurance of resolving solar irradiance fluctuations to better than 0.1 per cent, and thus promised to aid in resolving the raging debate on the constancy of the solar irradiance. This promise has evidently been fulfilled. Two distinct questions arise: first, is there any evidence for variation in the solar constant (such as integrating the solar bolometric flux at some fixed radius over 4π steradians)? Second, is there any evidence for variability in the solar irradiance (such as in the total flux as seen from ~1 AU)? The second question (which is the only one a single spacecraft can sensibly answer) is intimately connected with a classic problem of solar physics: where does the radiative energy 'missing' from sunspots go? Several alternative solutions have been proposed, all constrained by the long known fact that there is (to some photometric precision) no evidence for a bright ring surrounding sunspots (compare with refs 24-26 and refs therein). These alternatives include redistribution of the missing energy over a sufficiently large portion of the solar surface, redistribution in spectral range (to the far UV or X rays), redistribution in transmission mode (conversion of radiative flux to magnetohydrodynamic waves), redistribution in time (storage in the convection zone, followed by gradual release over a substantial portion of the surface) and, most recently, spatial and angular redistribution (by means of the solar faculae 27). The first observational problem is of course to detect the effect of the passage of a sunspot across the solar disk on the total solar irradiance. This goal has been admirably met by ACRIM: Willson and Hudson28 demonstrate fluctuations of the total solar irradiance of the 0.05 per cent level; but a correlation with specific solar surface activity could not be established in this preliminary analysis. The vagaries of publication are such that even before the appearance of the 'SMM' Astrophysical Journal Letters, Willson and his collaborators published29 a more extensive analysis of their observations in Science; thus it is already well known that the 'dips' in total irradiance seen by ACRIM correlate well with the passage of sunspots across the solar disk (confirming previous Nimbus data suggesting, but not establishing, a similar correlation). The next task will be to identify definitively the source of the 'peaks' in total irradiance; it should then be possible to answer the 'missing flux' problem of sunspots.

The preliminary results discussed above suggest that, as the SMM data are more fully analysed and their implications for theoretical work better appreciated, SMM will contribute significantly to solar research, particularly in the areas of spatially and/or temporally resolved spectroscopy, imaging at hard X-ray photon energies and solar irradiance variability. It is to be expected that the interpretation of SMM data, which understandably received relatively less weight in these presentations of preliminary results, will play a far greater part in the next round of SMM presentations. It is noteworthy that the new Japanese satellite, ASTRO-A, is observing solar atmospheric dynamics with instruments very roughly comparable with the BCS and HXRBS. Given the premature failure of SMM's pointing capability, it is fortunate that it may, after all, be possible to follow up some of the discoveries revealed by the studies of the SMM data set.

Rust, D.M. et al. Astrophys. J. Lett. 244, L179 (1981).

Sturrock, P.A. (ed.) Solar Flares: A Monograph from Skylab Solar Workshop II (Colorado Associated University Press, Boulder, 1980). Chipman, E.G. Astrophys, J. Lett. 244, L113 (1981)

Bohlin, J.D. et al. Solar Phys. 65, 5 (1980). House, L.L. et al. Astrophys. Lett. 244, L117 (1981)

Wagner, W.J. et al. Astrophys. J. Lett. 244, 1 123 (1981). Dulk, G.A. et al. Solar Phys. 49, 369 (1976).

Rust, D.M. et al. in Solar Flares: A Monograph from Skylab Solar Workshop II (ed. Sturrock, P.A.) 273 (Colorado Associated University Press, Boulder, 1980). 9. Tandberg-Hanssen, E. et al. Astrophys. J. Lett. 244, 1.127 (1981).

Woodgate, B.E. et al. Astrophys. J. Lett. 244, 1.133 (1981). 11. Canfield, R.C., Priest, E.R. & Rust, D.M. in Flare

related Magnetic Field Dynamics (eds Nakakawa, Y. & Rust, D.M.) (NCAR, Boulder, 1974)

Heyvaerts, J., Priest, E.R. & Rust, D.M. Astrophys. J. 216, 123 (1977).

Acton, L.W. et al. Astrophys J. Lett. 244, L137 (1981). Culhane, J.L. et al. Astrophys. J. Lett. 244, 1.141 (1981) Doschek, G.A. et al. Astrophys. J. Lett. 233, L167 (1979).

Feldman, U. et al. Astrophys. J. 241, 1175 (1980) Hoyng, P. et al. Astrophys. J. Lett. 244, 1.153 (1981)

van Beek, H.F. et al. Astrophys. J. Lett. 244, L157 (1981). Dennis, B.R. et al. Astrophys. J. Lett. 244, 1.167 (1981).

Orwig, L.E. et al. Astrophys J. Lett. 244, L163 (1981).

Ramaty, R. et al. in Solar Flares: A Monograph from Skylab Solar Workshop II (ed. Sturrock, P.A.) (Colorado Associated University Press, Boulder, 1980).

Chupp, E.L. et al. Astrophys. J. Lett. 244, L171 (1981).

Ryan, J.M. et al. Astrophys. J. Lett. 244, L175 (1981). Parker, E.N. Solar Phys. 40, 275 (1975)

Cowling, T.G. Mon. Not. R. astr. Soc. 177, 409 (1976) Spruit, H.C. Solar Phys. 55, 3 (1977).

Oster, L.F. et al. Preprint (1981). Willson, R.C. & Hudson, H.S. Astrophys. J. Lett. 244, 1.185 (1981).

^{29.} Willson, R.C. et al. Science 211, 700 (1981).

ARTICLES

Jupiter tail phenomena upstream from Saturn

F. L. Scarf*, W. S. Kurth†, D. A. Gurnett†, H. S. Bridge‡ & J. D. Sullivan‡

* Space Sciences Department, TRW Defense & Space Systems Group, Redondo Beach, California 90278, USA
† Department of Physics and Astronomy, The University of Iowa, Iowa City, Iowa 52242, USA
‡ Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Voyager 2 plasma wave and plasma probe measurements from February 1981 suggest that phenomena associated with a well defined tail of Jupiter have been detected at a distance of about 6,200 $R_{\rm J}$. This indicates that Saturn's magnetosphere will be affected by the jovian tail and that by comparing Voyager 1 and 2 observations information on the physics of Saturn's magnetosphere can be obtained.

BEFORE Voyager arrived at Jupiter, the possibility of encounters with Jupiter's extended tail during the subsequent Jupiter-to-Saturn phase was noted¹. It was shown that in the spring and summer of 1981, Voyager 2 and Saturn would both cross the expected region of tail or wake about $7,000-8,000\ R_{\rm J}$ downstream from Jupiter and that this tail might cause a change in characteristics of Saturn's magnetosphere². In August 1980, Voyager investigators found evidence that Jupiter's tail extends out to at least $700\ R_{\rm J}$ (ref. 3). Here, we report Voyager 2 plasma wave measurements from February 1981 which are evidence for a well-defined distant tail of Jupiter at least 3 AU downstream, indicating that Saturn's magnetosphere will be affected by the jovian tail^{1,2,4}.

Tail encounter at $R = 6,200 R_1$

Jovian continuum radiation was detected by the Voyager 1 plasma wave instrument as soon as the spacecraft entered the dayside magnetosphere5, but the most intense signals were observed when Voyagers 1 and 2 were both traversing the nightside tail lobes⁶⁻⁸. Indeed, we regard the detection of this continuum radiation from the Voyager 16-channel spectrum analysers as the single plasma wave measurement characteristic most clearly associated with a spacecraft location within Jupiter's magnetosphere. Therefore, when Voyager 1 and 2 detected roughly similar plasma wave emissions after leaving the nominal close-in magnetosphere, simultaneous measurements from the on-board plasma probes were used to identify whether the spacecraft had re-entered Jupiter's magnetosphere. One Voyager 1 event (with $R = 163 R_{\rm J}$, $\phi = 245^{\circ}$) and six Voyager 2 events (with $184 R_J \le R \le 706 R_J$, and $\phi = 224-225^\circ$) were recognized as true encounters with the extended tail (here, ϕ is the azimuth angle in a right-handed Jupiter-centred system. with $\phi = 0$ oriented towards the Sun). The other 19 events, detected when the Voyagers were still in the solar wind, were interpreted in terms of leakage from an extended tail into a low-density solar wind trough, which would act as a wave guide, allowing signals from the distant tail to reach the spacecraft.

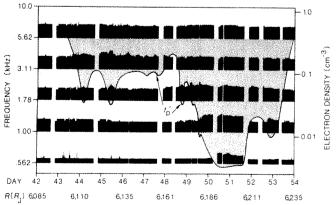
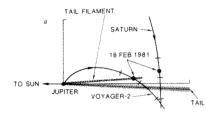


Fig. 1 Time profiles of average electric field strengths detected in five spectrum-analyser channels of the Voyager 2 plasma wave instrument. The scale on the right-hand side refers to the plasma density that gives an equivalent electron plasma frequency $(f_p^- = 9.000 \sqrt{N_e})$.



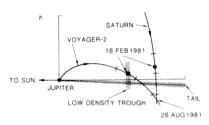


Fig. 2 Paths of Voyager 2 and Saturn with respect to the hypothetical extended tail of Jupiter (a Jupiter-centred Sun-oriented coordinate system is utilized). a, b, Different models that could account for the 18 February 1981 detection of continuum radiation from Jupiter's magnetosphere.

As Voyager 2 continued towards Saturn, the ϕ -value reached a maximum in summer 1980, and after that, the spacecraft moved progressively closer to $\phi = 175-180^{\circ}$, where the extended tail of Jupiter might again be encountered. One of the first very clear events indicative of an actual distant tail traversal occurred in mid-February 1981, and Fig. 1 shows the corresponding wave measurements for the interval 11-22 February 1981; the time profiles of the average electric field strengths detected in 5 of the 16 spectrum analyser channels are displayed. For each channel, the height of the black area is proportional to the logarithm of the electric field strength averaged over a 12.8-min interval, and the distance between the baselines for adjacent channels represents three orders of magnitude in amplitude. The scale on the right-hand side refers to the density that gives an equivalent electron plasma frequency $(f_p^- = 9,000)$ $\sqrt{N_e}$, where N_e is electrons cm⁻³ and f_p^- is in Hz).

Figure 1 shows that tracking was almost continuous during this 12-day interval and that there were several small but abrupt level changes from one relatively steady amplitude to another. The upper eight channels (1-56 kHz) are mildly affected by a failure in the flight data system^{3,8}, leading to small repetitive or quasi-periodic sequences of changes in background (see, for example, the 1-kHz data for days 43-45). Figure 1 also shows natural (or irregular) changes in levels for the channels from 562 Hz to 5.62 kHz, and the line f_p traces the lower envelope of these variations. We interpret this as detection of continuum radiation from Jupiter's extended tail, and we need to determine whether Voyager 2 was within the tail or connected to it by a solar wind density trough.

These two possibilities are shown in Fig. 2, which uses a Jupiter-centred Sun-oriented coordinate system to show the paths of Voyager 2 and Saturn with respect to the hypothetical

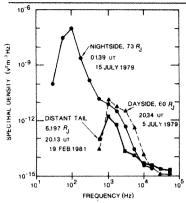


Fig. 3 Spectral densities of continuum radiation detected on Voyager 2 in 1979 (near Jupiter) and 1981 at 6,200 R. Lowfrequency cutoff varies as the local plasma density changes, but the striking similarities in the spectra at high frequencies suggest Voyager 2 was back in Jupiter's tail early in 1981.

extended tail of Jupiter. Bars on the Voyager and Saturn curves represent the positions on 1 December 1980, 1 April 1981 and 1 August 1981 (more trajectory information is given in ref. 1), and the locations for 18 February 1981 are marked with dots. In mid-February, Voyager 2 was still well above the nominal $\phi \simeq 177.5^{\circ}$ position for an aberrated anti-solar tail configuration, but had the tail fragmented as indicated in Fig. 2a, it is quite likely that Voyager 2 would have been within a tail filament at this time and could have been 'connected' to the tail by a low-density trough as indicated in Fig. 2b.

Voyager 2 plasma probe measurements suggest both phenomena were operative. For the early interval, days 43-48, the spacecraft was apparently immersed in streaming plasma which could have been low-density solar wind, or even a wake region around a tail filament; the same applies for the end of the interval (days 52, 53). However, for the period centred around 19 February (day 50) when the $f_{\rm p}^-$ -profile of Fig. 1 was drawn between f = 300 and 500 Hz, the Voyager 2 plasma probe detected no streaming ions, and we propose that at this time the spacecraft was back in Jupiter's tail. Besides the filament hypothesis it is possible that a solar wind disturbance displaced the main tail3.

During the 3-day period which includes this apparent crossing of the distant tail, Fig. 1 indicates that $f_{\rm p}$ (min) was ~400 Hz, leading to $N_e(\min) \approx 2 \times 10^{-3}$ electrons cm⁻³. This N-value yields a current higher than the plasma probe threshold for ion measurements in the streaming solar wind, but if the flux is lowered because the flow speed is also much reduced, then no plasma will be measured. Thus, the absence of detectable plasma during this period, when N_e was at a minimum is consistent with an actual entrance into the tail cavity.

A final test of the tail-crossing hypothesis involves searching the plasma wave noise spectrum for the characteristics associated with continuum radiation. Figure 3 contrasts characteristic close-in continuum radiation spectra for 5 and 15 July 1979, with a distant tail spectrum from 19 February 1981; for $f \ge 1$ kHz all three spectra have very similar shapes, but at low frequencies there are marked differences. The complete 1979 Jupiter measurements indicate that the continuum radiation has an essentially constant spectral shape throughout the magnetosphere⁶⁻⁸, and that the observed changes are primarily associated with variations in local plasma density, leading to shifts in the low-frequency cutoff. Thus, we associate the 5 July 1979 cutoff at $f \approx 1$ kHz with a local electron density of about $0.01 \,\mathrm{cm}^{-3}$, and the $f \approx 50 \,\mathrm{Hz}$ cutoff on 15 July 1979 with an extremely low local density value near 3×10^{-5} electrons cm⁻³ Because for $f \ge 1.0$ kHz, the continuum noise spectrum detected at $6,200 R_1$ has essentially the same shape as the spectrum measured on 15 July 1979, when Voyager 2 was at 73 $R_{\rm J}$ on its way to the initial exit from the magnetosphere, this strongly supports the concept that Voyager 2 was again in the magnetosphere. Indeed, Fig. 3 shows that the high-frequency tail spectra from 15 July 1979 and 19 February 1981 differ no more from each other than the two close-in noise spectra. These comparisons suggest that Voyager 2 was within Jupiter's tail on 19 February 1981, and that the tail confines the noise extremely well, or that the continuum radiation is generated within the distant tail.

The demonstration³ that many of the earlier tail-associated events involved leakage of continuum radiation into low-density solar wind troughs argues against an explanation based on noise confinement over a distance of $6,200 R_1 \approx 3 \text{ AU}$; thus mechanisms that produce continuum radiation far from Jupiter seem more promising. One such process involves production of electromagnetic waves by mode conversion from electrostatic upper hybrid resonance emissions. Observations related to the terrestrial plasmapause, the Io torus boundary, and the perturbations of Saturn's plasma disk at the positions of Tethys, Dione and Rhea¹⁰, suggest that strong mode coupling and intense electromagnetic radiation generally originate in regions with steep plasma density and temperature gradients.

That the magnetopause boundary also has steep gradients in plasma density and temperature suggests that continuum radiation could be generated all along the boundary. The initial Voyager 2 report⁶ clearly showed that intense upper hybrid resonance emissions and weaker continuum radiation were detected immediately after the spacecraft crossed the dayside magnetopause at $71.5 R_{\rm J}$ on 5 July 1979. Thus, we speculate that trapped continuum radiation at Jupiter is generated in the region of the magnetopause, so that its appearance at $6,200 R_J$ does not require strict confinement of the waves. This explanation can also readily account for the similarity in the noise spectra, as Barbosa¹¹ has proposed a wave-scattering mechanism with the spectrum of the continuum radiation controlled by fluctuations of the magnetopause surface.

Discussion

The filament picture in Fig. 2a is consistent with many aspects of the 1981 Voyager 2 measurements but it is useful to consider other sources of information. We can learn much about the possible configurations of extended magnetic tails in the solar wind by studying the structures of comet tails, as far downstream from any interplanetary object the only significant characteristic of the source region involves the close-in size of the obstacle. For visible comets in the inner Solar System, there is no firm knowledge of the true obstacle size, but the theoretical model of Biermann et al. 22 gives a contact surface or obstacle radius, $R_{\rm B}$, of $\sim 10^5$ km. As the subsolar Jupiter magnetopause is nominally located at $R_B \approx 56 R_J$, this means that the 18 February 1981 measurements were taken at $R \approx 110 R_B$. For the comet case, 110 R_B translates to a downstream distance of about 1.1× 10⁷ km, and many photographs of the visible ion tails of fresh comets show clear evidence of filaments extending to this distance or beyond (for instance, comet Kohoutek, Fig. 1 in ref. 13). This fact suggests that filaments may develop naturally in extended magnetic tails and that Voyager 2 will continue to encounter them.

If the extended tail of Jupiter has this type of structure, then Saturn itself is likely to have many brief encounters with the jovian tail cavity leading to significant differences between the Voyager 1 and 2 Saturn encounters. It will be of great interest to search Voyager 2 data for such effects, including variations in magnetosphere size, bow shock location, radio emission strength and trapped radiation belt population.

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- Scarf, F. L. J. geophys. Res. 84, 4422 (1979).
- Wolfe, J. H. et al. Science 207, 403 (1980).

- Wolfe, J. H. et al. Science 207, 403 (1980).
 Kurth, W. S. et al. J. geophys. Res. 86 (in the press).
 Grzedzielski, S., Macek, W. & Oberc, P. Nature 292, 615 (1980).
 Scarf, F. L., Gurnett, D. A. & Kurth, W. S. Science 204, 994 (1979).
 Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 206, 987 (1979).
 Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Geophys. Res. Lett. 7, 53 (1980).
 Scarf, F. L., Gurnett, D. A. & Kurth, W. S. J. geophys. Res. 86 (in the press, 1981).
 Siscoe, G. L. et al. J. geophys. Res. 75, 5319 (1970).

- Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Nature (20 August, 1981). Barbosa, D. D. Astrophys. J. 243, 1076 (1981).
- Biernan, L., Brosowski, B. & Schmidt, H. V. Solar Phys. 1, 254 (1976).
 Niedner, M. B. Jr, Rothe, E. D. & Brandt, J. C. Astrophys. J. 221, 1014 (1978).

Stratospheric aerosols properties from Earth limb photography

M. Ackerman, C. Lippens & C. Muller

Belgian Institute for Space Aeronomy, Circular Avenue, 3, B-1180-Brussels, Belgium

Balloon-borne observation at three wavelengths of visible sunlight scattered by the Earth limb allows the determination of aerosols abundances and size distributions at various altitudes in the stratosphere. The stratospheric aerosols are apparently still under the influence of the Mount St Helens volcanic eruption five months after its occurrence on 18 May 1980.

IT is only relatively recently that detailed studies of the stratospheric aerosols have been initiated. They are of importance because nucleating and catalytic agents are central in determining the radiation balance of the atmosphere. A survey of light scattering techniques used in the remote monitoring of atmospheric aerosols has been published recently. Twilight phenomena have been used both from the ground^{2,3} and from satellites, and Earth-limb observation from space has been based on sunlight scattering observed directly^{4,5} or through its absorption⁶. In situ sampling initiated 20 years ago⁷ has also provided much information on the composition of aerosols⁸.

We have previously reported the photographic observation, from a balloon gondola floating in the upper stratosphere, of the enhancement of stratospheric aerosols over Europe 23 days after the Mount St Helens volcanic eruption. Several other reports have now appeared 10-13 while details have been provided on the air trajectories which have caused the volcanic plume to move at various altitudes in various directions¹⁴ at the beginning of their many cumbersome revolutions around the Earth. Much information has been collected on the properties of the ejecta in relation to atmospheric effects¹⁵. On 15 October 1980, five months after the eruption, another photographic balloon flight took place. One of the first observations was finding that the enhancement of stratospheric aerosols below 20 km altitude has become very horizontally homogeneous. This means that it is now possible to determine the basic properties of volcanically influenced aerosols and of natural aerosols which seem to differ in several respects in the stratosphere.

Observation method

Photographic cameras onboard a balloon gondola simultaneously record at various wavelengths the light from the Earth limb below the horizontal line-of-sight while the solar elevation is low. This light is scattered direct sunlight with a contribution from the Earth albedo which is minimized when the Sun is low. The observation geometry is shown in Fig. 1. The limb radiance, R, can be expressed directly in solar radiance units because solar images are recorded simultaneously with a known attenuation factor. The solar elevation is calculated from the time that the picture was taken and from the geographical position. An angular scale is constructed from several consecutive shots. The depression angles at which radiances are measured are related to the line-of-sight altitude of closest approach to the Earth surface taking into account refraction effects¹⁶. The measured integrated radiances can be inverted taking into account absorption by ozone and air along the line of sight by the 'onion pealing' method to yield the in situ radiance, R^* , per unit length versus altitude.

The Sun-oriented gondola can be rotated about its vertical axis so that pictures can be taken at various azimuth angles, A, relative to the Sun's position. The scattering angle θ of direct

solar radiation can be computed from the relationship

$$\cos \theta = -\sin D \sin h_{\odot} + \cos D \cos h_{\odot} \cos A \tag{1}$$

where D is the depression angle at which the atmospheric radiance is measured and h_{\odot} is the solar elevation angle at the time of measurement. As expected from their strong forward scattering properties, aerosols re-emit little light at an azimuth angle 180° away from the direction of the Sun. The limb radiance observed in this case is used to subtract Rayleigh scattering and to isolate aerosols scattering at all azimuths taking into account the Rayleigh phase function 17 .

If direct solar radiation only is considered, an *in situ* monodisperse aerosol radiance R_a^* per cm of pathlength is related to the average solar radiance R_{\odot} by

$$R_a^* = R_{\odot} \pi \alpha^2 Q_s \sigma \varphi_{\theta} n / 4\pi \tag{2}$$

where α is the angular radius of the Sun's disk, 2.16×10^{-5} rad, φ the properly normalized particulate phase function, Q_s the scattering efficiency factor, σ the particulate scattering geometrical cross-section in cm² if the number density, n, of the particles is expressed in cm⁻³.

In practice, our data confirm the previous observation¹⁸ according to which the phase function can, within experimental uncertainties, be represented by the Henyey-Greenstein function. The asymmetry factor g of the phase function and the total scattering efficiency can then be determined. The variation

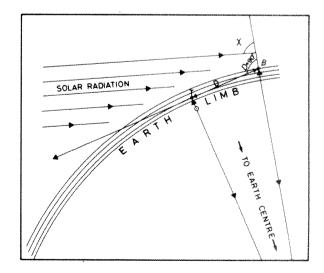


Fig. 1 Observational geometry: quasi parallel light falls on the atmosphere at a solar zenith angle χ , the atmosphere is seen at various depression angles D; through the rotation of the gondola, photographs are taken at various azimuth angles A from the Sun direction.

of these parameters with wavelength can be fitted to their theoretical variations using the effective size parameter of Hansen and Travis¹⁹. This procedure, which uses the Mie scattering computation programme of Wiscombe²⁰, leads to an evaluation of the effective particle size and provides information on other properties. From these two quantities and from the product $n\sigma$ obtained directly from equation (2) the value of n can be deduced.

The aerosol particle size is usually treated in terms of the wavelength, λ , of the interacting light through the Mie size parameter

$$x = 2\pi a/\lambda \tag{3}$$

where a is the radius of the spherical particle in a monodisperse aerosol. In a polydisperse aerosol the number of particles of radius r is expressed 19 by

$$n_r = Ar^{(1-3b)/b} e^{-r/ab} (4$$

where a becomes the effective radius while b is a measure of the width of the size distribution; A is a constant related to the total number of particles.

Comparing R_a^* with θ , the scattering angle, at various wavelengths provides information about the asymmetry factor, g. A knowledge of g and its variation with the wavelength of the interacting light leads to the unique determination of a and b. How the scattering efficiency Q_s varies with size parameter can then be determined. The behaviour of these quantities is known from the Mie scattering theory.

Experimental details

The balloon flight took place on 15 October 1980, the tropopause height being 11.4 km. The gondola floated at 37.6 km altitude and was equipped with seven Hasselblad EL 500 cameras with 80-mm focal length lenses and 70-mm films. Four of them were pointing forwards, one being loaded with colour EPR 475 Kodak film, and three in the opposite direction. The

six black and white cameras contained (each camera was paired with the one opposite): Wratten filters 47 (440 nm) and 25 (650 nm) with Plus X Kodak film and filters 87 with Kodak aerographic 2424 film (860 nm). To ensure that the Sun images were in the dynamic range of the film, neutral density screens made of exposed and processed sheet films were placed 60 cm from the lenses with their lower edge placed a few centimetres above the optical axis. Before flight, step wedges, inconel filters and samples of the neutral density screens illuminated in parallel light were recorded by each camera in the laboratory. After the flight, the Plus X films were processed using the Kodak D76 developer ($\gamma = 1$) and the IR-sensitive film was processed in the Kodak D19 developer ($\gamma = 2.3$). The dynamic range of this last film was too small to record usefully simultaneous Sun and limb images so that its absolute calibration was derived from the comparison of optical densities due to quasi-pure Rayleigh scattering observed 180° from the Sun in the three colours above 30 km altitude. The absolute calibration in blue and red light was based directly on solar images. The colour, blue, red and IR camera settings (aperture, speed) were respectively: f/11, 1/125 s; f/8, 1/250 s; f/8, 1/250 s; f/8, 1/125 s. As soon as the gondola could be Sun oriented, pictures were taken during ascent and later during the float period. With its Sun sensor remaining locked on the Sun, the gondola was then rotated about its vertical axis so that the cameras could record Earth limb images 65°, 115°, 180°, 245° and 295° from the solar azimuth. The whole horizon scan took place between 16.08 and 16.18 h GMT, during which atmospheric illumination conditions changed very little. The latitude and longitude were respectively 1° E and 44° N. The average solar azimuth and elevation angles were 248° and 9.3° respectively. The optical densities were measured on three tracks per frame normally to the horizon by means of a Jarrell-Ash micro-densitometer and subsequently converted into radiance, in units of solar radiance, versus altitude of the grazing line of sight every 200 m. Each photograph allows an horizontal angular coverage of about 34°.

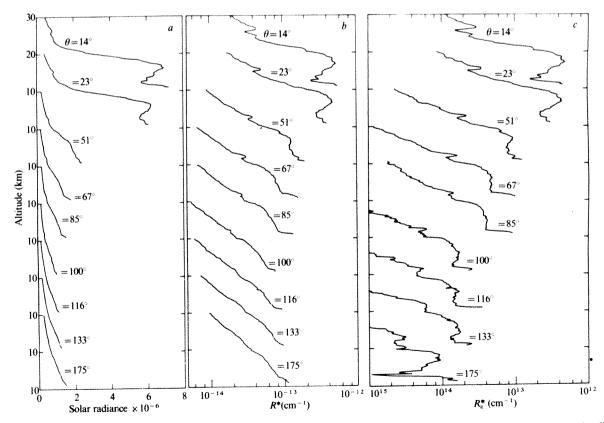


Fig. 2 Data at 650 nm versus altitude for various scattering angles, θ : a, integrated radiances, R, along the lines of sight; b, inverted radiances, R^* ; c, inverted radiances due to aerosols, R^* . Each successive lower curve is displaced by 10 km altitude from the one above. R^* and R^* are expressed in units of solar radiance.

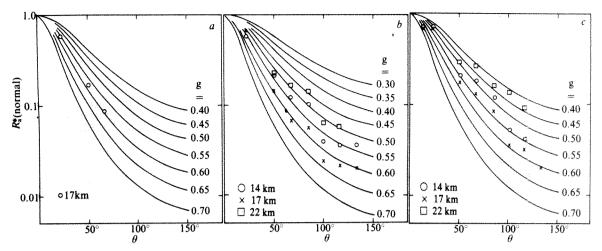


Fig. 3 Henyey-Greenstein phase functions of the radiance versus scattering angles, normalized at $\theta = 0^{\circ}$ for various values of the asymmetry factor, g. The experimental data points are shown at the three altitudes studied and for the three wavelengths: a, 440 nm; b, 650 nm; c, 860 nm.

Results

As an example, the observed limb radiance values are presented versus the altitude of closest approach of the line of sight to the Earth surface (sea level) for 650 nm and for various scattering angles in Fig. 2a.

The comparison of the vertical radiance profiles observed at small scattering angles in red light 11 days before, less than 1 month⁹ and five months after the Mount St Helen's eruption shows several characteristics. A radiance enhancement peaking at 17 km altitude still exists on 15 October 1980, by a factor of almost three relative to the pre-eruption value. Above 22 km altitude the radiance has returned to values close to what it was on 7 May 1980. This suggests that the heavy layer observed over England on 6 and 7 June¹⁰ and on 5 June⁹ over France constituted the main body of the material injected in the stratosphere by the volcano. Over 5 months, that layer would then have spread in altitude and its centre of gravity would have moved upwards by ~2 km, this being perhaps related to air heating through sunlight absorption by the aerosols.

The various photographs taken around the vertical axis of the gondola reveal the 17 km layer at all azimuth angles; above 20 km altitude a well defined 1-km thick layer is readily seen. Such thin, well separated features have been observed on each of our previous flights and most probably have no relationship with volcanic activity. They belong to the 'natural' stratospheric aerosol. In this particular case the layer is present towards the south from the gondola and absent towards the north. Its altitude is at 22 km from 125° azimuth counted clockwise from the geographical north to ~220° where it begins to rise up to ~25 km at 250° where it splits and becomes invisible. Particulate matter is also present above, what we call here, the 22 km layer. With respect to the horizontal homogeneity, the comparison of the aerosol direct sunlight scattering with the scattering angles is safe for the 17-km layer; for higher altitudes care must be taken.

For the angular study, along the lines-of-sight integrated radiances are inverted using standard ozone and air distributions²¹ and their respective absorption cross-sections^{17,22}. The *in-situ* radiance values, R^* , in units of solar radiance and per cm along the line-of-sight at the tangent altitude, are presented in Fig. 2b. The R^* values at 650 nm (Fig. 2b) show at $\theta = 175^{\circ}$ an exponential dependence versus altitude, as expected, on which some structure and noise is superimposed. At 860 nm in the backward hemisphere where aerosols are expected to bring a small contribution and particularly at 30 km altitude, the variation of R^* with θ fits well the Rayleigh phase function, giving confidence to the very low contribution from the Earth albedo to the observed signal. This is not surprising as the average cloud deck radiance measured at depression angles from 5 to 15° ranges from 7×10^{-7} to 6×10^{-7} of the solar

radiance at respective azimuths from 0 to 180° from the Sun. At $\theta = 14^{\circ}$ and 17 km altitude the aerosol contribution is about 14 times the Rayleigh contribution.

At 650 nm (Fig. 2b) the Rayleigh phase function can be fitted to the radiance variation observed in the backward hemisphere at 30 km altitude. In this case, however, the average cloud radiance varies from 6×10^{-6} near the Sun's azimuth to 6×10^{-7} elsewhere. In the forward direction the aerosol contribution to the radiance is 10.8 times the Rayleigh contribution at 17 km. At 440 nm, Rayleigh scattering dominates even if at $\theta=14^\circ$ it still seems to be five times smaller than aerosol scattering at 17 km. At $\theta=175^\circ$, R^* grows faster than exponentially at altitudes below 20 km. Multiple scattering here has an important role because the Rayleigh optical thickness on the tangent line of sight reaches unity at 20 km. This occurs at 9 km and at ground level for 650 and 860 nm respectively. In addition the average cloud deck radiance is very high in blue light varying from 1.6×10^{-5} near the Sun to 10^{-6} of the solar radiance elsewhere. The data obtained in blue light can then only be used

Table 1 Summary of balloon-borne results for three characteristic altitudes

Altitude	14 km	17 km	22 km
8440	*******	0.6	(m)minus
8 650	0.55	0.60	0.50
8 860	0.52	0.56	0.45
$\sum Q_{\rm s} n \sigma_{440} ({\rm cm}^{-1})$		3.2×10^{-8}	******
$(\sum Q_{\rm s} n \sigma_{440})_{\rm AM} ({\rm cm}^{-1})$		1.5×10^{-8}	ner-paner
$\sum Q_{\rm s} n\sigma_{650} ({\rm cm}^{-1})$	9.4×10^{-9}	1.0×10^{-8}	2.9×10^{-9}
$(\sum Q_{\rm s} n \sigma_{650})_{\rm AM} ({\rm cm}^{-1})$	6.0×10^{-9}	5.8×10^{-9}	1.5×10^{-9}
$\sum Q_{\rm s} n\sigma_{860} ({\rm cm}^{-1})$	1.9×10^{-9}	2.7×10^{-9}	9.3×10^{-10}
$(\sum Q_{\rm s} n\sigma_{860})_{\rm AM} ({\rm cm}^{-1})$	2.4×10^{-9}	3.1×10^{-9}	9.2×10^{-10}
Q_{s440}		0.8	modified
Q_{s650}	0.14	0.52	0.1
Q_{s860}	0.10	0.35	0.05
$\sum n\sigma_{440} (\text{cm}^{-1})$		4×10^{-8}	waterwidely
$(\sum n\sigma_{440})_{AM} (cm^{-1})$		1.9×10^{-8}	ecoloria
$\sum n\sigma_{650} (\text{cm}^{-1})$	6.7×10^{-8}	1.9×10^{-8}	2.9×10^{-8}
$(\sum n\sigma_{650})_{AM} (cm^{-1})$	4.3×10^{-8}	1.1×10^{-8}	1.5×10^{-8}
$\sum n\sigma_{860} (\text{cm}^{-1})$	1.9×10^{-8}	7.7×10^{-9}	8.9×10^{-9}
$(\sum n\sigma_{860})_{AM} (cm^{-1})$	2.4×10^{-8}	8.9×10^{-9}	8.8×10^{-9}
a (μm)	0.042	0.15	0.045
b	3.6	0.6	1.8
$V (cm^3)$	1.8×10^{-13}	1.5×10^{-13}	7.0×10^{-14}

g, The asymmetry factor of the phase function; $\sum Q_s n\sigma$, the optical aerosol scattering extinction deduced from observed radiances; $(\sum Q_s n\sigma)_{AM}$, the optical aerosol scattering extinction deduced from comparison with the molecular scattering extinction; Q_s , the scattering efficiency factor; $\sum n\sigma$ and $(\sum n\sigma)_{AM}$, the geometric scattering extinctions; a_s b, the size distributions parameters; V, the total volume of particles per cm³ of air at the respective altitudes.

with caution and at small scattering angle. The situation here is different from that in the balloon-borne aureole observation²³ due to the low solar elevation used.

Figure 2c presents the *in situ* radiance due to aerosols R_a^* obtained by subtracting the Rayleigh scattering R_M^* , due to air. R_M^* is evaluated from R^* at $\theta = 175^\circ$ following the 'clean air' procedure currently used in lidar work²⁴. R_M^* is then adapted for the various scattering angles considered according to the molecular phase function¹⁷.

Interpretation

The angular dependance of R_a^* has been fitted to the variation of the scattered light intensity with θ computed for various values of the asymmetry factor g of the Henyey-Greenstein function as shown for three wavelengths in Fig. 3. In blue light a few values of θ give useful results. For the reasons discussed above values for the 22-km layer have only been considered for θ between 51° and 116°.

The asymmetry factors so determined are listed in Table 1 with the optical scattering extinction coefficients $Q_s n\sigma$. The values at 440 nm have not been used because a model taking into account the measured cloud radiances indicates a nonnegligible contribution of the albedo to the limb radiance. But this contribution is negligible at the larger wavelengths. The observed g values, taking into account that it is probably slightly higher than 0.6 and 440 nm, tend to indicate that a value of the real index of refraction equal to 1.55 (see ref. 25) is observed here. This value will then be used to deduce the size parameter x, and its variation with λ from 650 to 860 nm leads to the values of a and b characterizing the size distribution listed in Table 1. Eventually, the scattering efficiency Q_s can be deduced from a and b following the Mie scattering theory¹⁹.

With the data available, there is, of course, another method of determining $\sum Q_* n\sigma$. From the air σ values¹⁷ and from the air number density $n_{\rm air}$ taken from a model²¹ (mid-latitude, spring-autumn) $(n\sigma)_{\rm air}$ can be evaluated. The $R_{\rm M}^{\rm M}$ values determined at $\theta=175^{\circ}$ by the 'clean air' method²⁴ are compared with the $R_{\rm a}^{\rm M}$ measured and by taking into account the air and aerosols phase

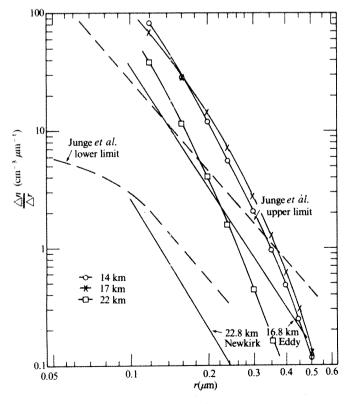


Fig. 4 Number of particles per cm⁻³ versus their radii and per micrometre size interval for three characteristic altitudes: 14, 17 and 22 km. Two previous data sets^{7,23} are shown for comparison.

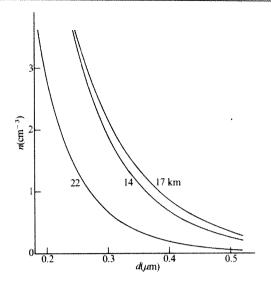


Fig. 5 Total numbers of particles with diameters larger than given values. The ratio of the number of particles with diameters $(d) > 0.3 \,\mu \text{m}$ to the number of particles with diameters $> 0.5 \,\mu \text{m}$ is larger for the layer at 22 km altitude than for 17 and 14 km altitudes indicating the volcanic influence in these two latter cases²⁹.

functions the values of $(\sum Q_s n\sigma)_{AM}$ for the aerosols are deduced. They are also listed in Table 1.

The absolute particle distribution shown in Fig. 4 for the three altitudes considered is based only on the geometrical extinction coefficient $\sum n\sigma$ at 860 nm for two reasons. In IR light, the albedo contribution to the measurement is negligible while it is a maximum in blue light and in the inversion of R leading to R^* the correction for the absorption by O_3 , of which a standard vertical distribution had to be used, is the largest in red light. This is supported by the discrepancy observed at shorter wavelength between the extinctions determined from the observed radiances and those determined by comparison with the molecular scattering. The agreement is very good at 860 nm. The Mie scattering theory also had the best chance to be valid, even if the particles do not exhibit a perfectly spherical and smooth shape, at small values of the size parameter.

Discussion

Our particle distributions are compared in Fig. 4 with the upper and lower limits deduced from in situ sampling and with two extreme values resulting from solar aureole measurements and the present results fall within the range of those data except for particles $< 0.2 \mu m$ radius where the upper limit given by Junge et al. sexceeded. As mentioned above the aerosols below 20 km are still in volcanically perturbed conditions. On the other hand, the collection efficiency of an in situ sampler might be reduced for small particles. Note also that our distributions are steeper especially at large radii. This effect is less pronounced in the comparison with the aureole data. This effect seems to be due to the small values of effective radii obtained here.

Another comparison of our data, shown in Fig. 5, can be made for the total number of particles with a radius $>0.15~\mu m$ obtained by balloon-borne optical particle counters²⁶. The number densities of these particles are 0.4-1.3 particle cm⁻³ in the 14-17 km altitude region and 0.1-0.9 particle cm⁻³ at 22 km. The data presented here for 22 km correspond to a low aerosol content above 20 km and lead to 0.7 particle cm⁻³ above $0.15~\mu m$. At 14 and 17 km altitude the values presented in Fig. 5 respectively imply 1.9 and 2.1 particle cm⁻³ of particles with radii $>0.15~\mu m$. A comparison between the two methods would be required to define a possible discrepancy as the aerosols are volcanically enhanced in the present case.

The optical extinction coefficient $Q_s n\sigma$ measured at 1 μ m wavelength by the SAM II satellite-borne instrument at low

aerosol load in July 197927 exhibits an almost constant value at altitudes from 14 to 20 km of 10^{-9} cm⁻¹. This compares very favourably with our 860 nm value at 22 km. At 17 and 14 km altitude, the extinction in the present case is 3 and 2 times larger. This can be expected from the volcanic influence which may also explain the larger effective particle radius a at 17 km altitude.

A value of the asymmetry factor g of 0.49 ± 0.7 has been measured recently at 633 nm, a wavelength close 650 nm used here, from 10.7 to 12.8 km altitude in the stratosphere 18. This value corresponds to our measurement at 22 km where the aerosol is considered to be purely 'natural'. Because g is larger in the present case at 14 and particularly at 17 km, one of the volcanic influences must then be to increase the g value and if the Mie theory is applicable to increase the particle size as we observe it at 17 km altitude.

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- Deirmendjian, D. Rev. Geophys. Space Phys. 18, 341–360 (1980).
 Bigg, E. K. Tellus 16, 76–83 (1964).
- Volz, F. E. & Goody, R. M. J. atmos. Sci. 19, 384-406 (1962).
- 4. Giovane, F., Schuerman, D. W. & Greenberg, J. M. J. geophys. Res. 81, 5383-5388
- Garriott, O. K. J. opt. Soc. Am. 69, 1064-1068 (1979).
- Rozenberg, G. V. & Nikolaeva-Tereshkova, V. V. Izv. Atmos. Oceanic Phys. 1, 228-232

- (1965).
 Junge, C. E., Chagnon, C. W. & Manson, J. E. J. Met. 18, 81-108 (1961).
 Cadle, R. D. & Grams, G. W. Rev. Geophys. Space Phys. 13, 475-501 (1975).
 Ackerman, M., Lippens, C. & Lechevallier, M. Nature 287, 614-615 (1980).
 Thomas, L., Chaloner, C. P. & Bhattacharyya, S. K. Nature 289, 473 (1981).
 Reiter, R., Jäger, H., Carnuth, W. & Funk, W. Geophys. Res. Lett. 7, 1099-1101 (1980).
 D'Altorio, A., Visconti, G. & Fiocco, G. Geophys. Res. Lett. 8, 63-65 (1981).
 Meixner, F. X., Georgii, H. W., Ockelman, G., Jäger, H. & Reiter, R. Geophys. Res. Lett. 8, 163-166 (1981).
 Papigleen, F. E. Science 211, 819-821 (1981).
- 14. Danielsen, E. F. Science 211, 819-821 (1981).

From an optical point of view the particle size distribution, of which the shape is of greater interest than the particle concentration for radiative transfer calculations25, is variable in the stratosphere. The asymmetry factor, g, is smaller than the constant value (0.7) currently used in models.

For photochemical models of stratospheric aerosols²⁸ the variable size distribution will have a part to play. The molecular content of the condensed phase thought to be oxidized sulphur, water vapour and perhaps other species is not negligable for the gas phase either as conversion into vapour would lead to volume concentrations in the 10⁸-10⁹ molecules cm⁻³ at altitudes from 15 to 20 km.

Our measurements therefore confirm recent data and provide new information on critical points which are of fundamental importance in the evaluation of the role that stratospheric aerosols in the size range from 0.04 to 0.4 µm radius can play.

- 15. Pollack, J. B. Science 211, 815-816 (1981).
- 16. Link, F. & Neuzil, L. Tables of Light Trajectories in the Terrestrial Atmosphere (Hermann, Paris, 1969).
- 17. Penndorf, R. J. opt. Soc. Am. 47, 176–183 (1957). 18. Grams, G. W. Geophys. Res. Lett. 8, 13–14 (1981)
- Hansen, J. E. & Travis, L. D. Space Sci. Rev. 16, 527-610 (1974).
 Wiscombe, W. J. Appl. Opt. 19, 1505-1509 (1980).
- U.S. Standard Atmosphere, 1976 (U.S. Government Frinting Office, Washington DC,
- 22. Ackerman, M. in Mesospheric Models and Related Experiments (ed. Fiocco, G.) (Reidel,
- 23. Newkirk, G. Jr & Eddy, J. A. J. atmos. Sci. 21, 35-60 (1964)
- Grams, G. & Fiocco, G. J. geophys. Res. 72, 3523-3542 (1967) Toon, O. B. & Pollack, J. B. J. appl. Met. 15, 225-246 (1976).
- 26. Hofmann, D. G., Rosen, J. M., Pepin, T. J. & Pinnick, R. G. J. atmos. Sci. 32, 1446-1456
- 27. McCormick, M. P. et al. Geophys. Res. Lett. 8, 3-4 (1981)
- Whitten, R. C., Toon, D. B. & Turco, R. P. Pageophysics 118, 86–127 (1980).
 Hofmann, D. J. & Rosen, J. M. J. atmos. Sci. 38, 168–181 (1981).

Extrachromosomal circular copies of the eukaryotic transposable element copia in cultured Drosophila cells

Andrew J. Flavell & David Ish-Horowicz

Imperial Cancer Research Fund, Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, UK

Circular copies of the transposable element copia have been isolated from cultured Drosophila cells. These molecules, which are present at approximately 1 copy per 10-50 cells, comprise a heterogeneous family of related species. Most are composed of a complete copy of the internal section of copia combined with either one or two copies of the flanking direct repeat sequences. Such structures are strikingly analogous to the circular proviral forms of retroviruses.

TRANSPOSABLE genetic elements were first defined genetically in maize1 and have since been found in a wide variety of organisms including bacteria^{2.3}, yeast³ and *Drosophila*³⁻⁵. They are capable of causing a wide range of genetic effects such as abnormally high mutation and reversion rates and chromosomal deletions or rearrangements. Recent experiments indicate that most, if not all, of these effects are connected with the ability of these elements to transpose to different sites in their host cell genomes. Although there is no direct evidence for analogous species in vertebrates, several observations strongly suggest that eukaryotic transposable elements and the integrated proviral forms of some retroviruses are related.

The following properties are shared by all eukaryotic transposable elements and integrated provirus retroviruses⁶⁻¹

• Their sequences are each composed of an internal DNA segment several thousand nucleotide pairs long flanked by a pair of identical DNA segments of several hundred base pairs (bp) which are both arranged in the same direct orientation on the element⁶⁻¹⁴ (Fig. 1). These repeat sequences are each bounded by small inverted repeats several nucleotide pairs long⁷

- The complete element is flanked by an identical pair of host DNA sequences, usually 4-6 bp long, that are present only once in the target site for integration^{7,10-12,14}.
- There is no detectable homology between the DNA sequences at the insertion site and the ends of the element.
- The terminal dinucleotides of a transposable element or integrated retrovirus provirus are 5'TG...CA3' and in some cases this sequence homology extends further into the elements,

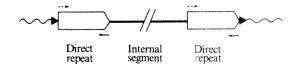


Fig. 1 Generalized structure of a eukaryotic transposable element or integrated retrovirus provirus. An internal segment of several kilobases is bounded by a pair of identical directly repeated sequences. These repeats carry small terminal inverted repeats several base pairs long which are normally imperfect (→ and ←). The complete element is flanked by an identical pair of host DNA sequences (>). The flanking genomic DNA is indicated (m).

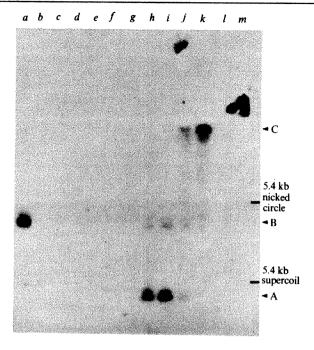


Fig. 2 Isolation of extrachromosc mal copia-specific DNA. Circular DNA was extracted from Drosophila cultured cells (Eschalier's Kc line²⁸; 1.5 × 10° cells) by a modificiation of the method of Hirt^{17,29}. The enriched sample was then subjected to CsCl-ethidium bromide ultracentrifugation. Nucleic acids were recovered from the fractionated gradient, dissolved in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA and treated with boiled RNase A (10 μg ml⁻¹ at 37°C for 15 min). 30% of each fraction was assayed for copia-specific sequences by agarose gel electrophoresis in 90 mM Tris-OH, 90 mM boric acid and 9 mM EDTA at 1.5 V cm⁻¹ 16 h, transfer to a nitrocellulose filter²⁰ and probing with ³²P-labelled nick-translated copia DNA³⁰ (a 5.1-kb Mspl fragment of the genomic clone cDm2056¹⁴ containing the entire copia element plus approximately 150 bp of flanking material). Dextran sulphate (9%) was used during hybridizatior³¹. The filter was exposed to Fuji Rx film with an intensifying screen at -70°C for 16 h. Lane a, 50 pg of the linear 5.1-kb copia Mspl fragment run in parallel, lanes b-m, gradient fractions 1-12 (fraction 1 is the bottom). The mobilities of supercoiled and nicked circular forms of the marker 5.4-kb plasmid pJB8 (ref. 29) are shown. Class A-C molecules indicate copia species which are described in the text.

for instance the *Drosophila* element *copia* shares with an avian retrovirus the sequence 5'TGT...TACAACA3' (refs 7, 13).

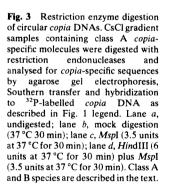
Although such shared properties are very unlikely to be coincidental, the exact relationship between transposable elements and integrated retrovirus proviruses is unclear. To probe further the similarities between eukaryotic transposable elements and retrovirus proviruses, we have searched for transposable element-encoded structures which are analogous to intermediates in the retroviral life cycle. One such intermediate is the circular double-stranded DNA copy of the viral RNA that is synthesized by the viral reverse transcriptase and is presumed to integrate into the host cell genome. The present report describes the isolation and characterization of closed circular DNA copies of the *Drosophila* transposable element copia whose structures closely resemble the circular forms of retrovirus proviruses. This observation further demonstrates the remarkable similarity between these classes of element.

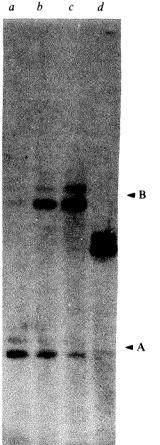
Isolation of circular copia DNA molecules

Extrachromosomal circular DNA was first described in *Drosophila* by Stanfield and Helinski¹⁵. *Drosophila* eggs and cultured cells contain a heterogeneous population of circular species with sizes varying from a few hundred nucleotides to 20 kilobases (kb). More than 80% of this DNA is homologous to middle repetitive DNA¹⁶ (sequences repeated ~100 times in the *Drosophila* haploid genome) of which the *copia* element is an example. It therefore seemed plausible that *copia* might be represented in *Drosophila* circular DNA.

Circular DNA was isolated from *Drosophila* culture cells by a modification of the method of Hirt¹⁷. The partially purified circles were separated from contaminating genomic DNA by CsCl-ethidium bromide isopycnic ultracentrifugation and the fractions from the gradient were assayed for *copia* sequences by agarose gel electrophoresis and Southern filter hybridization¹⁸. Three major size classes of *copia*-specific sequence were observed (Fig. 2): a fast migrating class (class A) which is shown below to comprise circular supercoiled molecules, minor amounts of nicked circular molecules with intermediate mobility (class B) and a slowly migrating class with a lower buoyant density containing high molecular weight genomic linear DNA (class C). The following experiments proved the assignments for class A and class B molecules; class C molecules were not studied further.

First, class A co-purifies with supercoiled mitochondrial DNA on the CsCl-ethidium bromide gradient (data not shown), strongly suggesting that class A is also supercoiled. Further evidence was provided by digestion of class A and class B molecules with restriction endonucleases. Gradient fractions containing the fast migrating class A copia species were pooled and digested with restriction enzymes which cut genomic copia elements once (HindIII) or not at all (MspI; Fig. 3). Mock digestion with no added enzyme led to partial conversion of class A (which in Fig. 2 is resolved into a doublet) into the more slowly migrating class B. DNA preparations of this purity usually contain low levels of contaminating nuclease which nick supercoiled circles to yield open circles. Thus, in the conditions of mock digestion, supercoiled class A circles were converted





partially into nicked class B circles. Digestion with MspI gave a similar result but addition of HindIII to this enzyme converted both classes of species into a doublet of slightly greater mobility than class B, presumably corresponding to linear molecules. We conclude that classes A and B in Fig. 2 are composed of supercoiled and nicked copia circles, respectively. Furthermore,

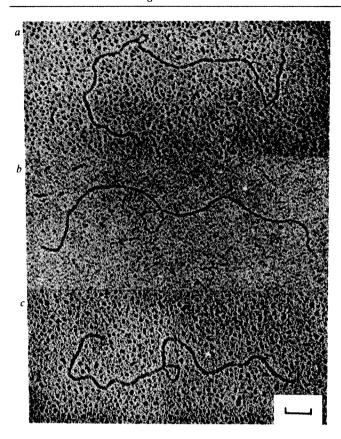


Fig. 4 Plasmids containing 5.0-kb (pBBl and pBB5), 4.7-kb (pBB3) and 4.2-kb (pBB6) inserts were digested with *Hin*dIII and *Msp*I restriction endonucleases to yield the linear insert DNAs (*Msp*I cuts the plasmid pAT153 to yield fragments smaller than 700 bp). Heteroduplexes between pair-combinations of these inserts were prepared and mounted ³². a, 5.0-kb and 4.7-kb inserts; b, 5.0-kb and 4.2-kb inserts; c, 4.7-kb and 4.2-kb inserts.

The bar indicates 0.1 µm.

there are at least two different sizes of circle, the smaller predominating.

The approximate yield of circular copia DNA recovered on the CsCl gradient was determined by comparison with known quantities of cloned copia DNA (Fig. 2). The hybridization signal from the circular copia DNA corresponds to about 50 pg of DNA from 4×10^8 cells, which is equivalent to 1 complete copia molecule per 50 Drosophila cells, assuming complete recovery. The actual recovery of circular DNA is difficult to establish but is likely to be ~25% (the value obtained from similar experiments by Smith and Vinograd)¹⁹, suggesting that the cultured cells studied contain 1 circular copia molecule per 10-50 cells.

Structure of cloned copia molecules

To analyse circular copia molecules more conveniently, these species were cloned into a bacteriophage λ vector. The circles were linearized by digestion with HindIII and then ligated into HindIII-cleaved λ Charon 21A. Approximately 1,000 copia-specific recombinant phage were detected; 13 of these were plaque-purified and phage DNA preparations were made. The size of the copia-specific DNA insert was determined in each of these purified phage by HindIII digestion followed by agarose gel electrophoresis. Two major size classes of insert were observed, 4.7 and 5.0 kb long. These two species are presumably derived from the two major size classes of copia circle visible in Fig. 2. Two examples of each of the size classes of recombinant, together with one phage containing a smaller 4.2-kb insert, were subcloned into plasmid pAT153²⁰ to simplify further analysis of the DNA.

To determine the relationship between these cloned DNAs, heteroduplexes between all three size classes of insert were visualized by electron microscopy (Fig. 4). Deletion loops were only observed between plasmids of different sizes, suggesting that the two members of each size class possess substantially similar sequences. Only single deletion loops were observed between plasmids containing inserts of different sizes, and the sizes of the loops correspond in all cases to the size difference between each pair of inserts. It therefore seemed likely that the 4.7-kb insert was a simple deletion variant of the 5-kb species and that the 4.2-kb species had a larger deletion in the same region.

Restriction mapping of the five plasmids (Fig. 5) confirmed this conclusion. All three classes of insert have similar restriction maps, the only difference between them being in the region between the unique PstI and PvuII sites. The 5.0-kb inserts have the restriction map predicted for an excised genomic copia element which has been circularized and cloned. In particular, these inserts contain the 280-bp BalI fragment predicted from the head-to-tail fusion of two direct repeats. The 4.7-kb inserts lack this BalI fragment and presumably contain only one direct repeat. The 4.2-kb insert has lost both the BalI fragment and a further 500 bp to the left of it.

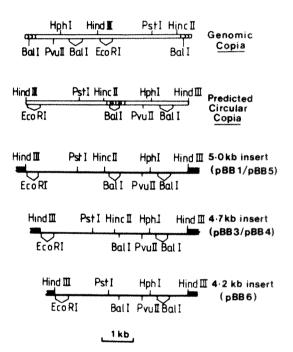


Fig. 5 Restriction maps of cloned copia circular DNA molecules. DNA of the vector phage & Charon 21A (a gift of R. A. Flavell) was treated with restriction endonuclease HindIII (Boehringer; fourfold overdigestion) and calf intestinal phosphatase (Boehringer, $0.02\ U$ per μg DNA for 30 min at 37 °C). The enzymes were inactivated at 70 °C for 60 min. Enriched circular DNA from Drosophila cultured cells (see Fig. 1 legend) was digested with HindIII and MspI restriction endonucleases, extracted with phenol and precipitated with ethanol. The cleaved vector DNA (2 µg) and insert DNA (~0.01 μg) were ligated in 0.01 ml 50 mM Tris-HCl pH 7.5, 10 mM dithiothreitol, 5 mM MgCl₂ and 1 mM ATP with T₄ DNA ligase (a gift from A. Udvardy) at 4 °C for 16 h. The mix was packaged into phage coats³³ and phage were plated on Escherichia coli K802 cells. Duplicate nitrocellulose filters were blotted from the plates³⁴ and hybridized to ³²P-labelled copia DNA as for Southern filters. Thirteen plaques showing hybridization were picked and the phage plaque-purified three times. Quick DNA preparations were made from suspension stocks of the phage³⁵. Insert DNAs from these phages were sized by agarose gel electrophoresis of *HindIII* digests. Eight phage contained 4.7-kb inserts, three contained 5.0-kb inserts, one contained a mixture of these two and one contained a 4.2-kb insert. Two examples of both the 4.7-kb and 5.0-kb inserts, together with the 4.2-kb insert, were subcloned into HindIII-digested pAT15320 and the resultant recombinants were used to transform HB101 cells to ampicillin resistance. Quick plasmid DNA preparations and large-scale preparations were by the method of Ish-Horowicz and Burke²⁹. A restriction map of a genomic copia element. (G. Rubin, unpublished) is shown and the location of the direct repeats (cm) is indicated. The predicted restriction map of a copia element, which has been perfectly excised from the recombinant plasmid cDm 2056¹⁴, cyclized and then cleaved at the unique HindIII site, is shown. Double cutting sites for Ball and EcoRI enzymes are indicated ()

Thus, the two major size classes of cloned circular copia DNA molecule seem to comprise intact copia elements circularized at the extreme ends of the element with or without the deletion of one direct repeat sequence. This result is very similar to that observed in cloned circular retroviral proviruses which also possess either one or two direct repeat sequences (usually termed long terminal repeats^{21,22}). The latter configuration is labile in bacteriophage vectors, with loss of one direct repeat sequence sometimes occurring by recombination during propagation²³. To prove that the cloned *copia* species containing one direct repeat were not derived from such artefacts, a sample of uncloned total Drosophila circular DNA was digested with three restriction enzymes (HindIII, HincII and PvuII) that easily distinguish between these two species. The copia-specific products of these digestions were identified by Southern filter hybridization. Fragments derived from molecules containing one direct repeat (0.9 kb) and two direct repeats (1.2 kb) in HincII/PvuII double digests of uncloned copia circles were observed (Fig. 6, lanes c, e), showing that both types of molecule exist in Drosophila cultured cells.

Restriction fragments characteristic of 4.2-kb circles were not detected in digests of uncloned circular DNA. This suggests that

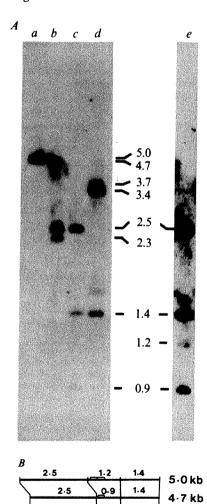


Fig. 6 Detection of circular copia molecules containing either one or two direct repeats in cultured cells. Samples of uncloned circular DNA from cultured cells were digested with restriction endonucleases and analysed for copia-specific sequences. A, lane a, HindIII plus MspI; lane b, HindIII plus MspI and HincII; lane c, HindIII plus MspI, HincII and PvuII; lane d, HindIII plus MspI and PvuII. Lane e is a longer exposure of lane c to reveal the 1.2-kb fragment characteristic of 5.0-kb circles. The sizes of copia-specific fragments were derived from comparison with parallel marker DNA fragments. B, the restriction maps of 5.0-kb and 4.7-kb insert DNAs for the enzymes used in these digests are shown. MspI does not digest either the uncloned or cloned copia DNAs but aids complete digestion in preparations containing uncloned copia circles for reasons that are unclear. The location of the direct repeats is indicated (

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either these elements are derived from a cloning artefact or they constitute a small minority of the circular *copia* species. We favour the latter interpretation because deletions associated with prokaryotic transposons^{2,3} and in circular retrovirus proviruses^{24,25} have often been observed previously.

Origin of circular copia elements

Circular copia elements might be derived in several ways. First, they may be self-replicating plasmids. We think that this is unlikely because of their low abundance. Second, they may be synthesized by reverse transcription of copia RNA. Such RNA could be derived either from infecting 'copia retroviruses' or possibly from the full-length intracellular copia RNA which is abundant in cultured Drosophila cells⁶. These hypotheses are supported by evidence of reverse transcriptase activity and the reported presence of retrovirus-like particles in Drosophila cells²⁶. However, we favour a third interpretation, that they are derived from the relatively infrequent excision of genomic copia elements.

Excision of genomic copia by homologous recombination between the terminal direct repeats would generate the observed 4.7-kb circles possessing one direct repeat. Such excision would leave single copies of the direct repeats behind in the Drosophila chromosomes. These single copies were not detected in embryonic cells¹³ but cultured cells, which possess approximately fourfold more copias per haploid genome, were not probed for such sequences. We therefore cannot exclude the possibility that the 4.7-kb molecules are derived from such a mechanism. Homologous recombination between direct repeats could not yield 5.0-kb molecules containing two repeat sequences, although recombination between the flanking host 5-bp repeats (Fig. 1) would create such species. However, if such a mechanism is implicated, there must be greater control of excision because any given pair of 5-bp sequences occurs about once in every 1,000 bp in the Drosophila genome. If the excision of 5.0-kb molecules is based on homologous recombination, one copy of the variable flanking 5-bp repeats might be present between the fused direct repeats in 5.0-kb circles. Alternatively, if the 5.0-kb circles are derived by reverse transcription, it is likely that all these molecules possess an invariant sequence at the junction between the two direct repeat sequences. The sequence of cloned 5.0-kb molecules in this region is now being determined to test these predictions.

Eukaryotic transposable elements and retroviruses

The experiments described here indicate that eukaryotic transposable elements and retrovirus proviruses share close structural similarities. The isolation of circular *copia* elements whose structures are closely analogous to circular proviruses extends these common properties. Such similarities could be a result of either a common ancestry or convergent evolution. Temin²⁷ has pointed out, however, that convergent evolution would be unlikely to produce such close structural similarities and such a high degree of sequence homology at the ends of these elements. It therefore seems likely that these sequences are evolutionarily related.

The degree of relationship between present day transposable elements and retrovirus proviruses is difficult to estimate. If these species are closely related it is possible that retroviruses can transpose without using RNA intermediates; there is no evidence to support or discredit this hypothesis. It is similarly possible that all eukaryotic transposable elements are retroviruses, although this seems highly unlikely in the case of yeast transposable elements. Furthermore, the presence of transposons in prokaryotes suggests that transposable genetic elements are more widespread than retroviruses and probably predate them evolutionarily.

We therefore favour the hypothesis that a broad spectrum of related species exists in eukaryotes between the transposable elements of yeast and the non-defective retroviruses of higher

vertebrates which usually, if not always, transpose via RNA intermediates. All these species may, however, still share common intermediates, such as circular DNAs, and all may use similar cellular enzymes in their integration. We are now investigating whether copia circles can integrate into the Drosophila chromosomes to create normal genomic copia elements. Of course, if circular copia molecules are derived by

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- McCEntock, B. Cold Spring Harb. Syrep. quant. Biol. 21, 197-216 (1957).
 Kleckner, N. Cell 11, 11-23 (1977).
 Calos, M. P. & Miljer, J. H. Cell 28, 579-595 (1980).
 Ijm, Y. V. et al. Cold Spring Harb. Syrep. quant. Biol. 42, 959-969 (1978).
 Green, M. M. A. Rev. Genet. 14, 109-120 (1980).
 Prinnegan, D. J., Rubin, G. M., Young, M. W. & Hogness, D. S. Cold Spring Hark. Syrepasser. Biol. 42, 1053-1063 (1978).
- Shanotohno, K., Muntani, S. & Tenm, H. M. Nasaw 284, 550–554 (1980)
 Sundrie, J. G., Shanotoh, T. M., Verna, I. M. & Lerner, R. A. Prec. asts. Acad. Sci. U.S.A.
- 9. van Beveren, C., Goddard, J. G., Berns, A. & Verms, I. M. Proc. nets. Acad. Sci. U.S.A. 77,
- 3307-3311 (1980). 10. Dhar, R., McClements, W. L., Enquest, L. W. & Vando Woude, G. F. Proc. nats. Acad. Sci
- Darr, R., McCaschents, W. L., Enquar, L. W. & Vance Woode, G. P. Prec. Add. Acad. S. U.S.A. 77, 3937-3941 (1980)
 Farsbeugh, P. J. & Pink, G. R. Netters 236, 352-356 (1980).
 Gafner, J. & Philippson, P. Netters 236, 414-418 (1980).
 Levis, R., Drussmir, P. & Rubin, G. M. Cell 21, 581-588 (1980).
 Dunsmir, P., Brorsin, W. J., Smoon, M. A. & Rubin, G. M. Cell 21, 575-579 (1980).

- Stanfield, S. & Heitraki, D. R. Cell 9, 333-345 (1976).
 Stanfield, S. W. & Langvel, J. A. Proc. natu. Acad. Sci. U.S.A. 76, 6142-6146 (1979).

excision of genomic material and if these circles can integrate in this manner, then they must be potential transposition intermediates.

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- 17. Hirt, B. J. molec. Biol 24, 365-369 (1967).

- Rutt, B. J. House. 28tol 28, 363-369 (1967).
 Southern, B. M. J. molec. Biol. 96, 503-517 (1975).
 Smith, C. A. & Vinograd, J. J. molec. Biol. 69, 165-178 (1972).
 Twigs, A. J. & Sherrett, D. Nature 283, 216-218 (1960).
 Shenk, P. R. et al. Coll 18, 1383-1395 (1978).

- Steink, F. R. et al. Call 18, 1363-1395 (1978)
 Yoshmora, F. K. & Weinberg, R. A. Call 16, 323-332-(1979);
 Goff, S. P., Gilbox, E., Witts, O. N. & Baltmora, D. C. Il 22, 777-785 (1980).
 Iu, G., Boons, L. & Skalka, A. M. J. Vivol 33, 1026-1035 (1980).
 Swanstrom, R., DeLoubs, W. J., Bashop, J. M. & Varmus, H. E. Froc. nats. Acad. Sci.
- U.S.A. 78, 124-128 (1981)
 26. Holne, C. W., Kelly, D. C. & Avery, R. J. J. gam. Vivol. 49, 385-395 (1980).

- Holms, C. W., Kelly, D. C. & Avery, R. J. J. gen. Virol. 49, 385-395 (1980).
 Tremm, H. M. Call. 21, 599-600 (1980).
 Bachaher, G. & Obanossian, A. Jr. Viero 6, 162-172 (1970).
 Izh-Horowacz, D. & Burks, J. F. Niclaio Acid Res. 9, 2989-2992 (1981).
 Rigby, P. W. J., Disckmann, M., Rhodes, C. & Berg, P. J. molec. Biol. 113, 237-251 (1977).
 Jackreys, A. J. et al. Cell. 21, 555-564 (1980).
 Daves, R., Simon, M. & Davidson, N. Mech. Enzym. 21, 413-428 (1971).
 Scalenghe, F., Turco, E., Edstrom, J. E., Prrotts, V. & Maill, M. Chremosome (Berl.) 82, 205-216 (1981).
 Berry, W. D. & Davidson, R. M. Simona 186, 180-181 (1977).
- Boaton, W. D. & Davis, R. W. Science 196, 180–181 (1977).
 Cameron, J. R., Philippeon, R. & Davis, R. W. Maciele Acada Res. 4, 1429 (1977).

Transformation of rat cells by an altered polyoma virus genome expressing only the middle-T protein

R. Treisman, U. Novak, J. Favaloro & R. Kamen

Transcription Laboratory, Imperial Cancer Research Fund Laboratories, London WC2A 3PX, UK

A modified polyoma virus genome has been constructed which can encode the middle-T protein, but not the large-T or small-T proteins. This was achieved, starting with the full length viral DNA inserted into a plasmid vector, by replacing a small genomic restriction fragment spanning the middle-T intervening sequence with the equivalent fragment from a cloned partial cDNA copy of the middle-T protein mRNA. Transfection of the modified viral DNA into cultured rat cells efficiently induced the formation of transformed cell foci which gave rise to cell lines that grew as tumours after injection into Fisher rats. The only viral early-region antigen synthesized by the cell lines was the middle-T protein. Expression of the middle-Tprotein is therefore sufficient to establish and maintain a transformed state. The viral mRNA produced by two of the transformed cell lines was structurally indistinguishable from the normal middle-T mRNA found in productively infected cells, suggesting that RNA splicing is not an essential step in the biogenesis of this messenger.

THE early region of polyoma virus DNA encodes at least three polypeptides, the large-T, middle-T and small-T proteins (refs 1-4; reviewed in ref. 5). Conclusive evaluation of the individual roles of these proteins in virally induced oncogenic transformation has been difficult because the DNA sequences^{6,7} which encode them overlap extensively (Fig. 1). It is known that expression of the large-T protein is insufficient to transform cells 5,9, and that the 3'-terminal half of the early region, which encodes only C-terminal sequences of large-T protein, is not required to maintain 10,111 or to establish 12-15 the transformed state. Attention has therefore focused on the 5'-terminal half of the early region, which encodes the small-T and middle-T proteins as well as the N-terminal portion of the large-T protein. As all previously reported mutations within this region of the genome affect at least two of the three T-proteins^{9,16-19}, rigorous assignment of the resulting phenotype to a change in any individual gene product has not been possible. We now report the construction of an altered polyoma virus early region which encodes exclusively the middle-T protein, and describe the mode and consequences of the expression of this DNA when it is introduced into cultured rat cells.

Construction of a viral early region encoding only middle-T protein

We constructed a modified viral early region encoding exclusively the middle-T protein by specific deletion of the middle-T protein intervening sequence (see Figs 1 and 2). We achieved this, starting with a recombinant plasmid containing full-length wild-type viral DNA (p53.A6.6), by replacing a small genomic restriction fragment spanning the small-T and middle-T protein intervening sequences with the corresponding shorter fragment from a cloned partial cDNA copy²⁰ of the middle-T protein mRNA (Fig. 2). From the structures of the Py early-region mRNAs (ref. 20; Fig. 1) we predicted that the plasmid produced, pPyMT1, would encode only middle-T protein. The polyoma virus early-region mRNAs are generated by the differential splicing of a common precursor. This involves the ligation of two splice donors (at nucleotides 409 and 746) to two splice acceptors (at nucleotides 795 and 809) in three of the four possible combinations (Fig. 1). The mature mRNA for middle-T protein (spliced from 746 to 809) lacks the acceptor (at 795) used in large-T and small-T mRNAs. Moreover, as the highly

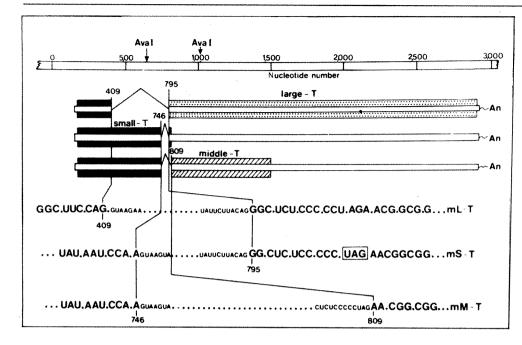


Fig. 1 The spliced structures of the principal Py early-region mRNAs. At the top of the figure is a linearized map of the Py early region, with conventional nucleotide numbers⁷ indicated. The three differentially spliced principal mRNAs²⁰ are aligned with this map. The coding regions of the mRNAs are boxed; solid boxes, dotted boxes and hatched boxes refer to reading frames (as defined in ref. 7) 1, 2 and 3 respectively. The nucleotide sequences across the splice joints²⁰ are shown in the bottom half of the figure.

conserved consensus sequences at splice donor sites lie largely within the intervening sequences 21.22, the splice donor at nucleotide 746 in the middle-T mRNA should also be inactive. The only potential splicing signal remaining in this mRNA would be the donor (at nucleotide 409) used in the large-T mRNA splice. The primary transcript of the viral early region in pPyMT1 (compare with sequence in Fig. 1) could therefore not be spliced to generate mRNAs encoding normal large- or small-T proteins, but it would encode the middle-T protein. A novel splice could perhaps join the large-T mRNA donor to an otherwise unused acceptor, but we considered this possibility to be improbable, and indeed such splices did not occur.

Transformation of rat cells with pPyMT1

We tested the biological activity of the viral genome in pPyMT1 by transfecting Fisher rat cells (F2408 cell line)²³, using the calcium phosphate technique²⁴, and quantifying dense foci overgrowing the normal monolayer which appeared after 2–3 weeks of incubation in the presence of 5% fetal calf serum (FCS). We used as a control the plasmid p53.A6.6, the parent of pPyMT1, which contains a normal polyoma virus genome (Fig. 2). In repeated assays, the transformation efficiency of pPyMT1 was 20–45% that of the control plasmid when non-saturating amounts of DNA were used (Table 1). No foci appeared when cells were transfected with carrier DNA alone, or with the plasmid vector and carrier DNA. Twenty foci induced by pPyMT1 and six induced by p53.A6.6 were picked and grown into cell lines for further analysis.

All the transformed cell lines were initially screened for the presence of viral T-antigens by indirect immunofluorescence using a serum from a Py tumour-bearing rat. Of the six control cell lines transformed by the unmodified parental plasmid, five showed the strong nuclear fluorescence pattern characteristic of the polyoma virus large-T antigen. None of the 20 cell lines derived from pPyMT1 transfections displayed such fluorescence, although many showed a weak specific immunofluorescence which appeared to be distributed throughout the cell.

Properties of rat cell lines transformed by pPyMT1

Eight pPyMT1 and two control lines were selected at random for more detailed study. The properties measured (Table 2) were saturation density, ability to form foci when grown with untransformed rat cells, ability to grow in semi-solid medium and morphology at low- and high-cell densities (Fig. 3). All 10 cell lines displayed, by these criteria, the usual range of phenotypes characteristic of polyoma virus-transformed rat cells. Those transformed by pPyMT1 could not be distinguished from the control cell lines. We conclude that from all of the parameters tested that the lines derived from pPyMT1-induced foci are transformed. We do not know whether differences between these cell lines and those transformed by genomic viral DNA might be demonstrable by measurement of other parameters of the transformed state. In particular, we have noted that many, but not all, of the pPyMT1-transformants grew poorly on plastic when seeded at very low cell densities in 5% FCS. We have also measured the ability of four pPyMT1-transformed (1.6, 1.7, 2.4 and 2.8) and two control (4.1 and 4.6)

Table 1 Transformation of F2408 rat fibroblasts by pPyMT1 DNA and control plasmid DNA

Expt no.	Plasmid	Amount (μg)	No. of foci	Foci per µg viral DNA	Ratio pPyMT1/ p53.A6.6
I	pPyMT1	0	0		
		0.1	59	1,000	0.40
		1.0	137	232	
	p53.A6.6	0	.0		
	•	0.1	149	2,530	
		1.0	409	693	
II	pPyMT1	0.05	100 (2.020	0.45
	• •	0.05	132∫	3,930	0.45
	p53.A6.6	0.05	248]	0.010	
	•	0.05	275	8,810	
III	pPyMT1	0	0	-	
		0.2	15	127	0.20
		1	82	139	0.28
		5	181	61	
	p53.A6.6	0	0		
		0.2	76	644	
		1	294	498	
		5	>500		
IV	pPyMT1	0	0		
		0.1	77	1,305	0.20
		1.0	324	549	
		5.0	361	122	
	p53.A6.6	0	0		
		0.1	375	6,360	
		1.0	>500		
		5.0	> 500		

Subconfluent monolayers of cells (90 mm cultures) were transfected with the indicated quantities of plasmid DNAs. Dense foci were counted 14-21 days later. In experiment II, the cells were trypsinized and divided among four dishes 24 h after transfection.

Table 2 Properties of pPyMT1- and control plasmid-transformed cell lines

Cell line	% Efficiency of focus formation*	% Efficiency of growth in agar†	Saturation density‡ (cells per cm ² × 10 ⁻⁶)
	Mination	iii agai	
pPyMT1 1.2	0.4, 2.2	4.4, 10	>0.99
1.6	2.7, 3.2	6.2, 96	>1.4
1.7	3.7, 93	5.4, 60	>1.0
1.8	1.0, 5.0	3.3, 1.2	>1.1
2.4	2.3, 1.6	0.8, 1.6	>1.3
2.6	1.0, 2.4	0.8, 0.9	>1.1
2.8	1.4, 12.8	2.5, 0.9	>0.97
2.9	1.4, 4.4	2.5, 0.9	>0.83
p53.A6.6			
4.1	3.0, 4.7	1.7, < 0.02	>0.70
4.6	1.7, 4.8	1.6, 20	>1.20
F2408 rat cells	none	< 0.01	0.28

^{*} Transformed cells $(5\times10^2 \text{ and } 5\times10^3)$ were plated in separate experiments with either 2×10^5 (first number) or 2×10^4 (second number) F2408 rat cells on 50-mm dishes. Foci were counted after 14 days of growth in Dulbecco's minimal essential medium (DMEM) containing 5% FCS.

† Transformed cells $(5\times10^2 \text{ and } 5\times10^3)$ were grown in DMEM, 5% FCS, 0.3%

cell lines to grow as tumours after injection into Fisher rats. Tumours were produced within 3 weeks of subcutaneous injection of 4×10^5 cells in all six cases; lines 1.6 and 2.4 produced tumours when only 4×10^4 cells were injected.

Viral proteins synthesized by pPyMT1transformed cell lines

The prediction that the viral early region in plasmid pPyMT1 would encode only the middle-T protein was confirmed by analysis of the viral proteins synthesized by the transformed cell lines. A randomly selected set of cloned cell lines transformed by p53.A6.6 or pPyMT1 were labelled with 35S-methionine and the proteins precipitable by polyoma virus anti-T serum were fractionated on SDS-polyacrylamide gels (Fig. 4). In eight of nine pPyMT1 cell lines tested, the only specifically immunoprecipitated protein detected co-migrated with the normal viral middle-T protein synthesized in mouse or rat cells infected with polyoma virus. One line, 2.9, additionally produced a smaller polypeptide which is probably a truncated middle-T protein. The amount of middle-T protein produced was within the usual range obtained with Py-transformed rat cells. No large-T, truncated large-T, or small-T proteins were found. These conclusions were strengthened by analysis of the viral mRNA present in the cell lines, as discussed below. Two control lines transformed by the control plasmid p53.A6.6 were similarly analysed. Both synthesized middle-T and small-T proteins. One (4.1) also produced a full-length large-T polypeptide, while the other (line 4.6, which was T-antigen negative by immunofluorescence) synthesized a shorter polypeptide which was probably a truncated large-T molecule, like those frequently found in other polyoma virus-transformed rat cell lines 10,11

Viral mRNAs synthesized by pPyMT1transformed cell lines

We investigated the structure of the viral mRNAs in several transformed lines for two reasons: first, to demonstrate unambiguously that the pPyMT1 transcripts were not spliced to produce mRNAs which could encode viral proteins other than middle-T; and second to know how the transformed cell lines generated stable cytoplasmic mRNA encoding middle-T

protein from the modified genome in pPyMT1. Previous reports²⁵⁻²⁷ had suggested that RNA splicing is an obligatory step in the expression of genes which normally contain intervening sequences. We therefore thought it possible that the transformed cell lines always contained viral genomes integrated such that the early region and a host intervening sequence could be co-transcribed. However, because several genes in higher eukaryotes are known to express unspliced transcripts as stable cytoplasmic mRNAs²⁸⁻³⁰, a direct mode of expression could not be discounted.

The S₁-gel mapping experiments shown in Fig. 5 (see legend for details) clearly established that, in three of the four pPyMT1transformed lines examined, the principal mRNAs had 5' termini mapping in the same position (around nucleotide 150; 73 map units) as those of productively infected cells or other cell lines transformed by genomic viral DNA^{11,31}, and that these mRNAs were colinear with the pPyMT1 DNA sequence throughout the region encoding the middle-T protein. This demonstrated that none of the usual splicing events had occurred. Three of the transformed lines also produced variable amounts of a transcript beginning somewhere in vector or host DNA sequence 5' to the early region, which was also unspliced. In other experiments not presented here, we used a 3'-labelled single-stranded DNA probe to detect specifically splices originating from the donor used in the large-T mRNA (see Fig. 1); control, but none of the pPyMT1-induced, cell lines used this

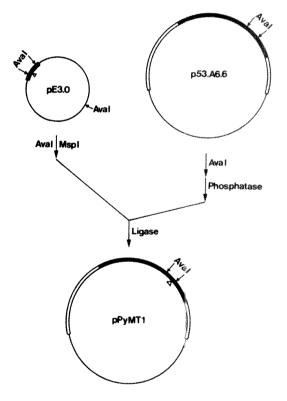


Fig. 2 Construction of a modified polyoma virus early region encoding only the middle-T protein. Plasmid pE3.020 consists of a partial cDNA copy of the middle-T protein mRNA (solid bar) inserted at the PstI site of plasmid pAT153⁴⁵. The absence of the intervening sequence is indicated by A The absence of the intervening sequence is indicated by \triangle . Plasmid p53.A6.6 consists of a complete wild-type Py genome inserted via the unique BamHI site into a derivative of pAT153 in which the plasmid AvaI site had been removed by cleavage with AvaI, S, nuclease treatment and recyclization. The Py early region is shown as a solid bar and other Py sequences as open bars. Plasmid pE3.0 was digested with AvaI+MspI. Plasmid p53.A6.6 digested with Ava I was treated with calf intestine alkaline phosphatase46 . The enzyme was inactivated by heating at 70 °C for 1 h, and the DNA used for ligation without further treatment. Equimolar amounts of the two plasmid digests were ligated at 25 µg mi⁻¹ p53.A6.6. After transfection into E. coli HB101, ampicillin-resistant colonies were picked and plasmid DNAs prepared⁴⁷. Recombinants were analysed for the substitution of the Py genomic Aval fragment by the pE3.0 cDNA Aval fragment using restriction endonucleases AvaI, AvaII, HinfI and MspI. One recombinant with the appropriate restriction map, pPyMT1, was chosen and large quantities of its DNA prepared⁴⁷.

[†] Transformed cells $(5 \times 10^2 \text{ and } 5 \times 10^3)$ were grown in DMEM, 5% FCS, 0.3% (w/v) agar. Microscopically visible colonies were counted after 14 days. Results of two independent assays are listed, done after extensive passage of the cell lines in culture (first number) or shortly after the original foci had become established as cell lines (second number).

[‡] Cells (5×10^5) were plated on 50-mm dishes and grown in DMEM/5% FCS, with medium changes every 24 h after the third day. The control F2408 cultures reached the indicated density at day 4 and increased no further. All transformed lines continued to divide until the cells came away from the plastic; thus the numbers listed are minima.

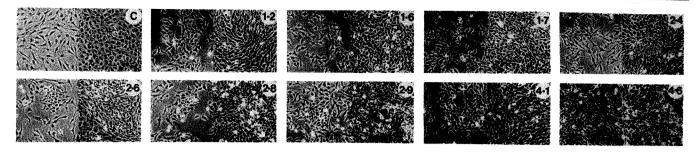


Fig. 3 Phase-contrast photomicrographs of pPyMT1- and p53.A6.6-transformed rat cell lines. Lines 1.2, 1.6, 1.7, 2.4, 2.6, 2.7 and 2.8 are pPyMT1 induced, 4.1 and 4.6 are p53.A6.6. 'C' is untransformed F2408 rat cells. Cells (5×10⁵) were plated on 50-mm dishes and incubated at 37 °C with DMEM containing 5% FCS for either 1 (left panels) or 4 (right panels) days.

splicing donor. Therefore, the only known early-region viral polypeptide which could be made from the mRNAs in pPyMT1-transformed cell lines is the middle-T protein.

The data shown in Fig. 5 also established that, at least in the two pPyMT1 lines mapped in detail (lines 2.4 and 2.8), viral mRNAs were produced which were colinear with the modified viral DNA sequence for the entire extent of the early region and had polyadenylated 3' ends mapping within viral sequence at the normal position. This strongly suggested, for reasons to be discussed below, that RNA splicing was not involved in the biogenesis of these messengers. Maps of the viral mRNAs produced by pPyMT1-transformed lines and by one p53.A6.6 line (4.6) are shown in Fig. 6. The third pPyMT1 line (1.2) examined in detail had mRNAs with 3' ends of colinear viral sequence which mapped within that portion of the early region which, in genomic DNA, uniquely encodes the C-terminal part of large-T protein. This has often been found in other Pytransformed rodent cell lines, and indeed is also the case with the one control line analysed here (line 4.6, see Fig. 6). It usually indicates the position of a virus-host integration junction 10,11 As such integration events remove the principal viral polyadenylation site, cell lines like 1.2 and 4.6 have often been found to produce relatively large amounts of mRNA terminating at the alternative viral polyadenylation site at 99 map units 10,20 illustrated in Fig. 6. One pPvMT1 cell line (2.8; see Fig. 6) also produced a continuous transcript of the L-DNA strand of the late region, extending from a 5' end at an undetermined point in the flanking vector or host DNA sequence to a polyadenylated 3' end mapping at the same position as that of late-region mRNAs produced in lytically infected cells.

The middle-T mRNAs synthesized by the pPyMT1-transformed cell lines 2.4 and 2.8, are identical in structure to the middle-T mRNAs produced during productive infection (Fig. 6). The mRNAs in the transformed lines, however, have not been spliced between their 5' ends and polyadenylated 3' termini. This strongly suggests that RNA splicing does not occur during the biogenesis of these messengers. We cannot rigorously exclude the alternative possibility, that the mRNAs derive from longer precursors which had been spliced and then subsequently processed to generate the 5' or 3' ends of the mature species, but we consider processing at the 5' end unlikely, because the ends detected are indistinguishable from those generated by normal use of the viral early promoter³¹; it is firmly established in several other systems^{32,33} that mRNA 5' ends correspond to transcriptional initiation points. We believe that cleavage and 3' polyadenylation after downstream splicing is equally improbable because polyadenylation has been shown to precede splicing during processing of Ad2 and simian virus 40 (SV40) RNAs^{34,35}. The efficiency with which the transformed lines produce cytoplasmic mRNAs may, however, be slightly impaired by the absence of splicing, as we have noted above that the transformation efficiency of pPyMT1 DNA is two to three times lower than that of the parental plasmid DNA. The level of expression obtained from integrated Py DNA has been shown to

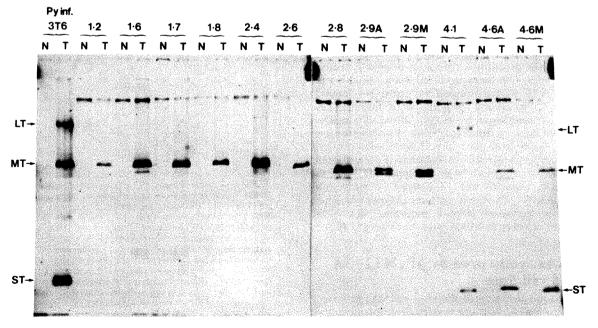


Fig. 4 Viral proteins synthesized by rat cells transformed by modified plasmid pPyMT1 (cell lines 1.2, 1.6, 1.7, 1.8, 2.4, 2.6, 2.8, 2.9A [after cloning in soft agar] and 2.9M, which had been cloned in Methocel) or by control plasmid p53.A6.6 (cell lines 4.1 and 4.6). Cultures grown in 50-mm plates were labelled with 35S-methionine (0.5 mCi per plate) for 3 h. Proteins were extracted and precipitated with Py anti-T protein serum (lanes T, immune serum; lanes N, non-immune serum) as described previously¹; they were then fractionated on 15% SDS-polyacrylamide gels, using the viral proteins precipitated from Py-infected mouse cells as markers (M).

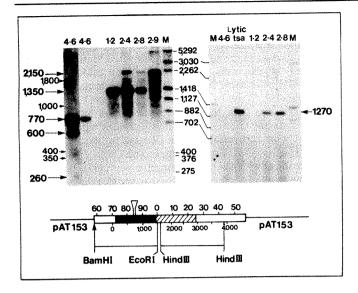


Fig. 5 Characterization of the viral mRNA in pPyMT1-transformed (1.2, 2.4, 2.8 and 2.9) and a p53.A6.6-transformed (4.6) cell lines by alkaline agarose gel electrophoresis of nuclease S₁-resistant hybrids. Experimental procedures were previously described⁴⁸ in detail. The diagram illustrates the structure of the Py DNA insert in the plasmids, with the middle-T proteincoding region in black, the remainder of the early region hatched and the restriction fragments used in the S1-mapping experiments indicated. The position of the deletion in pPyMT1 is also shown (∇). Left panel: S_1 resistant DNA from 100 µg of total cytoplasmic RNA from the indicated cell lines, hybridized to the unlabelled homologous DNA fragments extending from a BamHI to an EcoRI (58.0-100 map units; nucleotides 4,632-1,565; see diagram) site in plasmids pPyMT1 or p53.A6.6. After electrophoresis, the S₁-resistant DNA products were transferred to nitrocellulose and visualized by annealing to nick-translated viral DNA. The lane on the left is from a longer exposure of the same gel (on this exposure, the indicated 350nucleotide product was visible with the pPyMT1 transformants). Lane M shows Py restriction fragments run as size markers. The calculated lengths of S₁-resistant products are indicated to the left. Larger numbers refer to principal products. Smaller numbers refer to products resulting from S1 cleavage within DNA-RNA hybrids at 93 map units, a phenomenon always found in S₁ mapping of Py early-region mRNAs which is probably a methodological artefact²⁰. Product assignments are: lane/line 4.6: the 600and 260-nucleotide products represent the alternative 5'-colinear RNA segments, as shown in Figs 1 and 6; the 770-nucleotide product represents the 3'-colinear RNA segments, extending from the splice acceptors at nucleotides 795 and 809 to the end of the restriction fragment at 1,565; the minor 400- and 350-nucleotide products derive from the 770 by S₁ cleavage at 93 map units (~nucleotide 1,200). In lanes/lines 1.2, 2.4 and 2.8, the 2,150-nucleotide product represents a continuous transcript extending from the BamHI site to the EcoRI of pPyMT1 DNA; the 1,800-nucleotide product is the result of cleavage of the 2,150 at 93 map units; the major 1,350-nucleotide product represents a continuous transcript extending from the normal 5'-end position of Py early-region mRNAs (nucleotide 150; ref. 31) to the EcoRI site in pPyMT1 DNA; the minor 1,000- and 350nucleotide products represent cleavage of the 1,350 at 93 map units. Lane/line 2.9: pattern too complex for analysis. Right panel: polyadenylated cytoplasmic mRNA from the indicated transformed cell lines, or from mouse cells productively infected with Py thermolabile mutant sa^{20} , annealed to the unlabelled Py *HindIII* fragment illustrated in the diagram below. The transfer was annealed to a 32P-labelled probe48 which detects only transcripts of the E-DNA strand, and therefore the 1,270-nucleotide product maps the 3'-colinear segments of early-region mRNAs extending from the HindIII site (1.8 map units; nucleotide 1,650) to the previously determined polyadenylation site at 25.8 map units (~nucleotide 2,930). No bands were detected with RNA from cell lines 4.6 and 1.2 because the 3' ends of viral sequence in these mRNAs lie before (1.2) or shortly after (4.6) the HindIII site.

vary considerably among transformed cell lines, although each seems to use the viral early promoter¹⁰. This variation is probably a chromosomal position effect; similar observations have been made in avian sarcoma virus-transformed mammalian cell lines³⁶. Thus, we may have selected among the pPyMT1 transformants a subset of integrated genomes which are transcribed at a particularly high level to compensate for a defect in subsequent processing. Alternatively, the reduced trans_{D136} formation efficiency of pPyMT1 may have little to do with RNA₉₁₄₅

synthesis and instead reflect the absence of the small-T protein (see below).

To investigate this alternative, we have begun experiments comparing nuclear RNA with cytoplasmic RNA in the pPyMT1-transformed cell lines. We are also studying the expression of pPyMT1 DNA soon after transfection into rat cells; preliminary results indicate that the amount of viral cytoplasmic RNA synthesized from the modified genome is similar to that produced by unmodified DNA 60 h after DNA transfection, but better quantification is required to resolve the issue.

The production of stable, unspliced, cytoplasmic middle-T mRNA from the modified genome in pPyMT1 does not necessarily conflict with previous results^{25–27}. The conclusion that RNA splicing is obligatory could not be generalized from the few cases examined in these earlier experiments. Moreover, if instead of proposing that splicing in itself is essential, one hypothesizes that there is an RNA sequence, often but not always located within introns, which has a positive function in RNA maturation, all the available data could be accommodated. Given the pattern of differential splicing evolved by polyoma virus to produce three mRNAs from one precursor, it

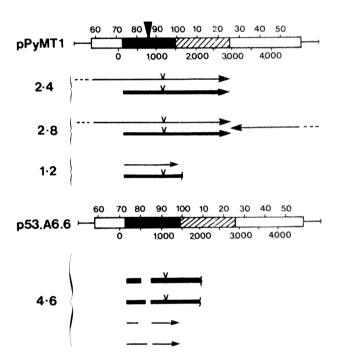


Fig. 6 Structures of Py mRNAs in pPyMT1- and p53.A6.6transformed rat cell lines, as deduced from S1-gel mapping studies such as those illustrated in Fig. 5 and other experiments (not presented) using one- and two-dimensional gel fractionation. The mRNAs are aligned below line diagrams of the viral DNA inserts in the plasmids used, but note that we have not determined the structures of the viral DNA actually present in the transformed cell lines. The middle-T protein-coding regions are shaded, and the rest of the early regions are hatched. mRNAs are represented by thick and thin lines to suggest their relative abundancies. The 5' ends of readthrough transcripts have not been mapped and are therefore indicated by dotted lines. Arrow heads are polyadenylation sites. The S₁-sensitive site at 93 map units is indicated by ♥. The 3' ends of viral sequence indicated by I probably represent the positions of virus-host joints, as frequently found in other Py-transformed cell lines 10. The four cell lines all contained detectable amounts of mRNAs ending at the 99-map unit alternative polyadenylation site 10,20, but these are only illustrated for lines 1.2 and 4.6 where they comprised significant proportions of the viral messenger. Note that in cell line 4.6 there must be mRNAs with the two different small splices (see Fig. 1) because this line produces both small- and middle-T proteins, but these cannot be distinguished by agarose gel analysis.

would not be surprising if such a sequence occurred somewhere outside the 63 nucleotides comprising the middle-T protein intervening sequence. This hypothesis would also better explain the expression of genes such as those for adenovirus protein IX2 and interferons 30 which lack introns, or the abundant production of unspliced late SV40 19S mRNA²⁸ in certain situations. The effect of deletion mutations interfering with the splicing of the SV40 small-T protein mRNA has also been investigated unspliced mRNAs were usually not expressed. It is possible, however, that most of the deletions caused preferential splicing of the precursor to yield large-T, rather than unspliced, mRNAs. Indeed, one deletion (dl 884) removing the small-T mRNA splice donor appeared to synthesize a truncated small-T protein, perhaps by translation of an unspliced messenger³⁷

Roles of polyoma virus early proteins in transformation

Our results strongly suggest that expression of the polyoma virus middle-T protein is sufficient to establish and maintain a transformed state. What of the other two viral early proteins? Viable deletion mutants (the hr-t mutants) have been isolated9 which synthesize normal large-T protein but no detectable small- or middle-T proteins^{4,38,39}. These mutants do not transform cells^{8,9}. Moreover, the integration of such DNA into the rat cell genome can result in cell lines which express the large-T protein but are phenotypically normal8. Deletion mutants lacking DNA encoding the C-terminus of the middle-T protein, but which encode normal small-T protein, are severely impaired in transforming activity⁴⁰, as are certain internal deletions mapping within the middle-T/large-T overlap region¹⁷⁻¹⁹ and a point mutant which causes premature termination of only the middle-T protein (D. Templeton and W. Eckhart, manuscript in preparation). These observations are consistent with our demonstration of the critical role of middle-T protein in transformation; they also suggest that expression of the small-T protein alone cannot transform cells. It is therefore clear that of the three early polypeptides, only individual expression of the middle-T protein can induce the dramatic changes in cell regulation identified as viral transformation. We found, however, that pPvMT1 DNA has a slightly reduced transformation efficiency, which may reflect either an impairment in middle-T gene expression or the absence of an additional gene product. It will be interesting to construct plasmids containing two modified early regions which

independently encode middle-T and one of the other T proteins and to determine whether any of these have a fully restored transformation potential. Furthermore, we have not vet systematically studied in the pPyMT1-transformed lines all relevant parameters of the transformed state. If the middle-T transformed lines prove to be different in some selectable property, it will be possible to assess the roles of the other T proteins by supertransfection with the appropriate cDNA recombinants.

Our results are in prima facie disagreement with the recent work of Cuzin and collaborators (Rassoulzadegan, M., Gaudray, P., Canning, M., Trejo-Avila, L. and Cuzin, F., submitted for publication) which suggested an involvement of the aminoterminal portion of the large-T protein in the maintenance of the transformed state. The disagreement may reflect differences in the way in which the transformants were obtained, although preliminary results indicate that pPyMT1 DNA does transform FR3T3 rat cells in the conditions used by these workers (F. Cuzin, personal communication). Collaborative experiments now in progress between the two laboratories using the plasmid DNA described here should resolve this issue.

The mechanism whereby middle-T protein causes transformation is unknown. Of the three polyoma virus T proteins, a unique property of the middle-T is its association with the plasma membrane 16,38, although efforts to detect it on the cell surface have been unsuccessful³⁹. Several groups have also found a protein kinase activity associated with the Py middle-T protein which results, in vitro, in the phosphorylation of a tyrosine residue in the middle-T protein itself⁴¹⁻⁴⁴. We tested four pPyMT1-transformed (1.2, 2.4, 2.6 and 2.8) and one p53.A6.6-transformed (4.6) cell lines for this kinase activity: they were all positive (data not presented), demonstrating that no viral protein other than the middle-T is required. However, it is not proved that the kinase is an activity of the middle-T protein rather than that of a bound host enzyme. Introduction of the modified viral early region from pPyMT1 into a bacterial expresion vector to obtain large amounts of the middle-T protein would therefore be important to produce a middle-T protein that could be used to assess directly whether it is a tyrosinespecific protein kinase and to assay other potential functions.

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- Ito, Y., Spurr, N. & Dulbecco, R. Proc. natn. Acad. Sci. U.S.A. 74, 1259-1263 (1977).
- Hutchinson, M. A., Hunter, T. & Eckhart, W. Cell 15, 65-80 (1978). Hunter, T., Hutchinson, M. A. & Eckhart, W. Proc. natn. Acad. Sci. U.S.A. 75, 5917-5920
- 4. Schaffhausen, B. S., Silver, J. E. & Benjamin, T. Proc. natn. Acad. Sci. U.S.A. 75, 79-83
- Tooze, J. (ed) The Molecular Biology of Tumour Viruses II. DNA Tumour Viruses (Cold
- Spring Harbor Laboratory, New York, 1980).

 Friedmann, T., Esty, A., La Porte, P. & Deininger, P. Cell 17, 715-724 (1979).

 Soeda, E., Arrand, J. R., Smolar, N., Walsh, J. E. & Griffin, B. E. Nature 283, 445-463
- 8. Lania, L., Griffiths, M., Cooke, B., Ito, Y. & Fried, M. Cell 18, 793-802 (1979)

- Lania, L., Grintins, M., Cooke, D., 10, 1 & Fried, M. Cell 18, 793-802 (1979).
 Benjamin, T. Proc. natn. Acad. Sci. U.S.A. 67, 495-498 (1970).
 Kamen, R. I. et al. Cold Spring Harb. Symp. quant. Biol. 43, 63-75 (1980).
 Lania, L., Hayday, A., Bjursell, G., Gandini-Attardi, D. & Fried, M. Cold Spring Harb. Symp. quant. Biol. 44, 597-603 (1980).
- 12. Israel, M. A., Simmons, D. T., Hourihan, S. L., Rowe, W. P. & Martin, M. A. Proc. natn. Acad. Sci. U.S.A. 76, 3713-3716 (1979)
- 13. Novak, U., Dilworth, S. M. & Griffin, B. E. Proc. natn. Acad. Sci. U.S.A. 77, 3278-3282 14. Hassell, J. A., Topp, W. C., Rifkin, D. B. & Moreau, P. E. Proc. natn. Acad. Sci. U.S.A. 77,
- 3978-3982 (1980).
- Bastin, M., Bourgaux-Ramoisy, D. & Bourgaux, P. J. gen. Virol. 50, 179-184 (1980).
 Silver, J., Schaffhausen, B. & Benjamin, T. Cell 15, 485-496 (1978).

- Griffin, B. E. & Maddock, C. J. Virol. 31, 645-656 (1979).
 Magnusson, G. & Berg, P. J. Virol. 32, 523-529 (1979).
 Griffin, B. E. et al. Cold Spring Harb. Symp. quant. Biol. 44, 271-284 (1980).
- 20. Treisman, R. H., Cowie, A., Favaloro, J. M., Jat, P. & Kamen, R. J. molec. appl. Genetics (in the press).
- Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. Proc. nam. Acad. Sci. U.S.A. 75, 4853-4857 (1978).

- 22. Seil, I., Khoury, G. & Dhar, R. Nucleic Acids Res. 6, 3387-3398 (1979).
- Freeman, A. E. et al. Proc. natn. Acad. Sci. U.S.A. 70, 2415-2419 (1973).
 van der Eb, A. J. & Graham, F. L. Meth. Enzym. 65, 826-838 (1980).
- 25. Gruss, P., Lai, C.-J., Dhar, R. & Khoury, G. Proc. natn. Acad. Sci. U.S.A. 76, 4317-4321
- 26. Hamer, D. H. & Leder, P. Cell 18, 1299-1302 (1979).
- Gruss, P. & Khoury, G. Nature 286, 634-637 (1980). Ghosh, P. K. et al. J. molec. Biol. 126, 813-846 (1978).
- Alestrom, P. et al. Cell 19, 671-681 (1980).
 Nagata, S., Mantei, N. & Weissmann, C. Nature 287, 401-408 (1980).
- Cowie, A., Jat, P. & Kamen, R. (in preparation). Contreras, R. & Fiers, W. Nucleic Acids Res. 9, 215-236 (1981).
- 33. Gidoni, D., Kahana, C., Canaani, D. & Groner, Y. Proc. natn. Acad. Sci. U.S.A. (in the press)
- Nevins, J. R. & Darnell, J. E. Cell 15, 1477-1494 (1978).
 Lai, C. J., Dhar, R. & Khoury, G. Cell 14, 971-982 (1978).
 Quintrell, N., Hughes, S. H., Varmus, H. E. & Bishop, J. M. J. molec. Biol. 143, 363-393
- Khoury, G., Gruss, P., Dhar, R. & Lai, C. J. Cell 18, 85-92 (1979).
 Ito, Y., Brocklehurst, J. R. & Dulbecco, R. Proc. natn. Acad. Sci. U.S.A. 74, 4666-4670 (1977)
- 39. Ito, Y. & Spurr, N. Cold Spring Harb. Symp. quant. Biol. 44, 149-157 (1980).
- Novak, U. & Griffin, B. E. Nucleic Acids Res. 9, 2055–2073 (1981).
 Smith, A. E., Smith, R., Griffin, B. E. & Fried, M. Cell 18, 915–924 (1979).
 Smith, A. E., Fried, M., Ito, Y., Spurr, N. & Smith, R. Cold Spring Harb. Symp. quant. Biol. 44, 141–147 (1980).
 43. Eckhart, W., Hutchinson, M. A. & Hunter, T. Cell 18, 925–933 (1979).
- Schaffhausen, B. S. & Benjamin, T. L. Cell 18, 935-946 (1979) Twigg, A. J. & Sherratt, D. Nature 283, 216-218 (1980).
- Weaver, R. F. & Weissmann, C. Nucleic Acids Res. 7, 1175–1193 (1979).
 Birnboim, H. C. & Doly, J. Nucleic Acids Res. 7, 1513–1523 (1979).
- 48. Favaloro, J. M., Treisman, R. H. & Kamen, R. I. Meth. Enzym. 65, 718-849 (1980).

Induction of 8-azaguanine resistance and sister chromatid exchange in human lymphocytes exposed to mitomycin C and X rays *in vitro*

H. J. Evans & Vijayalaxmi

Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK

Indirect evidence implies that 8-azaguanine-resistant (AG') lymphocytes in human periphera! blood are mutants associated with the loss of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus on the active X chromosome, the mutation frequency increasing linearly with age. AG' variants are readily induced in lymphocytes exposed to mitomycin C in vitro, their incidence correlating with induced sister chromatid exchanges (SCEs). Although SCE events and the development of an AG' phenotype may reflect a common type of DNA damage, mitomycin C-induced AG' variants are not mutants but are suggested to be cells having a transcriptional block at the HPRT locus. AG' variants are also readily induced by X rays in vitro, their incidence correlates closely with the incidence of aberrations induced in the X chromosome and they are considered to have a mutational origin.

A MAJOR requirement in attempts to measure the effects of environmental agents in inducing mutations in man is the development of systems to measure induced mutation frequency in readily accessible human somatic cells. In man, the enzyme hypoxanthine–guanine phosphoribosyltransferase (HPRT) (EC 2.4.2.8) salvages hypoxanthine and guanine by conversion to their respective nucleotides, but the presence of this enzyme, which is coded for by an X-linked gene, is not necessary for cell proliferation and is absent, or defective, in males with the Lesch–Nyhan syndrome¹⁻³. The enzyme also converts purine analogues such as 8-azaguanine (8-AG) or 6-thioguanine (6-TG) to their respective nucleotides and, as these are cytotoxic, their incorporation results in cell death^{4.5}.

Strauss and Albertini⁶ used the cytotoxicity of 6-TG to human blood lymphocytes possessing normal HPRT, to distinguish and select for viable variant cells resistant to 6-TG (TG^r). Autoradiography indicates that such cells undergo DNA synthesis and survive in the presence of normally toxic levels of 6-TG following their exposure to the mitogen phytohaemagglutinin (PHA). The frequency of TG^r cells in lymphocyte populations from normal individuals was $\sim 1.3 \times 10^{-4}$ at a 6-TG concentration of 2×10^{-4} M, whereas in cancer patients being treated with cytotoxic (and mutagenic) drugs, their frequency was increased, in some cases up to 20-fold.

Small lymphocytes have scanty cytoplasm, a low metabolism and poor conversion of purine bases into nucleotides⁷. However, exposure to PHA initiates de novo purine synthesis and activates the purine salvage pathways⁸. It seemed possible therefore that exposure of lymphocytes to mutagens in vitro might also yield variant ('mutant') cells that would undergo blast transformation and remain viable on exposure to PHA and toxic purine analogues, in contrast to normal cells. We show here that the spontaneous frequencies of human lymphocyte variants resistant to 8-AG (AGr variants) increase with the age of the blood donor; that variant frequency is increased in a dosedependent fashion following exposure of human lymphocytes in vitro to the alkylating agent mitomycin C (MMC) or to X rays; that, for MMC, their incidence is related to the incidence of induced sister chromatid exchanges (SCEs) and, for X rays, is correlated with the incidence of gross chromosomal aberrations.

Rationale

One of the preliminary experiments undertaken to develop the protocol outlined below was a comparison of the response of lymphocytes to 8-AG and 6-TG (Fig. 1). The spontaneous incidence of purine analogue-resistant cells (defined by their

ability to undergo transformation by PHA and proceed through to a DNA replication phase) decreased with increasing analogue concentration, reaching a plateau at concentrations of $\sim 2 \times$ 10⁻⁴ M. Factors favouring the use of selection in 6-TG rather than 8-Ag ^{15,16} were: differences in the stability of the two analogues ^{9,10}, the higher affinity of 6-TG than 8-AG for the substrate HPRT ^{11,12}, the influence of exogenous purines present in serum in reducing the selection pressure exerted by 8-AG, but not 6-TG ^{10,13}, and the fact that many mammalian cell mutants retrieved following selection in 8-AG may retain almost normal levels of HPRT whereas virtually all mutants following selection in 6-TG are HPRT (refs 14, 15). Nevertheless, similar mutant expression times and rates of spontaneous and X ray-induced mutations to AG^r and TG^r have been reported for human diploid fibroblasts¹⁷. In our study we were strongly influenced by the fact that 6-TG is incorporated into DNA^{5,18} and that inhibition of DNA synthesis by excess thymidine protects against the toxicity of 6-TG, but not of 8-AG³. In contrast, 8-AG is incorporated into RNA^{5,19}, and inhibition of RNA synthesis protects against the killing effects of 8-AG, but not 6-TG. The short-term culture of lymphocytes in vitro is inappropriate for selecting for resistance to cell killing after DNA synthesis, because the cells have a limited proliferation potential in vitro and colony-forming assays are not possible. In view of this, and because PHA-induced lymphocyte blast transformation requires considerable RNA synthesis, we used 8-AG $(2 \times 10^{-4} \text{ M})$ rather than 6-TG in all the experiments reported

Incidence of AG^r lymphocytes in relation to donor age and sex

The incidence of AGr lymphocytes was determined in blood samples from 26 healthy donors (14 male) aged 16-82 yr (Fig. 2). The variant frequency ranged from 0.80×10^{-4} to 3.99×10^{-4} and despite considerable scatter, there is, nevertheless, a clear age dependence: a similar scatter, but no obvious age dependence, was noted by Strauss and Albertini⁶. There was no evidence of any significant effect of sex ($t_{24}=0.76$, P>0.10) and Kendall's²⁰ coefficient of rank correlation for the pooled data was $y=0.325\pm0.140$ (t=2.33, P=0.02), indicating a significant age effect. The slope of the fitted line of least squares gives $b=0.0294\pm0.0099$, so that the AGr variant frequency increases by $\sim 3\pm1$ cells per 10^6 cells per yr. Interestingly, the two highest yields in the <45-yr age group were from blood samples from cigarette smokers, but almost equivalent yields

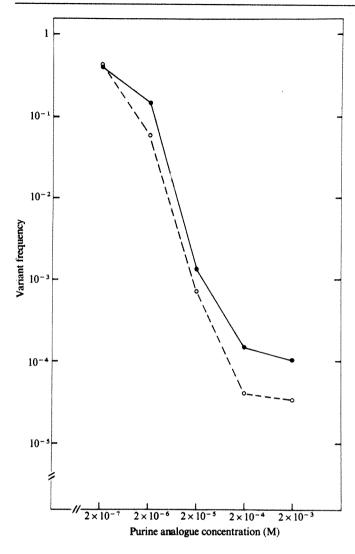


Fig. 1 The incidence of purine analogue-resistant variants in lymphocytes cultured in the presence of various concentrations of 8-azaguanine () or 6-thioguanine (O). In these and the other reported experiments, lymphocytes were separated by underlaying 3-ml aliquots of heparinized whole blood with 8 ml of Ficoll-Hypaque (10:5:2 of 12% Ficoll, 34% Hypaque and distilled water) and centrifuged for 15 min at 600 g. The WBC layer was removed, washed twice with phosphate buffered saline and the cells resuspended in RPMI 1640 medium with 12.5% fetal calf serum, 1.8% PHA (Wellcome), 1% glutamine, 100 μg streptomycin and 100 U pencillin. Duplicate sets of 5-ml cultures containing 2-3 \times 10⁶ lymphocytes were set up, one with and one without 8-AG or 6-TG, and incubated at 37 °C for 40 h. 3H -thymidine at 1 μ Ci ml $^{-1}$ (specific activity 18 Ci mmol $^{-1}$) was added to all cultures 12 h before collection. The procedure then followed that described by Strauss and Albertini⁶, including repeated syringing of resuspended pellets in small volumes of methanol/acetic acid (5:1) fixative using a 25-gauge spinal needle fitted to a 1-ml syringe to give free nuclei. Suspended nuclei were dropped onto clean microscope slides, air-dried and stained with carbol fuchsin (3% in ethanol) before being dipped in L4 liquid emulsion and exposed in the dark at 4°C for 72-96 h. As the work involved counting a considerable number of nuclei (up to 5 × 10⁶ in some experiments), use was made of an automated computer image analysis system to count the total numbers of nuclei on the slides to be scored and the coded slides were then analysed by one observer by high-power light microscopy to obtain a count for the number of labelled nuclei. For all paired treatments the incidence of labelled nuclei was then determined as a labelling index and the variant frequency (Vf) was calculated as

 $Vf = \frac{\text{labelling index in the presence of AG}}{\text{labelling index in the absence of AG}}$

were found in non-smokers and the limited numbers in our sample prevented any useful analysis of this parameter.

The incidence of 'spontaneous' and environmentally induced mutations in the somatic and germ-line cells of an individual would be expected to increase with increasing age and there is good evidence for an increased incidence of mutations transmitted to offspring in several studies on human populations^{21,22}.

Our results now provide evidence for an age-related increase in 'mutation' frequency in man's somatic cells. Ageing is associated with an increasing frequency of hypodiploid blood lymphocytes which in males consist largely of cells lacking the Y chromosome and in females of cells lacking an X chromosome, usually the inactive X 23,24. Ageing is also associated with smaller increases in the frequency of gross chromosomal structural aberrations (H.J.E., unpublished data), although aberration frequencies are of the order of 0.01 per cell whereas in ageing females more than 10% of the peripheral lymphocytes may be hypodiploid. Loss of an active X and consequent depletion of HPRT would result in the expression of an AGr phenotype. As the incidence of AG' lymphocytes is the same as between males and females, it seems more likely that a significant number of mutant AG' cells may be deficient for functional HPRT due to deletion or structural change at the HPRT locus. The gene is located in the terminal band (q27) of long arm of the X chromosome²⁵, so that all deletions involving this arm will result in the loss of the HPRT locus. If breakage sites are distributed between chromosomes in proportion to their length, 1/40th of such sites would be in the X chromosome in a male cell and some 60% of these would be in the long arm (Xq). With a deletion incidence of ~ 0.01 per cell, a deletion of the terminal region of Xq should therefore occur at a frequency of $\sim 1.2 \times 10^{-4}$ per cell, a value comparable with the observed incidence of AGr cells. In a study of G-banded chromosome preparations from individuals aged 60-90 yr we have identified a single Xq deletion in 2,131 cells—a frequency that again accords with the conclusion that the AG^r lymphocytes in vivo are probably cells with a deletion of Xa.

Induction of AG^r variants and SCEs following exposure to mitomycin C

Lymphocyte cultures were exposed to different concentrations of MMC for different time periods (see Table 1). Within each treatment time of 1, 5 and 10 h, the yield of MMC-induced AG' variants increased with MMC concentration, each doseresponse curve being of the form $y = \alpha + \beta d$, where y = variantfrequency, α = control frequency of $8.38 \pm 0.37~(\times 10^{-4})$ and $d = [\text{MMC}]^{0.33}$. Least squares analysis gave values of β_1 , β_5 and β_{10} , respectively, of 0.83 ± 0.21 , 0.92 ± 0.15 and 1.06 ± 0.13 . Although the product of MMC concentration and exposure time would, for certain combinations, be equivalent between the three sets of data, the effective concentration of MMC decreases with time in culture such that for a given variant frequency, a quadrupling of exposure time is equivalent to a halving of the initial concentration of mutagen. The maximum increase in AG' variant frequency is around 12-fold at the highest MMC concentrations, but significantly increased frequencies are evident following exposures to low doses, of the order of 10⁻⁸M, over short time periods.

The data obtained in the parallel cultures on SCE incidence (Table 1) show the very much higher frequencies of induced SCEs relative to induced AG^r variants per cell, but the shapes of the dose-response curves for the two end points are very similar. Least-squares analysis of the SCE data fitted to $y = \alpha + \beta d$ gave a value of $\alpha = 7.63 \pm 0.19$ and the slopes of β_1 , β_5 and β_{10} were, respectively, 0.53 ± 0.03 , 0.60 ± 0.03 and 0.81 ± 0.06 .

The similarities in the kinetics of response to AG resistance and SCE induction are shown in Fig. 3 where each datum on AG' variant frequency per cell has been multiplied by a factor of 7×10^3 and plotted against SCE frequency per cell. The resultant plot gives an excellent fit to a line of unit slope, indicating that for every induced AG' variant in the cell population there are some 7,000 induced SCE events. If the AG resistance involves an alteration on a single X chromosome, because that chromosome represents some 2-3% of the total chromatin per male cell²⁶, then for every event on an X chromosome that would result in an AG' cell there would be around 200 X-chromosome events that would yield SCEs. As SCE events may occur over almost the whole length of the chromosome, but

Table 1 Variant frequency and SCEs in human peripheral blood lymphocytes exposed to mitomycin C

		+Azaguanine			-Azaguanine				
Mitomycin C concentration	Exposure time (h)	Labelied cells	Cells counted	Labelling index (×10 ⁻³)	Labelled cells	Cells counted	Labelling index	Variant frequency $(\times 10^{-4})$	SCEs per cell
Control	1	20	229,492	0.087149	514	5,000	0.1028	8.48 ± 1.93	7.8 ± 0.31
10 ⁻⁷ M	•	22	248,372	0.088577	94	5,000	0.0188	47.12 ± 11.20	31.8 ± 1.11
10 M 10-6 M		10	108,928	0.091804	44	5,000	0.0088	104.32 ± 36.40	65.0 ± 1.94
10 ⁻⁵ M								dembis	waret-
Control	5	35	435,714	0.080328	469	5,000	0.0938	8.56 ± 1.50	7.4 ± 0.34
10 ⁻⁸ M		24	178.621	0.134363	223	5,000	0.0446	30.13 ± 6.47	19.6 ± 0.77
10 ⁻⁷ M		39	291,452	0.133813	126	5,000	0.0252	53.10 ± 9.73	38.5 ± 1.23
10 ⁻⁶ M		24	225,397	0.106479	50	5,000	0.0100	106.48 ± 26.30	70.2 ± 2.23
Control	10	52	627,603	0.082855	509	5,000	0.1018	8.14 ± 1.19	7.7 ± 0.33
10 ⁻⁹ M	10	126	866,525	0.145408	453	5,000	0.0906	16.05 ± 1.61	16.6 ± 0.82
10 M 10-8 M		86	481,292	0.178686	233	5,000	0.0466	38.34 ± 4.83	30.9 ± 1.43
10 ⁻⁷ M		84	576,284	0.145761	110	5,000	0.0220	66.26 ± 9.61	45.2 ± 1.46

The cells used were separated lymphocytes from 10-20-ml samples of blood from a single donor exposed to MMC at the time of culture initiation. They were washed after treatment and transferred to fresh medium containing PHA, with or without 8-AG, for 40 h at 37 °C and treated and processed as described in Fig. 1 legend. To determine SCE frequencies, a third set of cultures was set up following exposure to MMC, using complete medium containing 25 µM bromodeoxyuridine and these were collected after 72 h and slides stained to give harlequin-stained chromosomes³⁸.

alterations at, or associated with, the HPRT locus to give an HPRT⁻ phenotype would be localized to only a small region, the numerical association between induced SCE events and AG variant induction is quite striking. This, however, does not imply a common mechanism for these two end points, but may simply reflect the fact that both types of event are consequences of the interaction of MMC with the cellular DNA.

Carrano et al.²⁷ have also reported a positive linear correlation between SCE and AG^r induction in Chinese hamster fibroblasts exposed to MMC: calculations on their data imply some 5×10^5 SCE events for each induced AG^r cell—a relative variant incidence some two orders of magnitude less than in the present human lymphocyte experiments. Studies on spontaneous mutation rates to AG^r in cultured human fibroblasts^{17,27}, and to TG^r in human lymphoblastoid cells²⁸, give values of $5 \times 10^{-6} - 5 \times 10^{-5}$ per cell generation, although both higher and lower values have been reported for other cell systems^{29,30}. The reported⁶ spontaneous frequency of TG^r lymphocyte variants of 1.3×10^{-4} and our values of $8 \times 10^{-5} - 8 \times 10^{-4}$ for AG^r variants seem too high to reflect mutational events at a single locus, but, as we have seen, they are consistent with the notion that many of these spontaneous variants lack part of an X chromosome.

Although the spontaneous AG^r variants may be a consequence of chromosomal aberration or loss, such changes cannot be responsible for AG^r cells induced following MMC treatment, because cells identified as AG^r are transformed by PHA and pass through the G₁ into the S phase, whereas normal

cells are blocked in G_1 . However, the development of chromosomal aberrations following exposure to MMC occurs during the S phase and not before it³¹; moreover, chromosome loss could only occur at mitosis, long after cells are identified as AG^r cells. Similarly, induced variants are not base loss or substitution mutants at the HPRT locus following misreplication of damaged DNA at the S phase, although such changes might occur following DNA misrepair in G_1 . What, then are these variants?

The true nature of the induced AG^x variants can only be clearly ascertained by studying their progeny: unfortunately, PHA-transformed lymphocytes normally have a limited lifespan in culture, so that this is not a practical proposition. However, there is a wide phenotypic diversity among purine analogueresistant mammalian cells embracing variants with different mutations at the HPRT structural gene, or in associated regulatory loci³², as well as unstable cells with high reversion rates and considered to be 'non-mutant'33. If the MMC-induced AG' lymphocytes are not true mutants, an appealing possibility is that they represent cells in which MMC alkylation had occurred at sites on the HPRT locus where the large MMC-guanine adduct prevented transcription of the locus to give functional HPRT—and hence allowed the cells to survive in the presence of AG. Some of these hypothesized transcriptional blocks could well lead to mutation following replication, but this is not necessarily so. The transcriptional block hypothesis is not easy to test, but one approach would be to examine the response to AGr induction in these G1 cells by other mutagens.

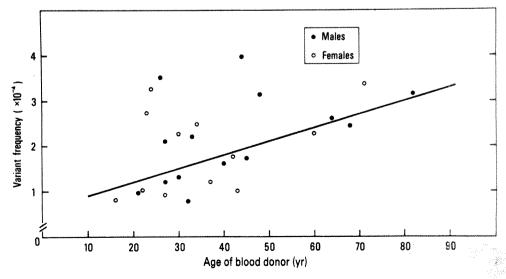


Fig. 2 AGr frequencies in blood lymphocytes of individuals of different ages.

Table 2 Variant frequency in human peripheral blood lymphocytes exposed to X rays

		+Azaguanine			-Azaguanine		
Radiation dose	Labelled cells	Cells counted	Labelling index (×10 ⁻³)	Labelled cells	Cells counted	Labelling index	Variant frequency (×10 ⁻⁴)
Expt I							
Control	72	454,777	0.158319	1.003	5,000	0.2006	7.89 ± 0.96
50 rad	109	676,089	0.161221	890	5,000	0.1780	9.06 ± 0.92
100 rad	58	328,998	0.176292	464	5.000	0.0928	19.00 ± 2.56
200 rad	44	241,749	0.182006	230	5,000	0.0460	39.56 ± 6.77
400 rad	35	231,676	0.151073	130	5,000	0.0260	58.10 ± 12.74
Expt II					· ·		
Control	89	533,180	0.166923	1,160	5,000	0.2320	7.19 ± 0.78
40 rad	115	715,109	0.160815	1,020	5,000	0.2040	7.88 ± 0.77
80 rad	151	849,436	0.177765	820	5,000	0.1640	10.84 ± 0.96
120 rad	151	726,107	0.207958	610	5,000	0.1220	17.05 ± 1.56
160 rad	131	556,885	0.235237	480	5,000	0.0960	24.50 ± 2.37
200 rad	114	510,800	0.223179	320	5,000	0.0640	34.87 ± 3.88

Two separate experiments were carried out using separated lymphocytes from a single blood donor. The cells were exposed to a graded series of X-ray doses (at 183 rad min⁻¹ from a Siemens Stabilipan 300 kV; HVL 3.1 mm Cu at 12 mA with Thoraeus I filter) at the time of culture initiation, were transferred to fresh medium containing PHA, with or without 8-AG, for 40 h at 37 °C and processed as described in Fig. 1 legend.

Induction of AG^r variants following X-ray irradiation

Two groups 15,17 have studied the induction of TGr and AGr mutants following exposure of human fibroblast cultures to X rays and report similar linear dose-response curves and mutation rates of 2.1×10^{-7} and 3.1×10^{-7} per viable cell per rad. At 50 rad the mutation frequency was about twice the spontaneous level and at 200 rad was increased four- to sixfold. In an experiment on AG' incidence in X ray-irradiated lymphocytes (Table 2), we observed no significant increase following exposure to 50 rad, but did so at higher dose levels. The best fit to a linear dose response gave an induced variant rate of 1.25×10^{-5} per rad, but the data give a better fit to the power law function $y = \alpha + \beta d^n$, with n = 1.4. This curvilinearity was investigated further in a second experiment (Table 2) where the inclusion of additional dose points emphasized the curvilinear nature of the response, the data fitting a quadratic with negligible linear term, or a power law function with n = 2.1. Assuming

n=2, then $\alpha=6.90\pm0.11~(\times10^{-4})$ and $\beta=6.81\pm0.20~(\times10^{-4})$. The X-ray results show that: (1) significant increases in AG variant lymphocytes occur following exposure to quite low doses

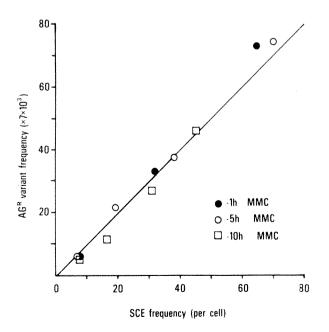


Fig. 3 SCE frequency per cell plotted against AG^r frequency per cell multiplied by 7×10^3 . The close fit of the experimental points to a line of unit slope indicates that over the range of doses and treatment times with MMC there are 7,000 SCE events for every single event resulting in an AG^r phenotype.

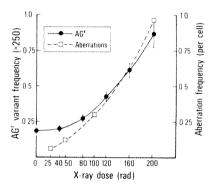


Fig. 4 AG' variant frequencies per lymphocyte versus X-ray dose (———) compared with aberration frequencies in X ray-irradiated lymphocytes (--—). The aberration frequencies are from Lloyd et al. 36 and include totals of fragments, centric rings and dicentrics, with the dicentric incidence doubled to account for equal numbers of undetected translocations (see ref. 37). The AG' frequencies have been multiplied by a factor of 250 for direct comparison with the aberration frequencies.

of X rays; (2) the dose response is curvilinear over 0-200 rad and approximates to a square law; (3) the kinetics of dose response for the induction by X rays of AG^r variant lymphocytes is remarkably similar to that for the induction of chromosome aberrations in lymphocytes (Fig. 4); (4) the rate of induction of AG^r lymphocytes is some 40 times that for AG^r fibroblasts, but is similar to mutation rates in human/hamster hybrid fibroblasts where mutant fitness does not influence mutant yield³⁴; (5) X ray-induced AG^r variants, in contrast to MMC-induced variants, are unlikely to be due to transcriptional blocks, but are more probably true mutations.

Cox and Masson¹⁵ have shown that some 40% of X rayinduced TG^r fibroblasts have stable X-chromosome aberrations (for example, deletions, translocations) whose location is consistent with the mapped position of the HPRT locus on the X chromosome. It seems probable therefore that most X rayinduced purine analogue-resistant mutants are associated with two types of gross structural change: those resulting in breakage of the X chromosome proximal to the HPRT locus, leading to loss of the gene at mitosis, and those resulting in breakage or damage directly at the locus itself. As the lymphocyte experiments involve no mitosis between X-ray irradiation and AG expression, direct damage to the HPRT locus seems the more likely. The relationship between the induction of chromosome aberrations in lymphocytes and X-ray dose is curvilinear35,36 with exchange aberrations increasing as approximately [dose]² and deletions as approximately [dose]^{1.4}. Much of the published data relate only to unstable aberrations, but in Fig. 4 published³⁶ dose-response data have been corrected to include all symmetrical interchange events, and the response curves for AG'

and aberration induction are seen to be similar in shape, with roughly one AG^r event for every 250 or so aberrations. Breakage sites are distributed in proportion to chromosome length so that some 2.5% occur in the X chromosome; thus, there are approximately six visible X-chromosome breaks for every variant induced. Our own studies of the total aberration spectrum in G- and R-banded lymphocyte chromosomes³⁷ from cells exposed to 200 rad of X rays reveal 150 breakage points in every 100 cells. At this dose there are 35 AG variants in 104 cells, or 35 variants per 15,000 breaks, that is, ~10 visible Xchromosome breaks for each variant.

Concluding comments

We have shown that the incidence of peripheral blood lymphocytes resistant to normally toxic levels of 8-azaguanine (AG' cells) increases with the age of the blood donor, is independent of sex and may well reflect the presence of cells with deletions of the terminal region of the X chromosome which contains the HPRT locus. In vitro exposure to MMC markedly enhances the incidence of AG' lymphocytes in a dose-dependent fashion and the rate of increase parallels the increasing incidence of induced SCEs. The high frequency of these MMC-induced AG^r cells and the fact that purine analogue resistance is expressed before the

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- Seegmiller, J. E. Adv. hum. Genet. 6, 75-163 (1976).
- DeMars, R. Fedn Proc. 30, 944-955 (1977)
- De Bruyn, C.H.M.M. Hum. Genet. 31, 127-150 (1976).
- Elion, G. B. Fedn Proc. 26, 898-904 (1967)
- Nelson, J. A., Carpenter, J. W., Rose, L. M. & Adamson, D. J. Cancer Res. 35, 2872-2878
- Strauss, G. H. & Albertini, R. J. Mutat. Res. 61, 353-379 (1979)
- Raivio, K. O. & Hovi, T. in Purine Metabolism in Man Vol. 2 (eds Müller, M. M., Kaiser, E.
- Raivio, K. O. & Hovi, T. in Purine Metabolism in Man Vol. 2 (eds Müller, M. M., Kaiser, E. & Seegmiller, J. E.) 448-455 (Plenum, New York, 1977).
 Hovi, T., Allison, A. C., Raivio, K. O. & Vaheri, A. in Purine and Pyrimidine Metabolism (eds Elliot, K. & Fitzsimmonds, D. W.) 207-224 (Elsevier, Amsterdam, 1977).
 DeMars, R. & Held, K. R. Humangenetik 16, 87-110 (1972).
 Van Zeeland, A. A. & Simons, J. W. I. M. Mutat. Res. 27, 135-138 (1975).
 Kong, C. M. & Parks, R. E. Molec. Pharmac. 10, 648-656 (1974).
 Donahue, T. F., Van Diggelen, O. P. & Shin, S. J. Cell Biol. 70, 312a (1976).
 Albertin, P. J. & DeMore, P. Science 169, 482, 485 (1970).

- Albertini, R. J. & DeMars, R. Science 169, 482–485 (1970).
 Albertini, R. J. & DeMars, R. Mutar. Res. 18, 199–224 (1973).
 Cox, R. & Masson, W. K. Nature 276, 629–630 (1978).
- 16. Meyers, M. B., van Diggelen, O. P., van Diggelen, M. & Shin, S. Somatic Cell Genet. 6, 299-306 (1980).

- de Ruijter, Y.C.E.M. & Simons, J.W.I.M. Mutat. Res. 69, 325-332 (1980).
 Ellis, D. B. & LePage, G. A. Cancer Res. 23, 436-443 (1963).
 Mitchell, J. H., Skipper, H. E. & Bennett, L. L. Cancer Res. 10, 647-649 (1950).

cells pass through a DNA replication phase, argue against a mutational origin for these cells. Their close correlation with the induction of SCEs is, however, considered to indicate that both AG' and SCE induction by MMC are a consequence of interaction of the mutagen with DNA and it is suggested that the AG' variants simply represent cells in which the large MMC-guanine adducts have prevented normal transcription of the HPRT locus to give functional HPRT.

 $\bar{\mathbf{X}}$ -ray damage, which does not require misreplication at the S phase to result in mutational change, is also effective in yielding AGr cells. The dose response in vitro is curvilinear and is very similar to that for X-ray-induced chromosome aberrations in lymphocytes. As chromosome, or fragment, loss cannot intervene between the time of irradiation and the expression of the AGr phenotype, it is argued that X-ray-induced AGr cells may be true mutants and our evidence indicates that for every induced AG' event in a cell population there are about 10 visible induced breaks in an active X chromosome.

Finally, we note that the methods used to detect and count purine analogue-resistant variants in human lymphocyte populations are amenable to automation by flow cytometry, so that this approach may offer a powerful method for studying the effects of mutagens on human cells.

- Kendall, M. G. Rank Correlation Methods 4th edn (Graffen, London, 1969).
- Murdoch, J. L. et al. Ann. hum. Genet. 33, 227-244 (1970).
 Vogel, F. in Chemical Mutagenesis in Mammals and Man (eds Vogel, F. & Röhrborn, G.) 16-68 (Springer, Berlin, 1970).
- Jacobs, P. A., Brunton, M., Court Brown, W. M. & Doll, R. Nature 197, 1080-1081 (1963).
- Galloway, S. M. & Buckton, K. E. Cytogenet. Cell Genet. 20, 78-95 (1978).
- Pai, G. S., Sprenkle, J. A., Do, T. T., Mareni, C. E. & Migeon, B. R. Proc. natn. Acad. Sci. U.S.A. 77, 2810–2813 (1980).
- 26. Paris Conf. (1971) Standardization in Human Cytogenetics, 7 (Birth Defects Original Article Ser. VIII, The National Foundation, New York, 1972)
- 27. Carrano, A. V., Thompson, L. H., Lindl, P. A. & Minkler, J. L. Nature 271, 551-553
- Jacobs, L. & DeMars, R. Mutat, Res. 53, 29-53 (1978)
- Jacobs, L. & Bernats, R. Mund. Res. 39, 29–31 (1976).

 Thilly, W. G., Deluca, J. G., Hoppe, H. & Penman, B. W. Mutat. Res. 50, 137–144 (1978).

 O'Neill, P. & Hsie, A. W. Mutat. Res. 59, 109–118 (1979).
- Evans, H. J. & Vijayalaxmi Nature 284, 370-372 (1980)
- 32. Kadouri, A., Kunce, J. J. & Lark, K. G. Nature 274, 256-259 (1978).
- Fox, M. & Radacic, M. Mutat. Res. 49, 275-296 (1978).
 Waldren, C., Jones, C. & Puck, T. T. Proc. natn. Acad. Sci. U.S.A. 76, 1358-1362 (1979).
 Evans, H. J. in Chromosomes and Cancer (ed. German, J.) 191-237 (Wiley, New York,
- 36. Lloyd, D. C., Purrott, R. J., Dolphin, G. W., Bolton, D. & Edwards, A. A. Int. J. Radiat. Biol. 28, 75-90 (1975)
- 37. Buckton, K. E. Int. J. Radiat. Biol. 29, 475-488 (1976).
- 38. Perry, P. & Wolff, S. Nature 251, 156-158 (1974)

Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex

Anita E. Hendrickson*, S. P. Hunt* & J.-Y. Wu*

* Department of Ophthalmology, University of Washington, Seattle, Washington 98195, USA † MRC Neurochemical Pharmacology Unit, Cambridge CB2 2QH, UK ‡ Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, USA

Neuronal cell bodies and synaptic terminals positive for glutamic acid decarboxylase, the enzyme responsible for synthesizing γ -amino butyric acid, have been located by immunocytochemical staining in all layers of the macaque monkey cortex. In layers II and III the staining pattern of periodic dots is identical with that seen in sections stained for cytochrome oxidase. The rows of dots run parallel with the ocular dominance columns, suggesting that the labelled neurones are preferentially related to each eye.

γ-AMINOBUTYRIC ACID (GABA) functions as an inhibitory neurotransmitter in the mammalian cerebral cortex including the visual cortex¹⁻⁴. We have localized the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) in monkey striate cortex and mapped the distribution of neuronal cell bodies and synaptic terminals positive for GAD (GAD+). Primate striate cortex has the advantage of being precisely laminated and has well characterized inputs and outputs⁵. Standard light microscopic immunocytochemical techniques were used6, with an antiserum to mammalian GAD which has been extensively characterized⁷⁻⁹. We have found that all cortical layers contain both neurones and synaptic terminals which are GAD⁺, but in different quantities. No variation in the distribution or number of GAD⁺ terminals was found in layer IVC which would correspond to the ocular dominance columns¹⁰, but in layers II and III a 375-µm periodic repeat of GAD⁺ dots arranged in rows was found. When the same brains were histochemically stained for the mitochondrial enzyme cytochrome oxidase ^{11,12}, the laminar density and distribution of cytochrome oxidase staining was the same as for GAD and an identical pattern of dots in rows was found in layers II and III.

Experimental procedure

Primary visual or striate cortex from four normal *Macaca fascicularis* monkeys was studied. In one animal the left eye was injected with 3 mCi of a mixture of ³H-proline and fucose 14 days before the animal was killed, to label the cortical ocular dominance columns by transynaptic transport¹⁰. In two animals the striate cortex received 1- μ l injections of colchicine (10 μ g μ l⁻¹) 1 day before they were killed to increase the GAD content of the neuronal cell bodies¹. GAD immunocytochemical and cytochrome oxidase staining methods are described in the legends of Figs 1 and 2.

In sections cut perpendicular to the cortical surface (Fig. 1b), the layers were strikingly outlined in the anti-GAD-incubated sections; sections incubated without specific anti-GAD serum showed no staining (Fig. 1a). All layers contained GAD staining

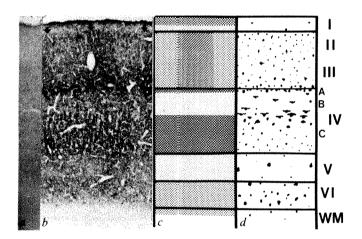


Fig. 1 Macaca monkey striate cortex with the layers numbered according to Lund¹³ on the far right of the figure. All monkeys were deeply anaesthetized and then perfused intracardially with phosphate-buffered 4% paraformaldehyde, pH 7.4, or a phosphatebuffered mixture of 4% paraformaldehyde, lysine and sodium periodate, pH 7.4. The brain was postfixed for 4-24 h, small pieces of cortex were sunk in 30% sucrose in phosphate buffer and serial frozen sections cut both perpendicular and parallel to the cortical surface. For immunocytochemistry, sections were incubated at 25 °C overnight in anti-GAD serum^{7.8} diluted 1/150 in phosphatebuffered saline, pH 7.4 containing 0.3% Triton and 1% normal sheep serum. This saline mixture was used subsequently for all antisera dilutions. After thorough rinsing, the sections were incubated in sheep anti-rabbit IgG serum diluted 1/10 for 30 min at 37 °C, and then in rabbit peroxidase-antiperoxidase⁶ diluted 1/50 for 30 min at 37 °C. The peroxidase was visualized by reacting the sections for 20 min at 25 °C in diaminobenzidine and hydrogen peroxide. Some sections were then lightly counterstained with thionine. Control sections were identically processed with the specific anti-GAD serum replaced by normal rabbit serum. a,b, Frozen sections processed for immunocytochemistry where the primary serum incubation used was (a) normal rabbit serum or (b) specific anti-GAD serum. In b all layers contain GAD synaptic terminals, but the amount varies between layers. In layers II and III periodic 375- μ m variations in density (b, arrows) are found. ×39. c, Schematic representation of the differences between layers in the density of GAD⁺ synaptic terminals. d, Schematic representation of the laminar distribution of GAD+ neuronal cell bodies; this drawing is based on many different sections from colchicinepretreated cortex.

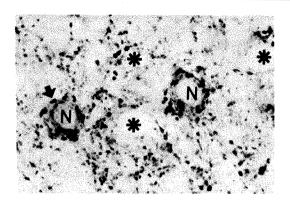
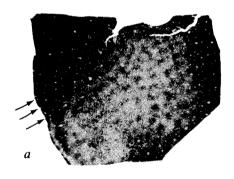


Fig. 2 Photomicrograph from layer IVC in a section reacted with specific anti-GAD serum. Many cell bodies do not stain (*) but are outlined by GAD⁺ terminals. Two GAD⁺ cell bodies show stained cytoplasm but an unstained nucleus (N) and are surrounded by GAD⁺ terminals (arrows). The neuropile also contains many GAD⁺ beaded processes and terminals. ×840.

but the intensity of labelling varied with the layer. The shifts in density corresponded to the laminar boundaries^{5,13} identified in thionine-stained sections. Under higher power (Fig. 2), the GAD staining was localized to cell bodies, beaded processes and small round densities which resembled synaptic boutons. Ribak¹ has shown in electron micrographs of GAD-stained rat visual cortex that these densities are synaptic terminals synapsing on cell bodies and dendrites. Because our preliminary electron microscopy has confirmed this finding for the monkey, we shall provisionally call these small densities terminals. There was no difference in laminar staining of central to mid-peripheral retinal representation in cortex; far-peripheral cortex was not examined.



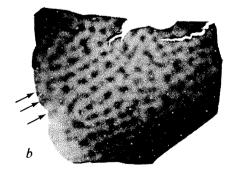


Fig. 3 a, A section cut parallel to the cortical surface passing through layers II and III which has been reacted in specific anti-GAD serum. An orderly pattern of dots aligned into rows can be seen (arrows). $\times 8.5$. b, An adjacent section stained for cytochrome oxidase by a 16-h incubation at 37 °C in phosphate-buffered diaminobenzidine and cytochrome $c^{11,12}$, pH7.4. The same pattern of dots aligned in rows is found. Compare the rows marked by the arrows in the two sections; $\times 80\%$ of the dots in these two sections directly superimpose on one another. $\times 8.5$.

Distribution of GAD⁺ terminals

As judged by both eye and photodensitometry, the upper halves of layers I, IVA and IVC were the most heavily labelled, II, III and VI slightly less so, and lower I, IVB and V were quite lightly labelled (Fig. 1b,c). The labelling in layer I was uniformly distributed and consisted of small, round GAD+ terminals in no obvious pattern. In all the other layers the labelling showed two patterns: GAD+ beaded processes run parallel or perpendicular to the cortical surface, and GAD+ terminals encircle both unstained and GAD+ cell bodies (Fig. 2). Most of the GAD+ terminals are ~ 1 μm in size, but occasionally there are clusters of much larger, 3-5-µm terminals. GAD+ terminals continue at least 50 µm into the white matter below the most inferior layer VI neurones.

Some GAD-reacted sections from the ³H-proline and fucose eye-injected monkey were coated for autoradiography, using Kodak NTB2 emulsion and 3-4-month exposures. Ocular dominance columns were apparent in layer IVC, but no





Fig. 4 Sections of striate cortex cut parallel to the cortex surface from the monkey whose left eye had been injected intravitrally with 3 mCi of equal parts ³H-proline and fucose 14 days before it was killed. The sections were first stained for cytochrome oxidase and then processed for autoradiography. In a the labelled ocular dominance columns from the left eve are visible in the dark-field photomicrograph as periodic repeats in IVC. In b this bright-field picture shows the same cytochrome oxidase-stained section. Layer IVC is darkly stained. Layer III and above contains periodic rows of dots. c, A drawing based on a short series of these doubly processed sections. Layer IVC contains alternating left evelabelled (solid black), right eye-unlabelled (white) ocular dominance columns. Beginning at layer IVA rows of cytochrome oxidase (or GAD⁺) dots lie at regular 350-400-µm intervals. The rows are schematically represented here; rows related to labelled columns are solid lines while rows related to unlabelled columns are dotted lines. There is one row for each column, running parallel to the axis of the column approximately along its centre. The three ocular dominance columns marked by arrows each became a continuous band in this series; all three ran parallel to the rows of dots. The ocular dominance columns and rows of dots related to the unlabelled eye were stained slightly darker in this monkey, possibly indicating some complication in the eye injection which was reflected by decreased cytochrome oxidase activity in cortex.

differential density of GAD+ terminals could be seen at either the column edges or centres, confirming the results from the other three animals. In all four monkeys a periodic repeat of GAD+ density was seen in layers II and III under low power (Fig. 3a; also see Fig. 1b arrows). This pattern consisted of fairly discrete dots arranged in rows formed by less dense connections between the dots. Along the rows the dots had a centre-tocentre spacing of $466 \pm 75 \mu m$ with the rows $378 \pm 55 \mu m$ apart. Under higher power it was apparent that the dots were formed by GAD⁺ cell bodies and a high density of GAD⁺ terminals. In adjacent sections stained for cytochrome oxidase (Fig. 3b), an

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- Ribak, C. J. Neurocytology 7, 461-478 (1978). Dichter, M. A. Brain Res. 190, 111-121 (1980).
- Iversen, L. L., Mitchell, J. F. & Srinivasan, V. J. Physiol., Lond. 212, 519-534 (1971).
 Sillito, A. M. J. Physiol., Lond. 271, 699-720 (1977).
- Lund, J. S., Lund, R. D., Hendrickson, A. E., Bunt, A. H. & Fuchs, A. E. J. comp. Neurol. 164, 287-304 (1975).
- Sternberger, L. in *Immunocytochemistry* (eds Osler, A. & Weiss, L.) (Foundations of Immunology Series, Prentice Hall, New Jersey, 1974).
- Wu, J.-Y. in GABA in Nervous System Function (eds Roberts, E., Chase, T. N. & Tower, D. B.) 7-56 (Raven, New York, 1976).

identical pattern of dots in rows was found. More than 80% of the GAD+ dots superimposed on the cytochrome oxidase dots in an adjacent section. When cortex sections from the 3H-proline/fucose eye-injected monkey were first stained for GAD or cytochrome oxidase and then processed for trans-synaptic autoradiography, the rows of dots in layer III ran parallel to the centres of the ocular dominance columns in layer IVC (Fig. 4).

Distribution of GAD⁺ cell bodies

Neuronal cell bodies have been considered to be specifically stained if they show an unstained nucleus with neuronal characteristics and a heavily stained cytoplasm with a sharp cellular outline (Fig. 2,N). In the two brains which received no colchicine, there were very few GAD+ cell bodies in layers I-IVC, but in layers V and especially VI, regularly spaced GAD neurones 15-20 µm in diameter were found. Small GAD+ cell bodies 8-10 µm in diameter lay in the lower edge of VI or in the white matter just under VI. In the two brains receiving colchicine injections, there was a striking increase in the number of GAD⁺ cell bodies, especially in layers I-IV. Even if our material is an underestimate because of the technical limitations inherent in these in vivo colchicine injections, our experiments indicate that monkey striate cortex contains many GAD+ neurones ranging in size from 9 to 30 µm. GAD neurones occur in every cortical layer and most appear to be various types of stellate neurone. Their distribution is schematically presented in Fig. 1d. In all layers some GAD+ neurones receive GAD+ terminals onto their cell bodies or dendrites. Most unstained cell bodies are outlined by GAD+ terminals in all layers.

Conclusions

Three findings emerge from this study. (1) The striate cortex of Macaca monkeys is very rich in both GAD⁺ cell bodies and synaptic terminals. Unlike rat visual cortex¹, there are clear laminar differences in the distribution of both GAD cell bodies and synapses. (2) There is a striking similarity between GAD distribution and cytochrome oxidase staining. Wong-Riley^{11,12} first pointed out the good correlation between cytochrome oxidase staining, GABA or GAD content and iron concentration in regions like cerebellum and basal ganglia. She suggested that inhibitory GABAergic cells are mitochondria-rich, energy-dependent cells which stain especially heavily for the haem enzyme cytochrome oxidase. This agrees with an earlier study14 where the nerve terminals which became labelled after exposure of rat brain homogenates to ³H-GABA were characterized by a high density of mitochondria within their cytoplasm. Our data support these suggestions and provide more direct evidence that cytochrome oxidase staining may be largely localized to GABAergic neurones. (3) The cytochrome oxidase and GAD⁺ pattern of rows of dots in layers II and III is identical to that shown in Macaca striate cortex using 2-deoxyglucose labelling15 in which similar rows of dots are parallel to the ocular dominance columns. In this study, sections doubly processed for GAD or cytochrome oxidase and trans-synaptic autoradiography also reveal that each row of dots is aligned parallel to the centre of each ocular dominance column. It therefore appears that there are rows of GAD+ inhibitory neurones in supragranular Macaca striate cortex which may be preferentially related to each eye.

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- 11. Wong-Riley, M. T. T. Brain Res. 108, 257-277 (1976)
- 12. Wong-Riley, M. T. T. Brain Res. 171, 11-28 (1979). 13. Lund, J. S. J. comp. Neurol. 147, 455-495 (1973).
- Lenn, J. S. Comp. Neuron. 147, 133–143 (1973).
 Iverson, L. L. & Bloom, F. E. Brain Res. 41, 131–143 (1972).
 Hendrickson, A. E. & Wilson, J. R. Brain Res. 170, 353–359 (1979).

Saito, K., Wu, J.-Y. & Roberts, E. Brain Res. 65, 277-285 (1974).
 Wong, E., Schousboe, A., Saito, K., Wu, J.-Y. & Røberts, E. Brain Res. 68, 133-139

^{10.} Hubel, D. H., Wiesel, T. N. & LeVay, S. Cold Spring Hurb. Symp. quant. Biol. 40, 581-589

LETTERS TO NATURE

Measurements of a solar flare-generated shock wave at $13.1 R_0$

Richard Woo & J. W. Armstrong

Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91109, USA

Most information on interplanetary shock waves generated by solar flares has come from in situ measurements near 1 AU and beyond $^{1-3}$, and little is known about the structure near the Sun. We report here the first measurements of the structure of wind speed, electron density and electron density fluctuations of a shock wave propagating through the acceleration region of the solar wind. The radio-scattering observations consisting of spectral broadening, mean phase and amplitude scintillations were made on 18 August 1979, 13.1 R_0 east of the Sun and near the ecliptic plane using the Voyager 1 2.3- and 8.4-GHz radio signals. The results show that the shock wave had a shock speed of \sim 3,500 km s $^{-1}$. When compared with the average speed based on its transit time to 1 AU, it is seen that substantial deceleration took place as the shock wave propagated outwards from the Sun, a result that is consistent with a blast wave $^{1.4}$.

Figure 1 shows the view of Voyager 1, which was 7.03 AU from the Earth, and the Sun as seen from the Earth on 18 August 1979. It is clear that the Voyager 1 radio path was ideally positioned for studying the shock wave which was apparently generated by a solar flare located at N 09 E 90 (ref. 5). The longitudinal extent of the interplanetary disturbance was large because the shock was measured 90° away near 1 AU by the ISEE-3 magnetometer at 05.50 UT on 20 August (E. J. Smith, personal communication). The average shock speed based on the transit time to ISEE-3 is 1,053 km s⁻¹. A type II radio burst associated with this shock wave was also tracked in the 30 kHz-2 MHz frequency range by the radioastronomy experiment on ISEE-3 (ref. 5).

The results presented here are derived from the bandwidth-reduced digitally recorded data of the 'open-loop' receivers at the 64-m NASA Deep Space station located in Goldstone, California⁶. The 2.3- and 8.4-GHz signals were recorded in bandwidths of 1 and 3 kHz. respectively. For studying the amplitude scintillations and mean phase, these signals were further reduced to 100 Hz using a digital phase lock loop procedure⁷.

The radio measurements and the inferred results are summarized in Figs 2 and 3, respectively. Shock arrival occurs at ~ 15.01 UT, and based on the transit time from the Sun to 13.1 R_0 , we obtain a shock speed of 3,509 km s⁻¹. The time histories of spectral broadening ^{8,9} of bandwidth B at 2.3 and 8.4 GHz for 1-min integration times are shown in Fig. 2a. B is the full width between frequencies enclosing half the area under the radio-frequency spectrum. The pre-shock bandwidth at 2.3 GHz is ~ 1 Hz and is consistent with previous measurements of the quiet solar wind ⁸. During shock passage the bandwidths increase

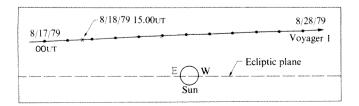


Fig. 1 View of Voyager 1 and the Sun as seen from the Earth on 18 August 1979.

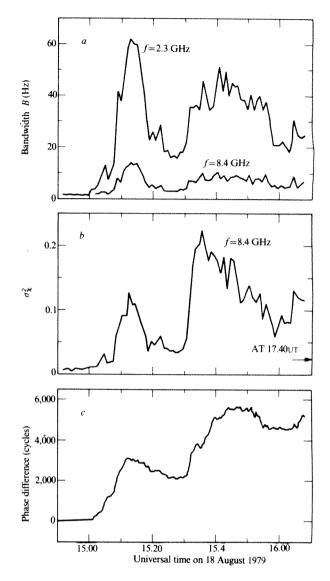


Fig. 2 Voyager 1 radio-scattering observations as a function of UT on 18 August 1979. a, Spectral broadening bandwidth B at 2.3 and 8.4 GHz. b, Variance σ_{χ}^2 of the log-amplitude scintillations at 8.4 GHz. The arrow shows the σ_{χ}^2 level at 17.40 UT. c, Difference between the 2.3 GHz and 3/11 of the 8.4-GHz phases.

considerably and two regions of enhanced bandwidth are observed. Although such transient events are rare during the solar minimum portion of the solar cycle⁸, they have been observed previously around the solar maximum^{9,10}. By examining the frequency dependence⁸ of B at 2.3 and 8.4 GHz we find that the average spectral index p of the three-dimensional power-law spatial wavenumber spectrum is 3.66 with a standard deviation of 0.11. The density spectrum, which is essentially Kolmogorov, is steeper than the quiet Sun spectrum for similar scales in this region of the solar wind¹¹, but consistent with other measurements during disturbed solar wind conditions¹⁰. Bandwidth increases are caused by enhancements in turbulence (electron density fluctuations) or acceleration of the solar wind⁸. These two effects can be separated by analysing the amplitude scintillations

The time history of the variance, σ_{xr}^2 of the log-amplitude scintillations at 8.4 GHz is shown in Fig. 2b. For weak scintillations ($\sigma_x^2 \ll 1$), σ_x^2 is related to the scintillation index m by $\sigma_x^2 = m^2/4$. The amplitude scintillations at 2.3 GHz are not

useful during shock passage because they are strong and saturated. The structure constant c_n of the refractive index fluctuations which characterizes the strength of the turbulence has been inferred from the 8.4-GHz scintillations and is shown in Fig. 3a. The corresponding scale for c_{n_e} , the structure constant for electron density fluctuations, is shown on the right. A spherical wave radio analysis has been used to interpret the scintillations 12. Before shock arrival c_n is assumed to vary as R^{-2} (R is heliocentric distance) along the radio path¹³. As the shock crosses the radio path, we assume that the contribution to σ^2 along the radio path outside the shock remains the same, and attribute the excess of the observed σ_x^2 to a uniform distribution of c, within the shock. The extent of the radio path traversed by the shock is defined by assuming that the shock is spherical in shape, emanates from the surface of the Sun and travels at a speed of $3,509 \text{ km s}^{-1}$. Figure 3a indicates that there are two regions of enhanced small-scale electron density fluctuations, corresponding to the two regions of increased spectral broadening. In these regions the turbulence representing scale sizes smaller than the Fresnel size (73 km at 8.4 GHz) increases by a factor of 3.7.

Two methods are used to deduce the solar wind flow speed (v) profiles shown in Fig. 3b. The log-amplitude scintillations are

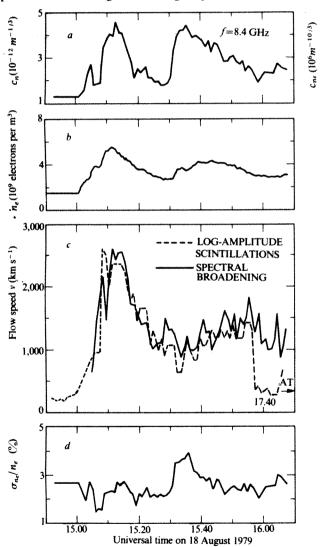


Fig. 3 Measurements of the shock wave inferred from the radio observations of 18 August 1979 shown in Fig. 2. a, Strength of turbulence as characterized by the structure constants c_n and c_{ne} of refractive index and electron density fluctuations, respectively. The results for c_n are for 8.4 GHz. b, Electron density n_e . c, Solar wind flow speed, v, as deduced using two methods (see text). The arrow corresponds to the flow speed measured at 17.40 UT. d, Ratio σ_{ne}/n_e in per cent, where σ_{ne} is the r.m.s. electron density fluctuation in scales smaller than the X-band Fresnel scale (73 km).

spectrum analysed in 1-min intervals so that velocity can be determined for these times from the location of the 'knee' or Fresnel cutoff using a spherical wave radio analysis 14. Although this method has considerable uncertainties when applied to quiet observations near the Sun because of the rounding of the knee due to fluctuations of the order of the mean11, it is useful in the case of the shock wave because the mean speed is large and velocity fluctuations are not significant. Generally, the correlation of multiple station intensity scintillations is the preferred radio method for measuring solar wind speed11,15. Near the shock front, two knees are present in the spectra, one due to the shock and the other to the slower ambient wind outside the shock. In these cases the higher velocity is always plotted. In a few instances around 15.00 UT, velocities could not be estimated due to the smearing of the knee by a rapidly rising velocity. At 15.58 UT the wind speed decreases quite abruptly. In this region the spectra again show two peaks. For these cases, however, the slower speed is chosen because it is clear from the level of the spectrum that it is due to a slowing of the flow speed along a radial cut through the shock and not to the slower ambient solar wind outside the shock.

The second method for estimating velocity involves using the measurement of σ_x^2 to remove the effect of turbulence from the spectral broadening observation in order to solve for velocity. This method requires that p be known. As this has been determined to be Kolmogorov, we can use the relationships that have been derived for a spherical wave ¹². The velocity profile inferred using this method is also shown in Fig. 3c. The agreement between the two independent methods is remarkable. When two velocities are present, the spectral broadening technique senses the faster one. This explains why this method gives correct estimates near the shock front but incorrect estimates beyond 15.56 UT.

As the shock crosses the Voyager radio path the flow speed rises sharply from 200 to 2,600 km s⁻¹, a factor of 13. Near the peak the velocities are fairly well determined and we estimate that the 2,600 km s⁻¹ measurement is accurate to within 20%. Elsewhere, the differences between the two curves in Fig. 3b probably represent the uncertainties in the velocity determination. Because the gross variation in velocity is substantial, it is easily recognizable and measured in spite of the uncertainties.

The final observation is phase at 2.3 GHz shown in Fig. 2c. This is formed by taking the difference between the S band and 3/11 (the frequency ratio of S and X bands) of the X-band phases¹⁶. During the phase lock loop procedures mentioned earlier, the S-band phase is non-coherently estimated using the X-band phase as a guide in the loop. In this way, any 2π ambiguities due to tracking errors are minimized. Because 'absolute' phase was not measured, Fig. 2c represents only the change in total electron content. We have deduced the electron density profile in Fig. 3b in the following manner. At 13.1 R_0 , we assume that the electron density $n_e = 1.5 \times 10^9$ electrons per m³. Before shock arrival we calculate the total electron content assuming a R^{-2} dependence for n_e . As the shock crosses the path we assume that the contribution to the total electron content outside the shock remains the same and attribute the excess of the observed electron content to a uniform distribution of n_e inside the shock. The extent of the shock is defined in the same way as that used for deducing c_n . Note that this inversion scheme used to obtain both c_n and n_e is most accurate near the shock front. Further back in the wake it becomes less accurate because, strictly speaking, c_n and n_e are not uniformly distributed along the radio path inside the shock. Nevertheless, it is a reasonable approximation and the general profiles for c_n and n_e should not be significantly affected. We note that the peak density increases by a factor of ~ 3.7 over the pre-shock value and is consistent with the theory for a strong shock 1,4. As expected for a shock wave, the peak in the turbulence characterized by c_n lags the peak in mean electron density.

Using the Rankine-Hugoniot relationships^{1,4}, pre-shock values of $n_e = 1.5 \times 10^9$ electrons per m³ and v = 200 km s⁻¹, post-shock values of $n_e = 5.5 \times 10^9$ electrons per m³ and

 $v = 2,600 \text{ km s}^{-1}$, we compute a shock speed of 3,500 km s⁻¹. which is consistent with the shock speed of 3,509 km s⁻¹ obtained from the transit time to 13.1 R_0 . According to time delay radio measurements¹⁷, electron densities in the solar wind at 13.1 R_0 may be as high as 3×10^9 electrons per m³. We have derived density profiles similar to Fig. 3b corresponding to 2×10^9 and 3×10° electrons per m³, and obtained shock velocities of 3,660 and 4,320 km s⁻¹, respectively. The fact that these shock speeds are higher than 3,500 km s⁻¹ indicates that some acceleration could have taken place between the Sun and $13.1 R_0$. This behaviour has been observed in white light coronograph measurements¹⁸, although shock speeds as high as 3,500 km s⁻¹ seem to be very rare 18,19. Questions do arise about the location of the shock fronts in the white light coronograph pictures 20 and simultaneous measurements with radio-scattering observations would be desirable. It is clear that the shock speed inferred from the Rankine-Hugoniot relationships has a rather high uncertainty because of two factors. First, the 20% accuracy in the flow-speed estimate translates approximately into a 20% accuracy in the deduced shock speed. Second, the uncertainty in the pre-shock electron density also leads to inaccuracy in the inferred shock speed. For example, by increasing the pre-shock electron density to 2×10^9 and 3×10^9 electrons per m³ we find that the shock speed increases by 5 and 23%, respectively.

We have plotted σ_{n_e}/n_e (σ_{n_e} is r.m.s. electron density fluctuations) in per cent in Fig. 3d. In this case σ_{n_e} has been computed for scale sizes smaller than the Fresnel size at 8.4 GHz (73 km). The fluctuations amount to several per cent before and after shock passage. It is interesting that this ratio shows a noticeable increase at about 15.35 UT. This represents the start of the second region of enhanced amplitude scintillations, and may correspond to the turbulent 'driver gas' of the shock wave. In contrast, the first region of enhanced amplitude scintillations corresponds to the compressed region of the shock wave.

The radio data end at about 16.08 UT before the completion of shock passage. However, some data were collected for a few minutes around 17.40 UT and yield a wind speed of 320 km s $^{-1}$ and $\sigma_x^2 = 0.0225$ at 8.4 GHz. These values, indicated by arrows in Figs 2b and 3c, show that the solar wind has almost returned to its pre-shock state.

It is clear that radio-scattering measurements using spacecraft radio signals complement similar measurements using natural radio sources21 and constitute a powerful tool for identifying and studying the detailed structure of coronal transients and disturbances. Plasma measurements of the 18 August shock wave were made by ISEE-3 at 1 AU. Information on shock speed beyond 13.1 R_0 but still close to the Sun will also be available from the ISEE-3 type II radio-burst observations. Future studies will correlate these measurements with the present results and will undoubtedly lead to an improved understanding of the evolution and deceleration of shock waves in the solar wind.

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- Hundhausen, A. J. Coronal Expansion and Solar Wind (Springer, Berlin, 1972). Dryer, M. Space Sci. Rev. 17, 277-325 (1975). Smith, E. J. & Wolfe, J. H. Space Sci. Rev. 23, 217-252 (1979).

- Parker, E. N. Interplanetary Dynamical Processes (Wiley, New York, 1963). Cane, H. V., Stone, R. G., Fainberg, J., Steinberg, J. L. & Hoang, S. NASA TM 82049 (Goddard Space Flight Center, 1980).

 Berman, A. L. & Ramos, R. IEEE Trans. Geosci. Rem. Sens. GE-18, 11-14 (1980).
- Woo, R., Kendall, W., Ishimaru, A. & Berwin, R. JPL TM 33-644 (Jet Propulsion Laboratory, Pasadena, 1973).
- Woo, R. & Armstrong, J. W. J. geophys. Res. 84, 7288-7296 (1979). Goldstein, R. M. Science 166, 598-600 (1969).
- Woo, R. & Armstrong, J. W. Space Sci. Rev. (in the press).

- Scott, S. L. thesis, Univ. California, San Diego (1979).
 Ishimaru, A. Wave Propagation and Scattering in Random Media Vol. 2 (Academic, New York, 1978).

- York, 1978).

 3. Armstrong, J. W. & Coles, W. A. Astrophys. J. 220, 346-352 (1978).

 4. Woo, R. Astrophys. J. 201, 238-248 (1975).

 5. Armstrong, J. W. & Woo, R. Astr. Astrophys. (in the press).

 6. Woo, R., Yang, F. C., Yip, K. W. & Kendall, W. B. Astrophys. J. 210, 568-574 (1976).

 7. Esposito, P. B., Edenhofer, P. & Lueneburg, E. J. geophys. Res. 85, 3414-3418 (1980).

 8. Gosling, J. T. et al. Solar Phys. 48, 389-397 (1976).
- 19. Dulk, G. A. in Radio Physics of the Sun (eds Kundu, M. R. & Gergely, T. E.) 419-433 (Reidel, Dordrecht, 1980)
- 20. Maxwell, A. & Dryer, M. Solar Phys. (in the press).
- 21. Rickett, B. J. Solar Phys. 43, 237-247 (1975)

Chemical composition of the atmosphere of Venus

V. A. Krasnopolsky & V. A. Parshev

Space Research Institute, Academy of Sciences. 117810 Moscow, USSR

Measurements onboard the Venera 11, 12 (refs 1-4) and Pioneer Venus^{5,6} spacecrafts stimulated us to study the chemical composition of the subcloud atmosphere of Venus in terms of the thermochemical equilibrium calculations, comparison of typical mixing and chemical times and a rule of heightindependent element mixing ratio in the absence of condensation7. The photochemistry of the atmosphere down to 50 km was calculated using transport effects and number densities of CO₂, H₂O, HCl, SO₂ and CO at the lower boundary and rate coefficients of 102 reactions. These reactions include catalytical cycles of COCl and COCl2 which accelerate O2 destruction and CO₂ formation. Altitude profiles of 27 components agree well with those measured in the upper and middle atmosphere. H2O and SO₂ mixing ratios are very similar and sharply decrease at 60 km due to SO₂ photolysis and sulphuric acid formation. Calculations show that sulphuric acid and sulphates are the main components of the second and third modes of particle size distribution in the upper and middle cloud layers. The lower cloud layer may consist of AlCl3 and FeCl3.

A model of atmospheric composition should produce vertical profiles of all atmospheric components from data on the abundance of parent molecules. This requires a knowledge of the chemical rate constants k_i , the values of temperature T and the eddy mixing coefficient K. As those constants are known in a few cases only, the number of components considered has to be limited and aeronomic analyses restricted to those parts of the atmosphere where these limitations apply.

The subcloud atmosphere (0-50 km) has a fairly high temperature and pressure (740 K and 90 atm near the surface) and is not exposed to solar UV radiation. Although thermochemical processes involving stable molecules dominate the region their rate constants are generally unknown. Hence we calculated only the thermochemical equilibria from the free enthalpy of the components. The comparison of the mixing times $\tau_m = H^2/K$ where H is the scale height, and of the chemical equilibrium, $\tau_c = (\sum_i k_i n_i)^{-1}$ where n_i is the concentration of a reagent considered, shows that mixing dominates chemical reactions in the whole lower atmosphere. The catalytic effect of the ground tends to accelerate chemical processes. For the order of magnitude comparison we used $K \approx 10^4$ $10^6 \text{ cm}^2 \text{ s}^{-1}$, $k_i = 10^{-10} \exp(-25,000/T) \text{ cm}^3 \text{ s}^{-1}$, mixing ratio $f_i \simeq 10^{-5}$; chemical processes on the ground were assumed to be accelerated by two orders of magnitude.

The atmospheric composition should then be approximately constant throughout the subcloud and can be determined by the thermochemical equilibrium near the surface (Qyama et al. assumed thermochemical equilibrium throughout the subcloud atmosphere). Calculations were based on the first data from Venera 11, 12 and Pioneer Venus experiments. The initial data chosen were concentrations of CO₂, N₂, SO₂ for which Soviet and US measurements had given similar results, concentrations of H₂O and S₂ (our estimate based on the spectroscopic

Table 1	The calculated com	position of the	subcloud atm	osphere of Venus

Gas	CO_2	N_2	SO_2	H_2O	S_2	HCl	HF	CO	COS	H_2S	H_2
Mixing ratio f_i	0.96	3.4×10^{-2}	1.3×10^{-4}	2×10^{-4}	1×10^{-7}	1×10^{-6}	1×10^{-8}	1.5×10^{-5}	2×10^{-5}	3×10^{-7}	2×10^{-8}

measurements⁴), of HCl and HF from ground-based observations⁸. Our calculations are summarized in Table 1 (inert gases are not taken into account).

Components whose calculated mixing ratio was lower than 10^{-10} are not given in Table 1.

Our calculations and the related estimates are confirmed by further processing of the observational data. The height variation of $f_{\rm CO}$ was determined from Venera 12 data³ for which $f_{\rm CO}$ ranges from 3×10^{-5} above 36 km to 1.5×10^{-5} near the surface. Gas chromatography measurements on Pioneer Venus⁵ first indicated that CO was not present in the lower atmosphere, but then produced a similar dependence on height. Small amounts of $\rm H_2S$ and $\rm H_2$ initially suggested by Venera 12 data have not been confirmed, while the indication of COS seems reliable³.

Similar calculations based on the data of H₂O and O₂ mixing ratios measured by the gas chromatograph on Pioneer Venus gave an abundance of H₂SO₄ which was much higher than the bulk of the cloud layer.

We now consider the experimentally-obtained height dependences of mixing ratios. Here we take into account that if condensation is absent in a certain atmospheric region and if eddy mixing dominates the molecular diffusion, the relative abundances of elements should be height-independent. This enables the altitude dependences observed to be considered qualitatively.

The height dependence of CO might explain its gradual conversion into COS with decreasing height. The sum $f_{\rm CO}$ + $f_{\rm COS}$ = 3.5×10^{-5} agrees well with the spectroscopic measurements⁸ of CO near the top clouds; $f_{\rm COS}$ should decrease with height and be $\sim 10^{-6}$ above 36 km. Its decrease should be accompanied by a slight ($\sim 10\%$) increase of $f_{\rm SO_2}$ up to cloud deck. But in this case $f_{\rm COS}$ should be $5-10\times 10^{-6}$ at 22 km, which exceeds the upper limit of 2×10^{-6} obtained by the third sample of Pioneer Venus gas chromatography. Because the drastic variation of sulphuric compound abundance at 20–25 km recorded by the Pioneer Venus mass spectrometer⁶ was an experimental artefact it was ignored.

It is difficult to explain height variations of $f_{\rm H_{2O}}$ (2×10⁻⁴ in the cloud layer and 2×10⁻⁵ near the surface) deduced from the spectroscopic measurements on Venera spacecraft⁴. While spectroscopic measurements of $f_{\rm H_{2O}}$ seem more convincing than the data of Pioneer Venus gas chromatography⁵ where there could be perturbation effects from the spacecraft (values of $f_{\rm H_{2O}} = 1-5 \times 10^{-3}$ were obtained), we believe that errors in the measurements⁴ and the initial data used for interpretation do

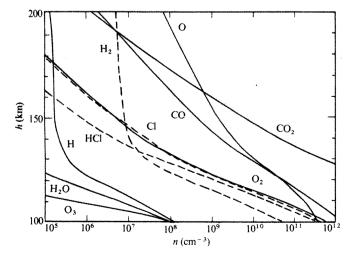


Fig. 1 Composition of the upper atmosphere of Venus. Dashed lines show the components for which the abscissa scale is enlarged 1,000 times.

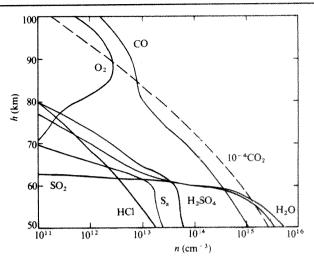


Fig. 2 Composition of the atmosphere of Venus between 50 and 100 km.

not preclude the possibility of $f_{\rm H2O}$ being independent of height. The other possibility is the presence of some hydrogen-bearing gas with mixing ratio $\sim 10^{-4}$.

We calculated the model composition in the atmosphere down to 50 km by solving a set of differential equations which describe the behaviour of 27 components (CO₂, H₂O, HCl, SO₂ and the products of their photolysis) involved in 102 reactions and include transport effects.

As our model takes into account both chlorine and sulphur compounds, the number of reactions compared with previous calculations⁹⁻¹¹ almost doubled. Our model uses an approximate description of the processes of radiation and matter transport in the cloud layer. We have developed a more universal method of imposing boundary conditions on daughter components, its special cases being photochemical and diffusion equilibrium.

We have considered not only chlorine oxides but also COCl and COCl₂ participating in cycles which are essential to O₂ destruction and CO₂ formation. Reactions of COCl and COCl₂ formation and removal and their rate coefficients¹² are shown in Table 2.

The reaction between H and Cl₂ provides the major sink for odd H* and Cl*. Our model also includes processes of sulphuric acid and sulphur aerosol formation from SO₂, four types of excited components and a long series of reactions.

Figure 1 shows the calculated composition of the upper atmosphere. The results of Pioneer Venus mass-spectrometric $^{13.14}$ and optical 15 measurements agree well with the calculations. Taking into account strong diurnal variability (our cacluations were made for solar zenith angle 60°) the comparison should be done for the altitudes at which CO_2 number densities are equal. Figure 2 shows the composition of the atmosphere and the cloud layer at 50–100 km, concentrations of many of minor components with $n < 10^{11}$ cm⁻³ are not included there. Figure 3 shows number densities of chlorine compounds. As we have not got room for a detailed analysis of all the components, we briefly describe some typical features of the H_2O , SO_2 , H_2SO_4 , aerosol sulphur S_a and O_2 distributions for which data are available.

The net photochemical transformation of SO₂ in the presence of H₂O can be described by

$$3SO_2 + 4H_2O \rightarrow 2H_2SO_4 \cdot H_2O + S$$

for 85% acid drops. Hence, the profiles of SO_2 and H_2O should be similar within most of the cloud layer and their concentrations at 50 km should differ by a factor of 1.3–1.8 for 80-85% acid drops. Our profiles agree well with measurements^{3,4} on

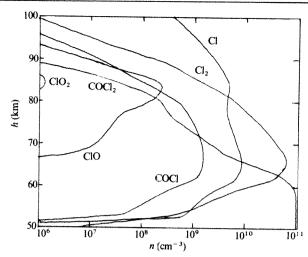


Fig. 3 Number density of chlorine compounds found in the atmosphere of Venus.

Venera spacecrafts and suggest that these measurements are correct and that H_2SO_4 formation is the basic drying agent in the above-cloud atmosphere.

Both profiles are determined by upward transport of SO_2 and H_2O from the subcloud atmosphere and their photochemical transformation into sulphuric acid drops through the SO_2 photolysis, SO_3 formation and absorption of H_2O . Analytically derived ratios and computations of H_2O and SO_2 number densities show that the eddy mixing coefficients should be $K = 2-5 \times 10^4 \text{ cm}^2 \text{ s}^{-1}$ at 50-60 km, to ensure a good fit with the spectroscopic ground-based and satellite measurements $^{16-18}$ of SO_2 and H_2O .

Eddy mixing determines not only H_2O and SO_2 number densities but also the amount of sulphuric acid: the higher K, the more H_2SO_4 should be in the cloud layer. The calculated amount of H_2SO_4 corresponding only to the second mode of the particle size distribution^{19,20} may be obtained for $K=100~\rm cm^2~s^{-1}$ which is not consistent with atmospheric dynamics and the observed SO_2 distribution.

A good agreement of the calculated values with the measured SO_2 number densities and the aerosol amount in the middle cloud layer may be obtained when $K=2\times10^4\,\mathrm{cm^2\,s^{-1}}$, in this case both the second and the third modes^{19,20} of particle size distribution in the middle cloud layer are dominated by sulphuric acid or the products of its further conversion (sulphates). For this conclusion, sulphuric acid must predominate drying in the above-cloud atmosphere.

Sulphur particles can constitute only a minor component of the cloud layer; their mass is an order of magnitude smaller than that of sulphuric acid and its products. The calculated concentration of sulphuric acid varies with height and is 80-87% at 55-70 km. The results of calculations are very sensitive to small variations of the $\rm H_2O/SO_2$ ratio at the lower boundary (50 km). A 10% increase of $f_{\rm H_2O}$ is followed by a sharp increase in humidity in the upper cloud layer, far beyond the limits of the ground-based spectroscopic measurements 14 ($f_{\rm H_2O}=10^{-(5-7)}$ at 62-65 km), by the much larger amounts of atomic hydrogen in the upper atmosphere.

The main paths of the formation of O_2 from atomic oxygen produced by CO_2 photolysis are the reactions

$$O+ClO \rightarrow O_2+Cl$$

 $O+O+CO_2 \rightarrow O_2+CO_2$
 $O+OH \rightarrow O_2+H$

Its destruction occurs through

$$COCl + O_2 \rightarrow CO_2 + ClO$$

 O_2 forms a layer 15-km thick at 90 km. The total amount of O_2 is 3.7×10^{19} cm⁻² which is a factor of 1.5 lower than the spectroscopic limit²¹. It is inversely proportional to K, hence $K \le$

 6×10^5 cm² s⁻¹ at 90 km. The above-cloud atmosphere cannot be a source of O_2 in the cloud layer where $f_{O_2} \simeq 4\times10^{-5}$, according to Pioneer Venus measurements⁵. The presence of such amounts of O_2 in the clouds should lead to another order-of-magnitude reduction of the aerosol sulphur abundance but it does not contradict the spectroscopic limit as the O_2 profile will be similar to the profiles of SO_2 and H_2O . However, this does not explain the appearance of O_2 in the cloud layer as the presence of O_2 is impossible in the lower atmosphere.

For the calculations and measurements to agree K must be 2×10^4 cm² s⁻¹ at 50-60 km, $K \le 6 \times 10^5$ at 90 km; the data on atomic oxygen in the upper atmosphere impose $K = 4 \times 10^6$ at Z > 110 km. At heights of 60-110 km the values obtained are well described by the dependence $K \sim n^{-1/2}$ which agrees well with the Pioneer Venus results¹⁴ and was assumed in our calculations.

The photochemistry of the atmosphere of Venus at 58-96 km was considered recently by Winick and Stewart¹¹. The main difference in input data between their model and ours is the absence of some chemical processes (53 instead of our 102 reactions); the most important of these are given in Table 2. The other difference is the assumption of height-independent H_2O mixing ratio $f_{H_2O} = 10^{-6}$ instead of $f_{H_2O} = 2 \times 10^{-4}$ at the lower boundary in our calculations, which shows very non-uniform distribution of H_2O (Fig. 2). Number densities of CO, O_2 , O_3 , SO calculated by Winick and Stewart¹¹ exceed the results of spectroscopic observations^{8,21-23}.

The X-ray fluorescence analysis 24 of the cloud-layer particles of Venera 11 and 12 indicated the presence of considerable amounts of chlorine ($\sim 2 \times 10^{-9}$ g cm $^{-3}$) in the aerosol. The presence of HCl drops is impossible because of the presence of sulphuric acid, and AlCl₃ is the most probable chlorine bearing material. If the Al₂O₃ abundance in the Venus lithosphere is similar to its abundance on Earth (15% in sediments), then the presence of HCl in the atmosphere implies a certain presence of aluminium chloride due to the heterogeneous thermochemical reaction

$$Al_2O_3 + 6HCl = 2AlCl_3 + 3H_2O$$

If its mixing ratio is 10^{-4} , it should be condensing at the lower cloud layer, and the calculated parameters of the layer (thickness, 2km; $\rho_{\rm max}=4\times10^{-7}~{\rm g~cm^{-3}}$ for $K=2\times10^4~{\rm cm^2~s^{-1}}$; size particle, 5 μ m) do not differ much from those measured ¹⁹.

Thus the third particle mode may have different composition in the middle and lower cloud layers. This is not a contradiction, because the nephelometer measurements²⁰ show that the middle cloud layer is very uniform and may have a photochemical nature while the lower cloud layer is variable and has a condensation origin.

The presence of chlorides in the cloud layer has recently been confirmed. The atmospheric albedo in the near UV is explained by the presence of two absorbers 18 : SO₂ and 1% FeCl₃ solution in the concentrated sulphuric acid. FeCl₃ condensation probably occurs near the lower cloud layer and the fine-fraction of the condensate with sizes of $\sim 0.5~\mu m$ moves through mixing in the upper cloud layer to act as sites of H_2SO_4 condensation. With

Table 2 Reactions of COCl and COCl₂ in cycles of O₂ destruction and CO₂ formation

No. Reaction	Rate coefficient (cm³ s ⁻¹ and cm ⁶ s ⁻¹ for two- and three- body reactions)
$ \begin{array}{lll} (1) & {\rm CO+Cl+CO_2} \to {\rm COCl+CO_2} \\ (2) & {\rm COCl+O_2} \to {\rm CO_2+ClO} \\ (3) & {\rm COCl+O} \to {\rm CO_2+Cl} \\ (4) & {\rm COCl+Cl_2} \to {\rm COCl_2+Cl} \\ (5) & {\rm COCl+Cl} \to {\rm CO+Cl_2} \\ (6) & {\rm COCl+CO_2} \to {\rm CO+Cl+CO_2} \\ (7) & {\rm COCl+H} \to {\rm CO+HCl} \\ (8) & {\rm COCl_2+O} \to {\rm CO_2+Cl_2} \\ \end{array} $	$3 \times 10^{-33} 2 \times 10^{-14} 10^{-13} 6 \times 10^{-13} \exp{(-1,400/T)} 6 \times 10^{-14} \exp{(-400/T)} 10^{-12} \exp{(-3,200/T)} 10^{-13} 10^{-14}$

this size ratio the resulting FeCl₃ solution concentration is close to 1%.

Thus aerosol particles in the upper cloud layer consist of drops of 80-85% sulphuric acid and a small amount of FeCl₃ (~1%). In the middle cloud layer the major components are sulphuric acid and its chemical conversion products such as sulphates.

The presence of nitrosylsulphuric acid²⁵ seems doubtful due to the smaller amount of lightning26 and its production of NO without further conversion to NO2, which needs large amounts

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- Istomin, V. G., Grechnev, K. V. & Kochnev, V. A. Pisma AZh 5, 211-216 (1979).
 Istomin, V. G., Kochnev, K. V. & Ozerov L. N. Cosmic Res. 17, 703-707 (1979).
 Gelman, B. G. et al. Pisma v AZh 5, 217-221 (1979); Cosmic Res. 17, 708-713 (1979); Space Res. (in the press).
- Grace Res. (in the press).

 Moroz, V. I. et al. Pisma AZh 5, 222-228 (1979); Cosmic Res. 17, 727-742 (1979).

 Oyama, V. I., Carle, G. C., Woeller, F. & Pollack, J. B. Science 203, 802-805 (1979);

 J. geophys. Res. 85, 7891-7902 (1980).
- geopnys. Res. 65, 7631-7502 (1900).
 Hoffman, J. H., Hodges, R. R., McElroy, M. B., Donahue, T. M. & Kolpin, M. Science 205, 49-52 (1979); J. geophys. Res. 85, 7882-7890 (1980).
 Krasnopolsky, V. A. & Parshev, V. A. Cosmic Res. 17, 763-771 (1979); 19, 87-103,
- 261–278 (1981); Preprints No. 590 and 591 (Space Research Institute, Moscow, 1980). Onnes, P., Connes, J., Kaplan, L. D. & Benedict, W. S. Astrophys. J. 147, 1230–1237 (1967); 152, 731–743 (1968).
- Sze, N. D. & McElroy, M. B. Planet. Space Sci. 23, 3763-786 (1975). Prinn, R. G. J. atmos. Sci. 32, 1237-1247 (1975).
- 11. Winick, J. R. & Stewart, A. I. J. geophys. Res. 85, 7849-7860 (1980).

of HO2 and is necessary for nitrosylsulphuric acid formation. The lower cloud layer most probably consists of aluminium and iron chlorides. The presence of ammonium chloride is less likely as thermochemical calculations preclude the possibility of ammonia appearance in the lower atmosphere, and the photochemical formation of ammonia is highly unlikely.

Note added in proof: Further data on the temperature and position of the lower cloud layer preclude AlCl₃ being its main component.

- 12. Kondratiev, V. N. Rate Constants of Gas Reactions (Nauka, Moscow, 1971).
- 13. Niemann, H. B. et al. Science 203, 770-772 (1979); 205, 54-56 (1979).
- Niemann, H. B. et al. Science 203, 70-772 (1979); 203, 20-20 (1979).
 von Zahn, U., Krankowsky, D., Manersberger, K., Nier, A. O. & Hunten, D. M. Science 203, 768-770 (1979); J. geophys. Res. 85, 7829-7840 (1980).
 Stewart, A. I., Anderson, D. E., Esposito, L. W. & Barth, C. A. Science 203, 777-778
- Fink, U., Larson, H. P., Kuiper, G. P. & Poppen, R. F. Icarus 17, 617-631 (1972)
- Esposito, L. W., Winick, J. R. & Stewart, A. I. Geophys, Res. Lett. 6, 601-604 (1979). Krasnopolsky, V. A. Moroz, V. I. & Zasova, L. V. Space Res. (in the press).
- Knollenberg, R. G. & Hunten, D. M. Science 205, 78-74 (1979) Blamont, J. & Ragent, B. Science 205, 67-70 (1979).
- Traub, W. A. & Carleton, N. P. in Exploration of Planetary Atmospheres (eds Woszyck, A. & Iwaniszewska, C.) 223-225 (Reidel, Dordrecht, 1974).
- Owen, T. & Sagan, C. Icarus 16, 557-568 (1972). Krasnopolsky, V. A. Planet. Space Sci. 27, 1403-1408 (1979).
- Surkov, Yu. A. et al. Pisma AZh. 5, 7 (1979)
- Watson, A. J. et al. Geophys. Res. Lett. 6, 743-746 (1979)
- Krasnopolsky, V. A. Cosmic Res. 18, 429-434 (1980).

Radiation-enhanced exhalation of hydrogen out of stainless steel

M. Ikeya*, T. Miki* & M. Touge†

*Technical College and †Faculty of Engineering, Yamaguchi University, Tokiwadai, Ube 755, Japan

The state and diffusion of hydrogen in solids have been studied extensively1,2, and the transport of hydrogen and its isotopes in metals has been found to involve classical diffusion and quantum effects such as tunnelling migration and small polaron hopping^{3,4}. The permeability and diffusion of hydrogen isotopes, particularly tritium (T), have been studied in a D-T fusion device5. Diffusion of tritium in the first wall of fusion reactor is expected to occur under high radiation fields due to plasma radiation, self β rays from the held-up tritium and neutroninduced radioactivity⁶. Heinrich et al. observed α -ray enhanced hydrogen permeation in an iron foil due to activated adsorption of the ionized hydrogen gas on the metal surface⁷. The γ -ray irradiation from a 1 mCi source, however, caused no appreciable change in permeation in 316 stainless steel⁵. No detectable loss of tritium was observed from a niobium sample containing 1% tritium⁸: the effect of the self β rays on tritium diffusion in niobium seems to be negative. We report here an experimental study of radiation-enhanced diffusion of hydrogen in hydrogencharged stainless steel using direct observation of hydrogen exhalation and lattice dilatation.

Hydrogen was charged into type 304 stainless steel of thickness ~100 µm by cathodic charging in 0.5 M H₂SO₄ solution at a current density of 30 mA cm⁻². Exhalation of gaseous hydrogen was detected using volume measurement of hydrogen gas bubbles by immersing the specimen in liquid glycerine and monitoring the increase of the liquid level in a glass microcylinder. Hydrogen bubbles on the surface were examined microscopically by coating the specimen with glycerine and hydrogen-induced lattice expansion was studied by X-ray diffraction. To enhance hydrogen evolution the X-ray tube was operated at 45 kV and 15 mA. The exposure rate of 30 ± 5 rad was determined with thermoluminescence dosimeters (TLD) of CaSO₄ taking the energy dependence of TLD into account. In some experiments γ -irradiation from a 60 Co source was also

Figure 1 shows the enhancement by X-ray irradiation of the gas exhalation rate out of stainless steel as a function of the time after 30 min of hydrogen charging. Exhalation rates, both with and without irradiation, decreased gradually with the time but the decrease was not monotonic presumably because of nonuniform distribution of hydrogen in the specimen. The exhalation rate of 4×10^{-6} cm³ cm⁻² s⁻¹ at 10 min is enhanced to 6×10^{-6} cm³ cm⁻² s⁻¹ by X-ray irradiation. The exhalation rate of hydrogen molecules deduced from the hydrogen gas volume exhalation rate is shown on the right coordinate of Fig. 1. The total volume of hydrogen gas exhaled was $\sim 3 \times 10^{-2} \text{ cm}^3 \text{ cm}^{-2}$ or 8 × 10¹⁷ H₂ cm⁻² indicating an initial average concentration of $\sim 1.6 \times 10^{20} \,\mathrm{H}$ atom cm⁻³ for a specimen $\sim 100 \,\mathrm{\mu m}$ in thickness—a number roughly two orders of magnitude smaller than that of f.c.c. unit cells.

Part of the hydrogen-charged specimen was covered with glycerine after the hydrogen exhalation had ceased and was exposed to X rays at room temperature. Hydrogen bubbles were observed on the surface of the specimen at the irradiated site as shown in Fig. 2a. No hydrogen bubble was detected at the non-irradiated site at this stage (Fig. 1b). The temperature of the same specimen was then raised to ~80 °C and new bubbles formed only at the non-irradiated site. Hydrogen atoms trapped and stabilized in stainless steel would have been released by X-ray irradiation.

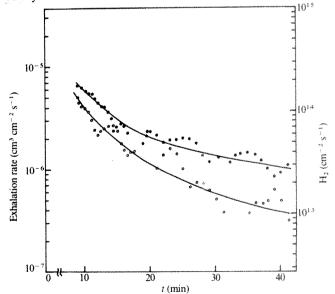
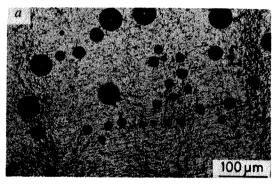


Fig. 1 The exhalation rate of hydrogen gas out of stainless steel 304 in liquid glycerine under X-ray irradiation () and without irradiation (O) as a function of time after the cathodic hydrogen charging in 30 min. The exhalation rate is enhanced by the radiation.

Hydrogen bubbles were not uniformly distributed on the specimen surface and their diameters were also random. The exhalation behaviour was different during irradiation; tiny bubbles appeared all over the surface and formed a 'film' of hydrogen gas on the surface. Presumably, exhalation from the grain surface by radiation-induced bulk diffusion dominates over grain boundary exhalation. No hydrogen bubble formed during X-ray irradiation if the stainless steel was not cathodically charged with hydrogen; confirming that the bubbles were not due to radiolysis of glycerine.

Enhancement of the exhalation rate of 2×10^{-6} cm³ cm⁻² s⁻¹ or 5.4×10^{13} H₂ cm⁻² s⁻¹ on exposure to X rays at 30 rad s⁻¹ indicates that $\sim 1.8 \times 10^{12}$ H₂ molecules are exhaled by 1 rad or $5 \times 10^{13} \, \mathrm{eV} \, \mathrm{g}^{-1}$. In terms of hydrogen atoms exhaled, $3.6 \times 10^{12} \, \mathrm{H \ cm^{-2} \ rad^{-1}}$ is greater than the number of the ion pairs produced by 1 rad in 0.03 cm³ gaseous hydrogen. The radiation energy absorbed by the 100-um thick stainless steel of density 8 g cm⁻³ is roughly 4.0×10^{12} eV for exposure to 1 rad. Thus, for one hydrogen atom exhalation the input energy was ~ 1 eV. The efficiency decreases so that ~4 eV per hydrogen atom exhalation is required 40 min after hydrogen charging. It is assumed that the probability of retrapping before hydrogen diffuses to the surface increases as the concentration of hydrogen is decreased. If the hydrogen concentration is high, the traps are almost all occupied by hydrogen. Most energy would be used to ionize the host stainless steel lattice atoms. The net energy absorbed at the unit cells with a hydrogen atom would contribute to causing the release of hydrogen in a cluster model, if the energy transfer process is absent. Thus, around 10^{-2} eV is required to detrap a hydrogen atom. It is not known how many retrapping and detrapping events are occurring, however, considering the high concentration, it seems that hydrogen comes out of stainless steel without an appreciable number of retrapping events. A similar radiation-enhanced exhalation rate of hydrogen out of stainless steel was observed on measuring pressure change with the specimen in an evacuated cell.

The lattice constant of a metal is increased by the dissolution of hydrogen. Figure 3 shows X-ray enhanced recovery of the lattice expansion measured from the shift of X-ray diffraction peaks for thin foils of the stainless steel with the thickness of



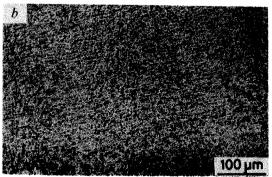


Fig. 2 Surface micrographs of electrolytically hydrogen charged stainless steel 304 coated with glycerine after 3.4 h ageing following the light charging for 5 min. a, Hydrogen bubbles induced at the irradiated site; b, no bubble was observed at the non-irradiated site.

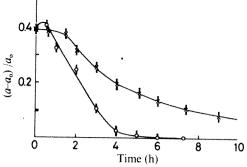


Fig. 3 X-ray enhanced recovery of lattice expansion induced by hydrogen in thin stainless steel foil. Fractional changes of the lattice constant determined from the X-ray diffraction peak are shown as a function of the time of X-ray irradiation (O) and of the ageing time for the non-irradiated specimen (X).

 ${\sim}35~\mu m$. The lattice expansion of ${\sim}0.4\%$ was decreased to 0.2% in ${\sim}4$ h and recovered in 12 h. On the other hand, it was reduced to 1.5 h for the fractional expansion to be the half and to 4.5 h for the complete recovery under X-irradiation. No hydrogen bubble was detected from the sample coated with glycerine after X-ray irradiation for 4.5 h although bubbles were still observed at the non-irradiated sample in agreement with the observation of the lattice expansion.

The lattice expansion persisted at 77 K without the X-ray irradiation for a long time. The X-ray or γ-ray irradiation at 77 K decreased the expansion and restored the lattice parameters. The result indicates that the hydrogen exhalation operates in the region where the monitoring X rays penetrate for the diffraction studies. The exhalation would not be caused by the radiation-induced decrease of the surface impedance at the surface barrier layer because the lowering of the surface impedance would not lead to the exhalation of hydrogen at 77 K where hydrogen is not mobile without irradiation. It also seems unlikely that specimens dipped in liquid nitrogen are heated by radiation. Thus, we conclude that the radiation has induced hydrogen diffusion in the bulk of the specimen. The present observation contrasts to that of Heinrich et al., in which surface impedance from gaseous hydrogen to the iron was found to be reduced by the α -ray ionization of hydrogen as in a permeation

It is still premature to assign the radiation-induced hydrogen diffusion a definite mechanism, but two possible mechanisms can be suggested tentatively. The electronic structure of hydrogen in metals has recently been calculated using a cluster molecule model involving the nearby metal electron orbitals. Hydrogen in metals also forms a stable quasimolecule, a diatomic complex, with C, N and O impurity on and with Ni in Fe-Ni alloys. Ionizing radiation might excite such quasimolecules or cluster molecules to antibonding or non-bonding states—such states were reported 1-2 eV above the valance band in studies of photoelectron emission and soft X-ray spectroscopy. Decomposition of the cluster might follow when antibonding or non-bonding states are reached. If this is the case, the input energy of ~1 eV per H atom exhalation was used effectively by some energy transfer process.

Destruction of the cluster or diatomic complex may also be explained by the core-hole Auger decay model for electron- or photon-induced desorption of gas molecules on the surface of solid¹⁴. However, the contribution of Auger process would be small considering the high efficiency of ~1 eV per hydrogen exhalation, and the model of cluster excitations remains the more likely explanation.

Energy transfer to hydrogen or to the proton by elastic collisions with photoelectrons and Compton electrons is also plausible. Like the electrotransport and heat transport mechanism for hydrogen migration², electron scattering at hydrogen causes a momentum transfer from electrons to hydrogen. No work has yet been done to prove the possibility of this

mechanism. However, it is conceivable that hydrogen is detrapped by the transfer of ~0.1-0.01 eV of energy from electrons by collisions.

The permeation rate was not affected appreciably by γ -rays from a 1 mCi source5. The dose rate in that case, assuming an average distance of 1 cm from the source, is only 10 rad h⁻¹, four orders of magnitude smaller than 10⁵ rad h⁻¹ in the present experiment. The 'hold-up' of tritium in the first wall of a fusion device is a severe problem. The hydride at the surface wall would be destroyed by radiation of the order of 10⁶-10⁸ rad h⁻¹ and also by the X-ray flux from the plasma. The reduction of tritium diffusion due to the blocking by transmutation-produced ³He (ref. 15) and to the trapping by defects or impurities might be compensated by the radiation-induced tritium diffusion. Further investigations are necessary to explain this radiation-induced hydrogen exhalation. Details of the X-ray diffraction studies will be published elsewhere with the result of isotope effects.

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- Alefeld, G. & Vökl, J. (eds) Hydrogen in Metals Vol. I (Springer, Berlin, 1978)
- Wipf, H. in Hydrogen in Metals Vol. 2 (eds Alefeld, G. & Vökl, J.) Ch. 7, 273 (Springer,
- Vökl, J. & Alefeld, G. (eds) Hydrogen in Metals Vol. 1, Ch. 12, 321 (Springer, Berlin, 1978).

Yosh, J. & Aleten, G. (eds.) raylogen in metals vol. 1, cli. 12, 321 (spring Stoneham, A. M. J. Phys. F2, 417 (1972).
 Maroni, V. A. & Van Deventer, E. H. J. nucl. Mater. 85/86, 257 (1979).
 Neef, M. & Carlson, G. A. J. nucl. Mater. 85/86, 17 (1979).

- Neef, M. & Carlson, G. A. J. nucl. Mater. 85/86, 17 (1979).
 Heinrich, R. R., Johnson, C. E. & Crouthamel, C. E., J. Electrom. Chem. Soc. 112, 1067
- 8. Chen, C. G., Birnbaum, H. K. & Johnson, A. B. J. nucl. Mater. 79, 128 (1979).

Adachi, H. & Imot, S. J. phys. Soc. Jap. 46, 1194 (1979)

- 10. Kronmüller, H. in Hydrogen in Metals Vol. 1 (eds Alefeld, G. & Vökl, J.) Ch. 11, 289 (Springer, Berlin, 1978).
- Ikeya, M., Miki, T., Touge, M. & Kamachi, K. Trans. Jap. Inst. Met. 21, Suppl. 633 (1980).
 Weaver, J. H., Knapp, J. A., Eastman, E. E., Peterson, D. T. & Satterthwaite, C. B. Phys. Rev. Lett. 39, 639 (1977).
- 13. Fukai, Y., Kazama, S., Tanaka, K. & Matsumoto, M. Solid State Commun. 19, 507 (1976).
- Knotek, M. L. & Feibelman, P. J. Phys. Rev. Lett. 40, 964 (1978).
 Emin, D., Baskes, M. I. & Wilson, W. D. Z. phys. Chem. 114, 231 (1979).

Expected immersion of Saturn's magnetosphere in the jovian magnetic tail

S. Grzedzielski, W. Macek & P. Oberc

Space Research Centre, Polish Academy of Sciences, Ordona 21, PL-01 237 Warsaw, Poland

Voyager 2 approaches Saturn (h) this month, August 1981, after the possible encounter^{1,2} of the planet with the tail or wake3 of Jupiter (24). With the magnetic flux in the tail of $\phi \cong 2 \times 10^{12}$ Wb (refs 4, 5) a simple model suggests that the tail is very long (7-15 AU) and wide enough (~0.6 AU) to engulf Saturn. This could result in a sudden drop (by a factor of \sim 40) of the ram pressure on the magnetosphere of Saturn. The ensuing inflation of the magnetosphere may cause effects observable from Voyager 2 and/or Earth-orbiting satellites, including a flare-up of kilometric radiation and enhancement of the Ly α limb brightening. Such events, if observed, could shed light on the magnetic and plasma nature of the jovian tail and on the electrodynamics of the saturnian magnetosphere.

The expected length of the jovian magnetic tail is 6-8 approx-

$$L_{t} = 2B_{1}r_{pc}^{2}/(\alpha B_{s}y_{c}) \tag{1}$$

where $2y_c$ is the width of the jovian magnetosphere at x = 0(centre of the planet, see Fig. 1 inset), B1, Bs are the ionospheric and the solar wind magnetic field intensities respectively, r_{pc} is the radius of the polar cap, and $\alpha < 1$ parameterizes the efficiency of reconnection⁷. Taking $y_c = 87 R_J$ (where R_J is the radius of Jupiter⁹), $B_1 = 1$ mT and $r_{pc} = 0.28$ R_1 (Voyager data^{4,5,10,11}), $B_s = 0.6$ nT (ref. 12) and assuming $\alpha = 0.1-0.2$ (as for Earth⁸) one obtains $L_t = 7-15$ AU. Thus, the jovian tail could be several times longer than the Jupiter-Saturn distance

Orbital considerations suggest that Saturn should miss the axis of the jovian tail by $\Delta \approx 380 R_{\rm J}$. This event (for a 400450 km s⁻¹ solar wind) would have been expected during the first week of May 1981. Immersion requires $\Delta < y(x_s)$, the half-width of the tail at Saturn's orbit (see Fig. 1 inset).

Recent Voyager 1 and 2 Jupiter flybys identified a tailwardoriented magnetic field (3-5 nT at $x_0 = 80-180 R_1$ (refs 5, 10, 11)) and two populations of circumjovian plasma: a cold one¹³⁻¹⁶ (densities¹³ $n \sim 10^{-5}$ cm⁻³) on the pre-dawn tail-like region of the magnetosphere, and a (denser, $n \sim 10^{-4}$ - 10^{-3} cm⁻³) hot plasma beyond $x \sim 160 R_{\rm J}$ streaming nearly antisunward¹⁷⁻¹⁹. Based on these data we have constructed a simple magnetohydrodynamical, one-dimensional model of the distant jovian tail.

We assume that the tail shape y(x) is determined by the condition of tail pressure equalizing total solar wind pressure

$$B_{s}^{2}/8\pi + p_{t} = m_{s}n_{s}v_{s}^{2}\cos^{2}\psi + p_{s} + (\mathbf{B}_{s} \cdot \hat{\mathbf{e}})^{2}/8\pi$$
 (2)

where $\hat{\mathbf{e}}$ is a unit vector ($\hat{\mathbf{e}} \perp \hat{\mathbf{n}}$ or $\hat{\mathbf{e}} \| \mathbf{B}_s$, where $\hat{\mathbf{n}}$ represents a unit outward vector normal to the tail boundary y(x), Fig. 1 inset).

The tail pressure consists of two components: magnetic $B_1^2/8\pi$ with the planetary magnetic field B_1 (and the plasma streams) parallel to the tail boundary y(x), and particle thermal pressure $p_t = 2n_t kT_t$. Conservation is also assumed of the magnetic flux, the mass flux, and the momentum

pure O were tried, see ref. 17).

The solar wind magnetic field pressure is due either to the tangential component only $(\hat{\mathbf{e}} \perp \hat{\mathbf{n}})$, which seems appropriate for v(x) in the ecliptic plane, or to the full field $(\hat{\mathbf{e}} | \mathbf{B}_s)$ which applies in the solar meridian plane. A standard Archimedean spiral model was used for \mathbf{B}_s (ref. 12) with a radial $(B_r \propto r^{-2}, r^2 =$ $(x+d)^2+y^2$, see Fig. 1 inset) and an azimuthal component $(B_{\varphi} \propto r^{-1})$. At Jupiter: $B_r = 0.08 \text{ nT}$, $B_{\varphi} = 0.58 \text{ nT}$. The solar wind is spherically symmetric with the thermal pressure p_s = $2n_s kT_s$, $(n_s \propto r^{-2})$, n_s (21) = 0.28 cm⁻³, T_s = constant = 0.25 × 10^5 K (ref. 12) and the ram pressure equal to $m_s n_s (\mathbf{v}_s \cdot \hat{\mathbf{n}})^2$, where $v_s = \text{constant} \approx 450 \text{ km s}^{-1}$ (ref. 12). Both the magnetic (tangential) pressure and the ram pressure depend on the angle of attack (defined in Fig. 1 inset: $\cos \psi = -\hat{\mathbf{v}}_{s} \cdot \hat{\mathbf{n}}$) which is related to the tail shape y(x) by the equation

$$dy/dx = \cot \alpha (\psi - \varphi), \quad \tan \varphi = y/(x+d)$$
 (4)

Equations (2)-(4) were numerically integrated for several cases given in Fig. 1 and Table 1, yielding y, v, n, T, p, B in the tail. The

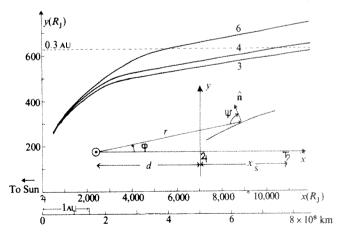


Fig. 1 Predicted boundary of Jupiter's distant magnetic tail in the jovicentric rectangular frame. The scale of ordinates is 10 times larger than the scale of abscissae. The shape of the tail was found by numerical integration of the pressure balance equation (2) for several cases, as shown in Table 1 and explained in the text. Assumed geometry is schematically shown in the inset. The most probable tail width (curve 4) at Saturn $2y_s = 1,212 R_J = 0.58 \text{ AU}$.

Table 1 Predicted jovian magnetic tail values at Saturn

		Solar wind pressure		Tail pressure		at Saturn [§] $(x = x_5)$					
	Ram*	Thermal	Full	lagnetic Tangential	Magnetic	The Cold [‡]	rmal Hot [‡]	$y_{s} = y(x_{s})$ (R_{J})	$ \begin{array}{c} nv \\ (cm^{-2}s^{-1}) \end{array} $	$\frac{mnv^2}{(\text{erg cm}^{-3})}$	p = 2nkT (erg cm ⁻³)
1	on [†]	off		off	on	o	ff	980			
2	on	on		off	on	o	ff	660		NACON TWO	Assistance .
3	on	on	on	****	on	on		579	1.8	9.9×10^{-18}	2.3×10^{-19}
4	on	on	on		on		on	606 [§]	8.1×10^{3}	7.5×10^{-12}	1.6×10^{-13}
5	on	on	-	on	on	on		659	1.4	7.7×10^{-18}	1.5×10^{-19}
6	on	on		on	on		on	693	6.2×10^3	5.8×10^{-12}	1.0×10^{-13}

 $x_s = 9,170 \ R_J$ (1 $R_J = 71,398 \ \mathrm{km}$): half-width y_s of the tail (in R_J , 1 AU = 2,095 R_J), tail plasma flux $n_t v_t$, tail wind momentum flux $m_t n_t v_t^2$ $(m_t = \text{proton mass})$, tail thermal pressure $2n_t k T_t$, for various terms in equation (2), 'on' or 'off'.

For a long tail in a radial flow the ram pressure contribution vanishes for $x > x_f \sim 3,500 R_1$ ($\psi(x_f) = \pi/2$).

The approximation $\psi \to 0$ in equation (+) (total direction of the solar wind parallel to the sum-suprier (5-24) line) allows an analytical solution $y^3 - y_c^3 = 2.73x(x/2 + d)$ (x, y in R_1 , d = 10,900 R_1) leading to $y_s = 730$ R_1 ; this approximation is, however, not applicable to a wide tail.

For the cold plasma ¹³ (few eV, $n_{t0} \sim 10^{-5}$ cm⁻³) we assume the thermal to magnetic field energy density ratio β_0 to be $\sim 10^{-7} - 10^{-6}$. The hot ¹⁷ plasma (~ 30 keV and $n_{t0} \sim 3 \times 10^{-4}$ cm⁻³) has $\beta_0 \sim 0.3$ (at $x_0 = 184$ R_1 and $y_0 = 181$ R_2 , Voyager 1 and 2 data ^{5,10,13,17}).

For anisotropic plasma (CGL) one obtains a slightly lower value $y_s = 603R_1$.

integrations started close to Jupiter, at $x_0 = 50 - 200 R_1$ with $y_0 = 150 - 200 R_1$ (refs 5, 10). We assumed the plasma initially (at $x = x_0$) always isotropic with $v_t(x_0)$ equal to the local sound velocity.

Our calculations show that the tail width 2y_s at Saturn is large, of the order of 0.6 AU. Practically, this result is sensitive neither to the initial data nor to the details of the assumed model (see Table 1 and Fig. 1) and we suggest that Voyager 2 and Saturn will not miss the jovian tail. These unique events may shed light on several questions.

Penetration by Voyager 2 into the jovian tail may clarify two aspects of the gradual loss of identity by the tail: (1) merging of the tail and the interplanetary magnetic fields when the ratio ${\mathcal R}$ of field polarity reversal time scale to the time scale of the solar wind flow past the tail is 10^{-3} – 10^{-2} , in contrast to the terrestrial case when $\Re \ge 1$; the spatial scale of the inhomogeneities of the plasma in the tail should be of the order of $\Re y_s$; and (2) filling-up of the tail with the solar wind plasma through the open field lines and/or diffusion across the field. These processes should be well in progress at Saturn's distance although still far from completion²⁷. Thus information on the relative roles of these two aspects could be available well before NASA's OPEN programme²⁰

Inside the tail, Saturn will encounter unusual conditions: a tail wind rather than a solar wind. The total pressure $(nmv^2 + p)$ may drop by a factor of 30-50 and the plasma flux (nv) by a factor of 400-600 for the hot tail plasma (cases 4 and 6). This will lead to (3) a weakening or disappearance of the bow shock and to (4) an inflation of the saturnian magnetosphere by a factor of ~ 2 . The inflation could be much stronger for the cold tail plasma (this seems, however, unrealistic). These effects could be directly observed by Voyager 2 if Saturn's immersion persisted till the third decade of August 1981.

Strong pressure gradients at the tail boundary crossing on a time scale of 1-2×10⁵ s may trigger a gigantic substorm followed by (5) a flare-up of kilometric radiation²¹⁻²³ and (6) an enhancement (by particle precipitation) of Saturn's Lya limb brightening²⁴. On the other hand, during prolonged immersion, the external magnetic field will become parallel to the plasma velocity which will lower the efficiency of magnetic field reconnection and magnetospheric dynamo.

Several observable effects are then to be expected: plasma and magnetic field effects (1), (2) (and with luck (3) and (4)) could be detected from Voyager 2; the kilometric radio wave flare (5) could be seen from Voyager 2 (and the Earth's orbit?) and the possible UV effect (6) could be detected from Voyager 2 and with some effort from the Earth's orbit25

Received 22 January; accepted 30 June 1981.

- Scarf, F. L. J. geophys. Res. 84, 4422-4424 (1979).
 Wolfe, J. H. et al. Science 207, 403-407 (1980).
- Van Allen, J. A. in COSPAR Space Res. Vol 17 (ed. Rycroft, M. J.) 719-731 (Pergamon, Oxford, 1977).

- 4. Connerney, J. E. P., Acuna, M. H. & Ness, N. F. Modeling the Jovian Current Sheet and Inner

- Connerney, J. E. P., Acuna, M. H. & Ness, N. F. Modeling the Jovian Current Sheet and Inner Magnetosphere (NASA preprint/Goddard Space Flight Center, 1980).
 Ness, N. F. et al. Nature 280, 799-802 (1979).
 Dungey, J. W. J. geophys. Res. 70, 1753 (1965).
 Kennel, C. F. & Coroniti, F. V. in The Magnetospheres of Earth and Jupiter (ed. Formisano, V.) 451-477 (Reidel, Dordrecht, 1975).
 Kennel, C. F. & Coroniti, F. V. in Solar System Plasma Physics Vol. 2 (eds Kennel, C. F., Lanzerotti, L. J. & Parker, E. N.) 105-181 (North-Holland, Amsterdam, 1979).
 Smoluchowski, R. in Jupiter (ed. Gehrels, T.) (University of Arizona Press, 1976).
 Ness N. F. et al. Science 206, 966-972 (1970).

- Ness, N. F. et al. Science 206, 966-972 (1979) Ness, N. F. et al. Science 204, 982-987 (1979)
- Smith, E. J. & Wolfe, J. H. Space Sci. Rev. 23, 217-252 (1979). Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 206, 987-991 (1979).
- Bridge, H. S. et al. Science 204, 987-991 (1979). Bridge, H. S. et al. Science 206, 972-976 (1979).
- Bagenal, F., Sullivan, J. D. & Siscoe, G. L. Geophys. Res. Lett. 7, 41-44 (1980). Krimigis, S. M. et al. Science 206, 977-984 (1979).

- Krimigis, S. M. et al. Science 204, 998-1003 (1979).
 Krimigis, S. M. et al. Science 204, 998-1003 (1979).
 Cowley, S. W. H. Nature 287, 775-776 (1980).
 Mutch, T. A. NASA Announcement of Opportunity (OPEN) A.O. No. OSS-1-79 (16 October 1979).
- 21. Brown, L. W. Astrophys. J. Lett. 198, L89-L92 (1975)
- Kaiser, M. L. & Stone, R. G. Science 189, 285-287 (1975).
- Kaiser, M. L. et al. Science 209, 1238–1240 (1980).

 Judge, D. L., Wu, F.-M. & Carlson, R. W. Science 207, 431–434 (1980).

 Weiser, H., Vitz, R. C. & Moos, H. W. Science 197, 755–756 (1980).

 Krimigis, S. M. et al. Geophys. Res. Lett. 7, 13–16 (1980).
- 27. Intriligator, D. S. et al. Geophys. Res. Lett. 6, 585-588 (1979).

Extended hopanes up to C_{40} in Thornton bitumen

Jürgen Rullkötter

Institute of Petroleum and Organic Geochemistry (ICH-5), Kernforschungsanlage Jülich GmbH, D-5170 Jülich 1, FRG

Paul Philip

CSIRO, Division of Fossil Fuels, Institute of Earth Resources, North Ryde, New South Wales 2113, Australia

Gas-chromatographic and mass-spectrometric investigation of the saturated hydrocarbon fraction of a distillation cut of Thornton bitumen has revealed the presence of diastereomeric pairs of side-chain-extended $17\alpha(H)$ -hopanes with carbon numbers ranging up to C40. Pentacyclic triterpanes of the hopane type are ubiquitous in sediments and related fossil fuels1. Commonly, besides the parent hydrocarbon itself, 17α (H)-hopane (C₃₀), compounds with degraded (C₂₇, C₂₉) and extended side-chains (C₃₁-C₃₅) are detected². While hopanes with 30 or fewer carbon atoms can easily be interpreted as diagenetic products of C₃₀ hopanoids, for example, hop-22(29)ene, known in several living organisms³, the extended hopanes $(C_{31}-C_{35})$ have recently been related to a C_{35} precursor, bacteriohopanetetrol, which was found to be a constituent of several microorganisms^{1,4-6}. We report here that the new C₃₆-C40 hopanes are of the same structural type as the well-known extended hopanes ranging up to C35.

The approximation $\varphi \to 0$ in equation (4) (local direction of the solar wind parallel to the Sun-Jupiter (\bigcirc -2 \downarrow) line) allows an analytical solution

For $\beta_0 = 1$ one obtains $y_s = 772 R_J$, suggesting that for very hot plasma $(\beta > 1)$ supported by more recent Voyager data²⁶, the tail should be considerably wider.

Adopted solar wind values at Saturn: $n_s v_s = 3.7 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$, $m_s n_s v_s^2 = 2.8 \times 10^{-10} \text{ erg cm}^{-3}$, $p_s = 2n_s k T_s = 5.7 \times 10^{-13} \text{ erg cm}^{-3}$ (ref. 12).

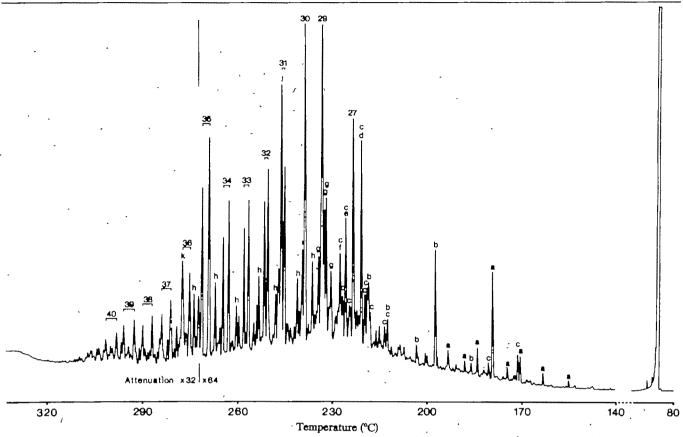
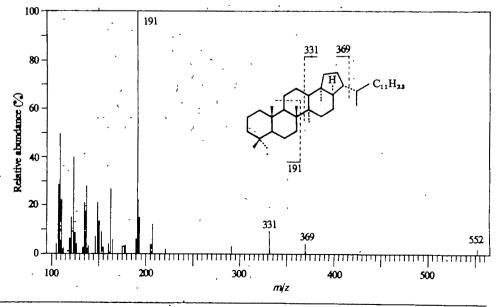


Fig. 1 Capillary column chromatogram of the saturated hydrocarbon fraction of a Thornton bitumen distillation cut. Numbers indicate $17\alpha(H)$ -hopanes (carbon atoms); a, tricyclic terpanes (C_{20} – C_{25}); b, tetracychic terpanes (C_{23} – C_{25} , C_{29}); c, steranes (C_{21} – C_{22} , C_{26} – C_{28}); d, $18\alpha(H)$ -trisnorneohopane; e, pentacyclic C_{28} -triterpane; f, C_{29} -sterane; g, 24-ethyl- $5\alpha(H)$ -cholestanes $[14\alpha(H),17\alpha(H),(20S)$ -, $14\beta(H),17\beta(H),(20S)$ -, and $14\alpha(H),17\alpha(H),(20S)$ - isomers in order of elution]; h, moretanes (C_{29} – C_{35}); i, pentacyclic C_{30} -triterpane; j, gammacerane (?); k, perhydro- β -carotene (?). A Varian 3700 gas chromatograph was used with FID-detector; 25 m×0.3 mm i.d. glass capillary column was coated with high-temperature SE 54 (Carlo Erba), injector, 300 °C; detector, 300 °C; carrier gas, helium; temperature programme, 80–330 °C (3 °C min⁻¹).

Thornton bitumen was obtained from the Thornton Quarry, just south of Chicago (Illinois). The bitumen is found in the Niagara dolomité deposited during Silurian times, and it is thought that it was formed simultaneously with the rock and thus may also be of Silurian age⁷. Rounded hillrocks of the ancient dolomite now protrude glacial clay. The rock is highly fossiliterous and contains unicellular species, and plant and animal skeletons⁷. A previous study of the saturated hydrocarbons from the bitumen by Marschner et al. revealed the absence of n-alkanes and a prominent contribution of steranes and triterpanes although no specific identifications were made.

As can be seen from the capillary column chromatogram in Fig. 1, the saturated hydrocarbon fraction of Thornton bitumen is enriched in pentacyclic triterpanes, most of which have a hopane skeleton. The most abundant compound is 17α (H)-hopane (C_{30}). 17α (H)-norhopane (C_{29}), 17α (H)-trisnorhopane (C_{27}) and 18α (H)-trisnorneohopane (C_{27}) were found as major degraded hopanes. Further tetra—and tricyclic terpanes, based on their mass spectral base peaks at m/z 191, may have structures related to hopane and may, at least in part, be derived from hopanoid precursors by degradation during diagenesis and catagenesis. Smaller amounts of steranes in Thornton bitumen

Fig. 2 Electron impact mass spectrum of the first-cluting C_{40} 17α (H)-hopane (see Fig. 1). A Varian Mat 112 S mass spectrometer was used with sonizing energy 70 eV, source temperature 260 °C, gas chromatographic conditions as given for Fig. 1.



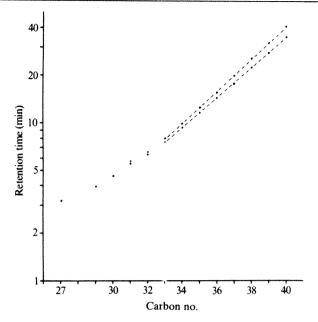


Fig. 3 Log/normal plot of retention times versus carbon number for C₂₇-C₄₀ hopanes obtained from isothermal gas chromatography at 260 °C (other conditions as given in Fig. 1). The C₃₃-C₄₀ hopanes show linear increase of log retention time, different for the C-22 diastereomers, indicating the presence of homologous series.

point to an advanced maturity level of the organic matter well within the oil window, as indicated by the dominance of 14β (H), 17β (H)-steranes⁸; this can easily be seen in Fig. 1 for the C₂₉ steranes.

Extended 17α (H)-hopanes in Thornton bitumen are pairs of C-22 diastereomers⁹ (Fig. 1). The carbon number maximum within this series at C35 either indicates a particular stability of this species or, more likely, a C₃₅ precursor for most of the C31-C35 extended hopanes as mentioned above. In contrast to hopane series reported so far, the extended hopanes in Thornton bitumen range up to C40. Diastereomeric pairs are observed as in the C₃₁-C₃₅ range together with a slight predominance of the first-eluting diastereomer (Fig. 1; the second C₃₆ isomer coelutes with a dicyclic tetraterpane, probably perhydro- β carotene). The mass spectrum of the first-eluting C_{40} hopane in Fig. 2 shows a fragmentation pattern consistent with a hopane structure with an extended side-chain. From the log/normal plot of retention times obtained at isothermal conditions against carbon number (Fig. 3), which shows a linear increase for the C₃₃-C₄₀ hopanes, the conclusion may be drawn that the sidechain extension is linear, as found for the C_{31} – C_{35} hopanes⁶, and no branching occurs.

The question now is whether the C₄₀ hopanes represent another end member in this series related to a specific precursor or whether this series extends beyond C40? This cannot be unequivocally answered from the analysis of this distillation cut. No hopanes above the C_{40} members marked in Fig. 1 could be detected in this sample, which may be due to the limiting experimental conditions (temperature) or the way the distillation cut was taken (exact temperature range unknown). If there were a C₄₀ precursor, the latter argument could explain the lower abundance of the C₄₀ hopanes relative to the C₃₆-C₃₉

As there is no precursor yet known for the C₄₀ hopanes, another mechanism for the formation of the C₃₆-C₄₀ extended hopanes is suggested. It has recently been found 10 that highly alkylated porphyrins in petroleum contain alkyl-chains up to C11. These porphyrins seem to be catagenetic products originating from porphyrin moieties bound to the kerogen matrix and released only at higher maturation levels by thermal cracking¹¹ A similar mechanism³ for the extended hopanes seems possible if one assumes that bacteriohopanetetrol (C35) is specifically

bound into the kerogen matrix during early diagenesis through a covalent carbon-carbon bond.

In addition to the extended $17\alpha(H)$ -hopanes, a series of moretanes $(17\beta(H),21\alpha(H))$ -hopanes has been tentatively identified in Thornton bitumen (Fig. 1). The series seems to include C₃₁-C₃₅ moretanes although only the C₃₁ compound in the mass spectrum clearly showed the slight dominance of the D/E-ring fragment (m/z 205) over the A/B-ring fragment (m/z 191) (ref. 12). Trace amounts of these compounds have been reported¹³ for a Toarcian shale from the Paris Basin. Unlike the $17\alpha(H)$ -hopanes, however, the extended moretanes were not detected as diastereomeric pairs (R and S at C-22) in Thornton bitumen; it seems possible that they are not separable in the given conditions2.

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- Ourisson, G., Albrecht, P. & Rohmer, M. Pure appl. Chem. 51, 709-729 (1979).
 Van Dorsselaer, A., Albrecht, P. & Ourisson, G. Bull. Soc. chim. Fr. 165-170 (1977)
- Arpino, P., Albrecht, P. & Ourisson, G. in Advances in Organic Geochemistry—1971 (eds von Gaertner, H. R. & Wehner, H.) 173-187 (Pergamon, Oxford, 1972).
- Förster, H. J., Biemann, K., Haigh, W. G., Tattrie, N. H. & Colvin, J. R. Biochem. J. 135, 133-143 (1973).
- Langworthy, T. A. & Mayberry, W. R. Biochim. biophys. Acta 431, 570-577 (1976). Rohmer, M. & Ourisson, G. Tetrahedron Lett. 3633-3636 (1976).
- Marschner, R. F., Duffy, L. J. & Winters, J. C. Trans Ill. State Acad. Sci. 68, 263-277
- Mackenzie, A. S., Patience, R. L., Maxwell, J. R., Vandenbroucke, M. & Durand, B., Geochim. cosmochim. Acta 44, 1709-1721 (1980).
- Ensminger, A., van Dorsselaer, A., Spyckerelle, Ch., Albrecht, P. & Ourisson, G. in Advances in Organic Geochemistry—1973 (eds Tissot, B. & Bienner, F.) 245-260 (Editions Technip, Paris, 1974).

 10. Quirke, J. M. E., Shaw, J. G., Soper, P. D. & Maxwell, J. R. Tetrahedron 36, 3261-3267
- 11. Mackenzie, A. S., Quirke, J. M. E. & Maxwell, J. R. in Advances in Organic Geochemistry-1979 (eds Douglas, A. G. & Maxwell, J. R) 239-248 (Pergamon, Oxford, 1980). Van Dorsselaer, A. thesis, Univ. Strasbourg (1975).
- 13. Ensminger, A. thesis, Univ. Strasbourg (1977).

Nitrous oxide cycling in Lake Vanda, Antarctica

W. F. Vincent, M. T. Downes & C. L. Vincent

Freshwater Section, Ecology Division, DSIR, PO Box 415, Taupo, New Zealand

Nitrous oxide (N2O) is a key intermediate for several steps of the aquatic nitrogen cycle. In oxygenated oceanic waters, bacterial oxidation of amino- and ammonium-N is a dominant source of N_2O (ref. 1); in regions of intense nitrification ΔN_2O values (difference between observed N2O and air-equilibrium concentration) typically rise to 40-100 nmol l⁻¹ (ref. 2). By contrast, anoxic waters are often undersaturated in N2O because of respiratory consumption by denitrifying bacteria3. We describe here an extreme accumulation of nitrous oxide (ΔN_2O of >2,000 nmol l⁻¹) in the saline bottom waters of Lake Vanda (77°35'S, 161°40'E), a warm meromictic water body in the Dry Valley region of Antarctica. From in situ experiments assaying various components of the nitrogen cycle, we conclude that this N₂O is produced by a narrow band of nitrifiers lying well above the oxycline. Nitrous oxide is lost from this zone at slow rates by diffusion, ultimately to the atmosphere above, or below to the upper anoxic zone where it is consumed by a finely stratified population of denitrifying bacteria.

Lake Vanda was sampled at a mid-lake deep-water site (maximum depth 68 m) during the austral summer of 1980–81. Holes were bored through the 3-3.5-m permanent ice-cap and 1-l samples removed with a discrete-depth water sampler. The unusual temperature and salinity profiles of Lake Vanda have been described elsewhere^{4,5}. Temperatures rise with increasing depth to a warm maximum (23.5 °C) in the region of high salinity (up to three times the conductivity of seawater) at the bottom of the lake (Table 1). Over the season of study the lake was well oxygenated to 59 m (~0.7 mmol l⁻¹ throughout, S. Nakaya, unpublished data) but anoxic at 60 m and below.

Table 1 Distribution of nitrogen and other characteristics of the mid-lake water column of Lake Vanda

		Forms	of nitrogen (µg	-atom l ⁻¹)	N.O.NO	Dissolved reactive P	Temperature	Conductivity	
Depth - (m)	NO ₃ -N	NO ₂ -N	N ₂ O-N	NH ₄ -N	Particulate-N	$- N_2O:NO_3$ (molar ratio × 10 ³)		(°C)	$(10^3 \mu\text{S cm}^{-1})$
3.25	3.5	0.10	0.05	0.9	0.7	7.1	0.02	4.5	0.6
5	4.1	0.11		0.6			0.01	5.0	0.7
15	2.2	0.08	0.15	0.2	0.3	34.1	0.03	5.5	1.1
25	2.1	0.04	0.18	0.1	0.7	42.8	0.01	7.0	1.2
35	2.3	0.05	0.19	0.1	0.5	41.3	0.01	7.0	1.2
45	4.4	0.02	0.51	0.7	0.4	57.9	0.02	9.0	2.3
47.5	7.3	0.07	0.72		_	49.3	0.02	9.5	- Medicine
50	47.0	0.17	1.96	16.9	0.6	20.9	0.01	12.5	8.0
51	89.7	0.27	2.18	19.1		12.1	< 0.01		-one-date.
52	133.5	0.39	2.78	27.8		10.4	0.02		*******
53	203.9	0.79	3.94	71.8	annexador .	9.7			nonless.
54	223.8	0.96	4.29	119.3		9.6	_	- Application	Milliante
55	232.7	1.14	3.80	144.1	0.8	8.2	< 0.01	1.8.5	39.5
56	165.4	1.90	3.51	269.2		10.6	0.02	nagewir	vulsame.
57	106.3	0.46	2.91	363.3		13.7	0.04		enginer
58	56.6	0.27	2.53	458.2	1.3	22.3	0.17	2 9.0	56.0
59	13.2	0.33	1.04	599.4		39.4	0.10	*variant*	- washing
60	3.1	0.10	0.05	686.9	1.9	8.1	0.15	22.0	60.9
62.5	<1.0	< 0.1	< 0.003	1005.6			2.21	22.5	79.5
65	<1.0	< 0.1	< 0.003	1346.5			5.24	23.5	80.6
67.5	<1.0	< 0.1	< 0.003	1747.4			8.15	23.5	80.8

Samples were collected 21 and 27 December 1980. All depths are relative to the water table, ~0.2 m below the ice surface. Water samples for nutrient analysis were filtered immediately on collection through acid-washed glass-fibre GF/C filters and stored frozen. Analyses were performed on a Technicon AutoAnalyzer II. Lakewater controls (sample without colour reagents) were run at all depths to correct for changes in refractive index. Further control samples were run with standard nutrient additions to correct for variable recoveries at increasing salt concentrations. Nitrite was measured by automated diazotization¹⁸. Nitrate was reduced to nitrite through a cadmium wire column¹⁹ at pH 8 (ref. 20) and then analysed by standard diazotization¹⁸. NH₄ was determined by an automated phenol-hypochlorite method²¹; high values in the bottom waters of Vanda were checked manually, after 100-fold dilution, by oxidation to nitrite and diazotization²². Dissolved reactive phosphorus was by an automated molybdenum blue method²³. Particulate nitrogen was filtered on to acid washed GF/C filters and stored frozen. Material was Kjeldahl-digested and the ammonium determined colorimetrically²⁴. For gas analysis, 15-ml lakewater samples were removed from the water sampler by hypodermic syringe and injected into 30-ml Hypovials that had been sealed with neoprene²⁵ stoppers and flushed with O₂-free, N₂O-free nitrogen. These samples were immediately preserved with glutaraldehyde (2% v/v final concentration) and stored at 0°C for up to 5 weeks. Before analysis, the samples were shaken vigorously for 1 h to ensure equilibration between liquid and headspace. 1-ml subsamples of the gas were injected into a Perkin-Elmer Sigma 4 electron-capture gas chromatograph fitted with a 2-m (3 mm o.d.) stainless steel column of Chromosorb 102. The GC was operated at column temperature of 55 °C with a carrier gas (95% Ar 5% CH₄) flow of 24 ml min⁻¹. The ⁶³Ni detector was run at 375 °C with a standing current of 3.5 × 10⁻⁹ A

N₂O concentrations were well above air-equilibrium values at all depths down the oxygenated portion of the water column (Table 1). Air-equilibrium concentrations of N₂O, the saturation value for water in equilibrium with atmospheric nitrous oxide, range from 17 nmol l⁻¹ at 4.5 °C to 9 nmol l⁻¹ at 23 °C (calculated from the solubility data of Markham and Kobe⁶ and measured values for N₂O in Antarctic air⁷). Immediately beneath the ice N_2O levels were 48% above air-saturation $(\Delta N_2O=8.2~\text{nmol}~\text{l}^{-1})$. Concentrations rose steadily with increasing depth, but sharply increased below 47.5 m to a maximum at 54 m (>20,000% air-saturation; ΔN_2O >2,000 nmol l⁻¹). N₂O tensions rapidly declined below the peak, and the gas was undetectable at 62.5 m and below. The deep N₂O maximum closely followed a nitrate peak previously reported by Torii et al.8 and confirmed in the present study (Table 1). NO₃ and N₂O profiles are strongly correlated ($r^2 =$ 0.909, P > 0.001) but an accumulation of nitrite in the lower depths was displaced 1-2 m downwards relative to the other forms of oxidized nitrogen (Table 1; N2O compared with NO2 $r^2 = 0.606$).

At least four biological processes are thought to involve nitrous oxide⁹. N₂O is an intermediate in the oxidation of ammonia to nitrate by nitrifying bacteria:

$$NH_3 \rightarrow NH_2OH \rightarrow N_2O \rightarrow NO_2^- \rightarrow NO_3^-$$

It is also an intermediate in the reduction of nitrate to nitrogen gas by denitrifying bacteria:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Nitrous oxide has been considered a possible intermediate in assimilatory nitrate reduction by algae (reverse of the nitrifier sequence). Finally, N_2O is an alternative substrate for nitrogenase, the enzyme of N_2 fixation. This fourth process cannot be an important control on nitrous oxide levels in Lake Vanda. Acetylene reduction assays¹⁰ on lakewater samples throughout

the profile failed to demonstrate measurable nitrogenase activity at any depth.

The well-defined and coincident maxima of NO_3 and N_2O provide circumstantial evidence of a deep band of nitrifying bacteria. More conclusive evidence comes from experiments conducted in situ from 3.25 m to 57.5 m, to measure nitrypyrinsensitive CO_2 fixation (Fig. 1). Significant differences (P < 0.05) between duplicates with and without this nitrification inhibitor were recorded only from 50 to 57.5 m. Rates were highest at 52.5 m and 55 m, in the region of abundant oxidized nitrogen.

There is some evidence from marine systems^{11,12} that nitrous oxide and nitrite may accumulate as byproducts of nitrate assimilation by algae. In Lake Vanda, as in other Dry Valley lakes¹³, algal production is low throughout most of the water column but rises to a sharp maximum just above the oxycline (Fig. 1). This photosynthetic peak lies well below the zone of N₂O accumulation, and algal assimilation is therefore unlikely to be a large source of nitrous oxide.

Denitrification has been invoked² to explain accumulation of N_2O in low-oxygen regions of the sea. Denitrification in Lake Vanda was assayed by in situ acetylene blockage¹⁴ experiments at depths of 55, 58, 59, 59.5, 60, 61, 62.5 and 64 m. Significant denitrifier activity was recorded only in the upper anoxic depths of 59.5–62.5 m (Fig. 1). Nitrous oxide was very low or undetectable in this region, and therefore the upper anoxic zone must be a region of N_2O consumption rather than a net source.

A comparison of the molar ratio of N_2O to NO_3 provides a further guide to the processes controlling nitrous oxide production and loss. Lowest ratios were recorded in the region 52–56 m, the zone of peak nitrifier activity. The minimum (8.2×10^{-3}) falls within the range reported for nitrifying bacteria in culture¹⁵, although it is high relative to typical accumulation ratios in the field—Elkins et al.¹ report an average ratio of 1.4×10^{-3} mol of N_2O produced per mol of NH_4 oxidized by aquatic nitrifiers. At higher and lower depths in Vanda the ratio

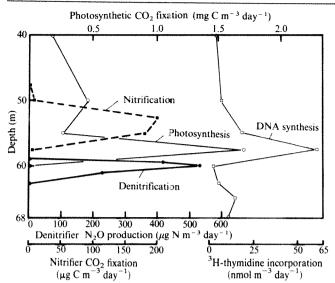


Fig. 1 Distribution of microbial activity at 40-68 m in Lake Vanda; nitrifier CO₂ fixation (■), photosynthetic CO₂ fixation (○), denitrifier N₂O production (•), 3H-thymidine uptake (□). All rates were determined by in situ incubations. Nitrifier activity (18 December 1980) was estimated by the difference between dark CO2 fixation with and without the nitrification inhibitor nitrypyrin²⁷. Samples were preincubated with inhibitor (10 mg l final concentration) for 1 h at the depth of collection, and then incubated for a further 5 h with ¹⁴C-HCO₃ (0.6 µCi ml⁻¹). Dissolved inorganic carbon was determined by IR gas analyser. Photosynthetic rates (21 December 1980) were measured by 24-h in situ ¹⁴C-HCO₃ (0.6 μCi ml⁻¹) incubations in light and dark bottles. Denitrifier activity (13 January 1981) was measured by the accumulation of nitrous oxide in samples of lakewater sealed in Hypovials and incubated at the depth of collection for 24 h with 10% acetylene1 inhibitor of nitrous oxide reductase. Samples were incubated with 3Hthymidine (20 nmol l⁻¹, 16 December 1980) to estimate microbial rates of DNA synthesis¹⁶. At the end of the 5 h incubation, samples were filtered on to 0.22 µm Millipore membranes which were washed several times with 10% trichloracetic acid and air-dried. The filters were later counted by liquid scintillation spectrometry and corrected for adsorption of label (glutaraldehyde-killed controls) and self-absorption of radiation.

of N₂O to NO₃ increased, probably reflecting the selective loss of nitrate with time by algal uptake and sedimentation. Particulate nitrogen, and by inference seasonal NO₃ removal by the plankton, is low throughout the water column (Table 1). Movement of N₂O and NO₃ by turbulent diffusion away from the peak must therefore proceed very slowly for algal uptake to affect significantly the molar ratio of the two forms of oxidized N. This ratio decreased again from 25 m towards the ice, which would be consistent with selective loss of N₂O to the atmosphere by diffusion through the ice-cap, and at the lake edge where the ice melts away each summer to produce a moat of open water 1-10 m wide.

Denitrifier activity was not detected at depths greater than 62.5 m, where N₂O and NO₃ were also absent: denitrification rates in Vanda may ultimately be limited by the rate of transfer of oxidized N to the upper anoxic zone. Ammonium substrate is relatively abundant in the region of nitrifier activity, and it is possible that phosphorus (Table 1) rather than nitrogen availability exerts an overall control on this component of the N₂O cycle. Phosphate values increased with depth towards the oxycline (Table 1), and at these lower aerobic depths total microbial activity (as estimated by ³H-thymidine incorporation into DNA¹⁶) increased to a water column maximum (Fig. 1). It remains unclear why the nitrifiers do not similarly peak in this superficially favourable environment at 57-59 m. A possible explanation is the steep ammonium gradient observed through this zone (Table 1). Nitrifying bacteria are inhibited by high levels of ammonia. Nitrobacter (nitrite oxidizer) is more sensitive than Nitrosomonas (ammonium oxidizer)¹⁷, and it is of interest that nitrite accumulates towards the bottom of the nitrification layer where ammonia becomes increasingly concentrated.

The relative importance of denitrifier and nitrifier activity for nitrous oxide accumulation in oceanic environments has caused considerable debate². In Lake Vanda, N₂O accumulated in the region of highest nitrifier activity where denitrification was

undetectable. Conversely, N₂O concentrations were greatly reduced in the region where in situ denitrification rates were maximal. In this body of water nitrification would seem to be a net source and denitrification a net sink for nitrous oxide. These different components of the N2O cycle occupy discrete bands of the stratified water column. This layered community may thereby prove to be a useful test of future models describing nitrous oxide production and loss in natural aquatic environments.

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- 1. Elkins, J. W., Wofsy, S. C., McElroy, M. B., Kolb, C. E. & Kaplan, W. A. Nature 275,
- Periotti, D. & Rasmussen, R. A. Tellus 32, 56-72 (1980)
- Cohen, Y. Nature 272, 235-237 (1978). Armitage, K. B. & House, H. B. Limnol. Oceanogr. 7, 36-41 (1962).
- Hoare, R. A. et al. Nature 202, 886-888 (1964). Markham, A. E. & Kobe, K. A. J. Am. chem. Soc. 63, 449-454 (1941)
- Rasmussen, R. A., Khalil, M. A. K. & Dalluge, R. W. Science 211, 285-287 (1981). Torii, T. et al. Geochemical and Geophysical Studies of the Dry Valleys, South Victoria Land
- in Antarctica, 5-29 (National Institute of Polar Research, Tokyo, 1975).

 9. Knowles, R. Microbiology 367-371 (1978).

 10. Hardy, R. W. F., Holsten, R. D., Jackson, E. K. & Burns, R. C. Pl. Physiol. 43, 1185-1207
- 11. Hahn, J. Meteor A16, 1-14 (1975)
- Wada, E. & Hattori, A. Limnol. Oceanogr. 16, 766-772 (1971).
- Vincent, W. F. Ecology (in the press).
 Yoshinari, T., Hynes, P. & Knowles, R. Soil Biol. Biochem. 9, 177-189 (1977).
- 14. Tosninari, I., Hynes, F. & Knowies, R. Soit Biol. Biochem. 9, 177–189 (19.
 15. Goreau, T. J. et al. Appl. envir. Microbiol. 40, 526–532 (1980).
 16. Fuhrman, J. A. & Azam, F. Appl. envir. Microbiol. 39, 1085–1095 (1980).
 17. Focht, D. D. & Verstraete, W. Adv. Microbiol. Ecol. 1, 135–214 (1977).
 18. Downes, M. T. Wat. Res. 12, 673–675 (1978).

- Stainton, M. P. Analyt. Chem. 46, 16 (1974). Nydahl, F. Talanta 23, 349-357 (1976).
- Reuschberg, B. & Abdullah, M. I. Wat. Res. 11, 637-638 (1977). Matsunaga, K. & Nishimura, M. Analyt. chim. Acta 73, 204-208 (1974). Downes, M. T. Wat. Res. 12, 743-745 (1978).
- Searle, P. L. N. Z. Jl agric. Res. 18, 183-187 (1975)
- Macgregor, A. N. & Keeney, D. R. Envir. Lett. 5, 175-181 (1973). McAuliffe, C. Chem. Technol. 1, 46-51 (1971).
- Somville, M. Wat. Res. 12, 843-848 (1978)

An opiate system in the goldfish retina

Mustafa B. A. Djamgoz* & William K. Stell*

Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California 90024, USA

Chen-An Chin & Dominic M. K. Lam

Cullen Eye Institute and Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030, USA

Recently, in addition to conventional neurotransmitters such as acetylcholine, dopamine, glycine and y-aminobutyric acid (GABA), putative neuroactive peptide transmitters have been localized to specific retinal amacrine cells1. In particular, opiate receptors^{2,3}, assayable enkephalin immunoreactivity⁴ enkephalin-immunoreactive neurones^{1,5} have been described in avian and mammalian retinae. However, little physiological evidence has been obtained for the involvement of neuropeptides in retinal function. Here we report that exogenous opiates affect both the release of GABA from GABAergic amacrine cells and the firing patterns of ganglion cells in the goldfish retina⁶. Our results show that the output of the retina is modulated by an opiate system whose neural organization and pharmacological properties resemble those described elsewhere in the vertebrate central nervous system.

^{*}Present addresses: Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, UK (M.B.A.D); Department of Anatomy, University of Calgary Faculty of Medicine, 3330 Hospital Drive, NW, Calgary, Alberta, Canada T2N 1N4 (W.K.S.)

Ganglion cell unit activity was recorded from the isolated, inverted retina of goldfish using metal microelectrodes. The retina was stimulated alternately at 6-s intervals for 0.7 s with a small red light spot (1 mm diameter, 650 nm, $2\mu W\ mm^{-2}$), or annulus (1.2 mm i.d., 3.2 mm o.d.) centred on the electrode. Ganglion cell responses were classified as ON, OFF (those responding to light with an increase or decrease of firing rate, respectively), or ON-OFF (those responding to light with bursts of spikes at onset and offset of stimulus). The retina was maintained at 22 °C in moist gas (95% $O_2+5\%$ CO_2), and drugs were applied to the photoreceptor side of the retina by nebulizer; no wash-out was possible. Further details are given in Fig. 1 legend.

The synthetic opioid peptide, D-Ala²-Met⁵-enkephalinamide (DALA, Peninsula), at concentrations of 0.1-1.0 μM, increased the spontaneous and light-evoked firing of 20 ON-centre units and suppressed the post-stimulus inhibition (Fig. 1a). DALA also abolished the opponent surround response to an annulus. In contrast, spontaneous and light-evoked firing in 9 of 12 OFFcentre units, was reduced by DALA; in the other three units, this inhibition was preceded by a transient period of excitation during which the cells gave ON responses to light (not shown). ON-OFF units were less predictably affected by DALA, and their activities were modified only by concentrations greater than 1.0 µM, from which they often did not recover. Morphine (0.1-1.0 µM), like DALA, excites ON-centre cells, but the excitation is prolonged (generally irreversible) and less readily modulated by light (Fig. 1b). Naloxone (4-40 µM) inhibits the dark- and light-evoked activities of ON cells, and blocks the response to DALA or morphine when applied simultaneously (Fig. 1c). Dextrorphan (4-40 \(mu\)M) has no effect (not shown). These results indicate that the endogenous opioid transmitter may be released in the dark, although the possibility that naloxone could be blocking ON cell activity by acting at any synapse cannot be excluded.

To test whether DALA acts on ganglion cells directly or through intermediary neurones, all synaptic transmission to ganglion cells was first blocked by treating the retina with cobalt chloride (1-2 mM), then DALA was applied. In the presence of cobalt ions, both dark- and light-activated firing of ON-centre cells was suppressed, and the response to DALA was blocked (Fig. 1d). Thus, the receptors for DALA and morphine may be located on an intermediary neurone presynaptic to ganglion cells rather than on ganglion cells themselves. Note, however, that neuronal responses to enkephalins may require calcium currents⁷ that would be blocked by cobalt ions, thus a direct interaction is also possible.

Preliminary experiments indicated that enkephalinimmunoreactive neurites are scattered throughout the inner synaptic layer. As the dendrites of ON-centre ganglion cells and neurites of GABA-accumulating amacrines both ramify in the innermost sublamina of this layer⁸, the pathways of these cells were investigated further. The GABA antagonist bicuculline causes an excitation of ON-centre cells and abolition of their surround responses similar to that caused by DALA or morphine; subsequent application of DALA, however, produces no significant further increase in the firing rate (Fig. 1e). We also examined the effects of opiates on the uptake and release of GABA in the retina. We have previously shown⁸ that the GABA amacrines in goldfish can be selectively loaded by incubation with ³H-GABA in the dark, whereas type H1 cone horizontal cells are loaded by incubation in the light. Release of ³H-GABA can be induced by increasing the concentration of K⁺ in the bathing medium, and the influence of opiates and their analogues on GABA release can be tested by adding these to the medium.

Dark-adapted goldfish eye cups were cut in half. The retina was isolated from each half under dim red light and incubated at 22 °C for 15 min in the dark with 0.2 ml oxygenated Ringer's solution containing 1 μ M ³H-GABA (39.2 Ci mmol⁻¹, NEN). The retinae were then incubated separately in 10 ml of unlabelled saline for 60 min with gentle shaking and a change of solution every 10 min. The efflux of ³H-GABA from each piece

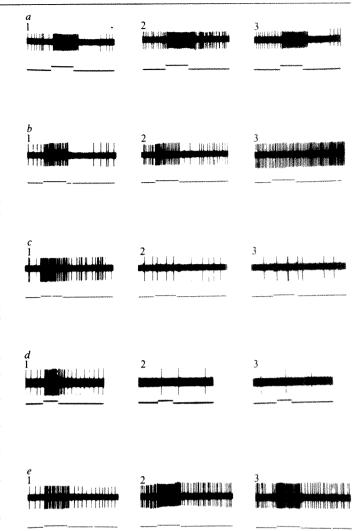


Fig. 1 Ganglion cell activity recorded extracellularly from the isolated retina of goldfish, Carassius auratus, placed in a chamber receptor side upwards and maintained and stimulated as described in the text. Concentrated stock solutions in cyprinid fish saline solution (120 mM NaCl, 2.5 mM KCl, 1.2 mM MgSO₄, 2.2 mM CaCl₂, 10 mM dextrose, 3 mM HEPES, pH 7.8) were run through an atomizer and sprayed on to the photoreceptor side of the retina; a number of solutions could be applied in turn. The dilution factor for the atomizer was estimated by spraying ³H-thymidine onto small pieces of filter paper at various dilutions and durations of application, counting in a liquid scintillation counter, and averaging the total radioactivity over a volume of retina (a circular patch 8 mm in diameter and 0.4 mm thick). Assuming that the atomized drug diffuses uniformly through the tissue, that it is neither destroyed nor taken up into retinal cells, that a steady-state concentration is achieved and that the drugs used in the physiological studies do not adhere more strongly to the stock bottles and nebulizing apparatus than ³H-thymidine, the minimum effective concentration for a readily detectable effect of DALA on ganglion cell activity was 0.1-1.0 µm. As these assumptions are certainly not valid and as each maximizes concentrations, the true minimum effective dose may be substantially lower than that estimated. The figure shows the action potentials of single ONcentre ganglion cells in the dark and in response to small, centred red light spots (square pulses below the response trace each denote $0.7 \,\mathrm{s}$). The pharmacological conditions were as follows: $a_{\rm s}$ (1) before application of DALA (control response); (2) during maximal effect of 1.0 µM DALA; (3) 5 min after application of DALA. b, (1) Control; (2) during 1.0 μM DALA; (3) during 1.0 μM morphine sulphate, after recovery from DALA. c, (1) Control; (2) after 40 µM naloxone; (3) after 40 µM naloxone + 1 µM DALA. d, (1) Control; (2) after 1 mM CoCl₂; (3) after 1 mM CoCl₂+1 μM DALA. e, (1) Control; (2) after 10 µM bisuculline methiodide; (3) after 10 µM bicuculline methiodide + 1 µM DALA. Results shown in a-e are from different ganglion cells; 1, 2, 3 in each row show responses of the same unit.

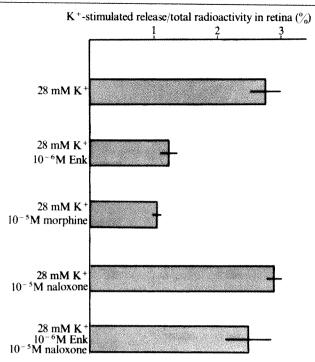


Fig. 2 The effects of enkephalin (Enk), morphine and naloxone on K⁺-stimulated release of ³H-GABA from amacrine cells of the goldfish retina. The GABAergic amacrine cells were pre-loaded by incubating the retinae in the dark for 15 min in saline containing ³H-GABA. Then the retinae were incubated with unlabelled saline for 60 min and the release of ³H-GABA into the solution was measured by scintillation counting ^{13,14} every 3 min until it became constant. Each piece of retina was then transferred to K⁺-rich Ringer's solution (28 mM K⁺) to which was added Met⁵-or Leu⁵-enkephalin (10^{-6} M), morphine (10^{-5} M) or naloxone (10^{-5} M), alone or in combination. After incubation for 6 min (2×3 min) in these solutions, each retina was again transferred to normal unlabelled saline. The percentage of ³H-GABA released from the retina by K⁺-stimulation was calculated using the following formula: K⁺-induced ³H-GABA release (d.p.m.) – ³H-GABA efflux in normal Ringer's solution (d.p.m.)/³H-GABA remaining in the retina (d.p.m.). Each value is the mean \pm s.e.m. of at least seven experiments.

of retina was measured using methods described previously 10,11 except that the K+-rich saline contained only 28 mM instead of 56 mM K⁺, and while one half was incubated in saline, the other half was incubated in saline containing enkephalin, morphine or naloxone, alone or in combination (for details see Fig. 2 legend). Using this method, we have previously shown that H-GABA accumulated by goldfish retinal cells is released in response to high external K⁺ concentrations and that this release is inhibited by 1 or 10 mM Co2+ in the medium. Figure 2 shows that enkephalin and morphine suppress the K⁺-stimulated. Ca²⁺dependent release of 3H-GABA from goldfish GABA amacrines (loaded in dark) but not from H1 cone horizontal cells (loaded in light; not shown). Both Met⁵- and Leu⁵-enkephalin have similar dose-dependent effects on GABA release, the inhibition of which ranges from 60% to 0% at 10^{-6} and 10^{-10} M enkephalin, respectively. Naloxone blocks the effect of enkephalin on GABA release from the amacrine cells but has no effect by itself in these incubation conditions. Furthermore, the suppression of K⁺-stimulated ³H-GABA release by enkephalin is not due to inhibition of the GABA uptake system, as retinae accumulate the same amount of 3H-GABA in the presence or absence of 10⁻⁵ M enkephalin, and enkephalin inhibits ³ GABA release from amacrines even in the presence of 10⁻⁴ M nipecotic acid, a specific inhibitor of GABA uptake (Chin and Lam, in preparation).

Our results show that in the inner synaptic layer of goldfish retina there are pharmacologically identifiable opioid pathways12. The pathway to ON-centre ganglion cells seems to consist of at least an intrinsic peptidergic (opioid) neurone. which may release an enkephalin, and an intrinsic GABAergic amacrine cell which is inhibited by the opioid peptide and in turn inhibits the ganglion cell. The simplest scheme for such a pathway is shown in Fig. 3. Additional elements, such as excitatory interneurones between enkephalinergic amacrine (AFNK) and GABAergic amacrine (A_{GABA}) or between A_{GABA} and the ON-centre ganglion cell, are possible. An overall effect of the presumed endogenous opioid transmitter, then, is to excite the ON-centre ganglion cells through disinhibition (opioid pathways to other ganglion cells may differ). This role of opioids in retinal ON pathways resembles their role elsewhere. For example, the immediate postsynaptic effect of enkephalins is generally inhibitory¹³, and the cells postsynaptic to enke-

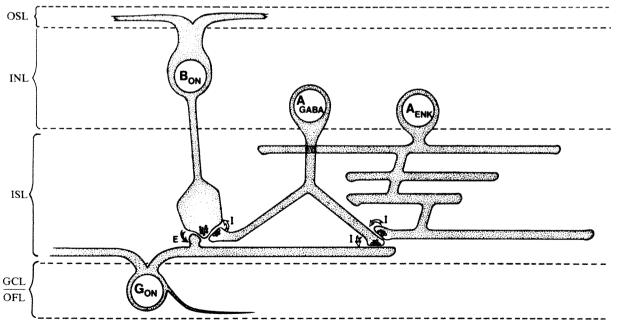


Fig. 3 The simplest probable hypothetical circuit of opioid and GABAergic neural pathways affecting ON-centre ganglion cells in the inner synaptic layer (ISL) of goldfish retina. Open arrows indicate inhibition (I); solid arrows indicate excitation (E). The presumed enkephalinergic amacrine (A_{ENK}) is inhibitory to the GABA amacrine (A_{GABA}) , which in turn is inhibitory to the depolarizing bipolar (B_{ON}) . The ON-centre ganglion cell (G_{ON}) may be inhibited by the GABA amacrine either directly or through excitatory input from the depolarizing bipolar. The nature of the synaptic input(s) to the presumed enkephalin cell are unknown. OSL, outer synaptic layer; INL, inner nuclear layer; GCL/OFL, ganglion cell/optic fibre layer.

phalinergic neurones may themselves often be inhibitory14. In specific cases described recently, exogenous opiates inhibited the activity of GABA interneurones in the hippocampus 14-16 and olfactory bulb¹⁶; the K⁺-evoked, Ca²⁺-dependent release of GABA from rat brain synaptosomes¹⁷, and the induced release in vivo of GABA from rat sensorimotor cortex18. These features are particularly clear in the goldfish retina which, because of its well-known neural organization and convenience as a natural 'brain slice', may be ideal for further testing of the organization of transmitter-specific neuronal assemblies such as these.

These results suggest interesting interpretations of the functional organization of the vertebrate retina. The precise function of amacrine cells is unknown, although they are generally supposed to have a critical role in controlling retinal output (ganglion cell responses)19. For example, in the inhibi-

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- 1. Stell, W., Marshak, D., Yamada, T., Brecha, N. & Karten, H. Trends Neurosci. 3, 292-295
- Medzhiradsky F. Brain Res. 108, 212-219 (1976).
- 3. Howells, R. D., Groth, J., Hiller, J. M. & Simon, E. J. J. Pharmac. exp. Ther. 215, 60-64
- 4. Brecha, N., Karten, H. J. & Laverack, C. Proc. natn. Acad. Sci. U.S.A. 76, 3010-3014
- Humbert, J., Pradelles, P., Gros, C. & Dray, F. Neurosci, Lett. 12, 259-263 (1979).
- Djamgoz, M. B. A. & Stell, W. K. Soc. Neurosci Abstr. 6, 613 (1980).
 Mudge, A. W., Leeman, S. E. & Fischbach, G. D. Proc. natn. Acad. Sci. U.S.A. 76, 526-530
- Marc, R. E., Stell, W. K., Bok, D. & Lam, D. M. K. J. comp. Neurol. 182, 221-245 (1978). Lam, D. M. K. et al. Neurochemistry 1, 183-191 (1980).
- 10. Sarthy, P. V. & Lam, D. M. K. J. Neurochem. 32, 1269-1277 (1977).

tory interneurone, the GABAergic amacrine is connected and colour-coded in such a way as to impose a double colouropponent surround upon the centred ganglion cell^{20,21}. The purpose of a second inhibitory interneurone, the presumed enkephalin amacrine, in modulating the effectiveness of this surround, is unclear. However, experiments on the role of enkephalin-releasing amacrine cells in the processing of visual information may elucidate the role of enkephalinergic neurones elsewhere in the central nervous system.

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- Chin, C. A. & Lam, D. M. K. J. Physiol., Lond. 308, 185-195 (1980).
 Simon, E. J. & Miller, J. M. A. Rev. Pharmac. Tox. 18, 317-394 (1978).
 North, R. A. Life Sci. 24, 1527-1546 (1978).

- 14. Zieglgansberger, W., French, E. D., Siggins, G. R. & Bloom, F. E. Science 205, 415-417
- 15. Lee, H. K., Dunwiddie, T. & Hoffer, B. Brain Res. 184, 331-342 (1980).
- Nicoll, R. A., Alger, B. E. & Jahr, C. E. Nature 287, 22-25 (1980).
 Brennan, M. J. W., Cantrill, R. C. & Wylie, B. A. Life Sct. 27, 1097-1101 (1980).
 Coutinho-Netto, J., Abdul-Ghani, A.-S. & Bradford, H. F. Biochem. Pharmac. 29, 2777-2780 (1980).
- Miller, R. F. in The Neurosciences, 4th Study Program (eds Schmitt, F. O. & Worden, F. G.)
 227-245 (MTT Press, Cambridge and London, 1979).
 Daw, N. W. J. Physiol., Lond. 197, 567-592 (1968).
 Marc, R. E. in Colour Vision Deficiencies V (ed. Verriest, G.) 15-29 (Adam Hilger, Bristol,

Tonic in vivo inhibition of rabbit myometrial adrenergic receptors

Lawrence E. Cornett*, Alan Goldfien & James M. Roberts

University of California School of Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Medicine and Cardiovascular Research Institute. San Francisco, California 94143, USA

With the development of radioligand binding assays for identifying hormone receptors directly, it has become evident that the number of receptors in a particular cell or tissue does not remain constant but can in fact, be regulated by a variety of agents1. Receptor concentrations decrease with exposure to homologous hormone (called densitization or down-regulation) but may be affected differently by heterologous hormones. Thus in the adrenergic contractile response of rabbit myometrium, mediated by α -adrenergic receptors², the concentration of α -adrenergic receptors is regulated by sex steroids^{3,4}. This oestrogen treatment increases the concentration of myometrial α -adrenergic receptors approximately threefold over that measured in myometrium from immature rabbits, and, furthermore, is accompanied by an increased contractile response to catecholamines5. We report here that, after 24 h in organ culture. rabbit myometrium is more sensitive to α adrenergic stimulation in vitro, and that particulate fractions prepared from cultured myometrium have a threefold increase in α -adrenergic receptors but not β -adrenergic receptors when compared with myometrium that has not been cultured. Our results suggest that the myometrium α -adrenergic receptor, in addition to being regulated by oestrogen, seems to be under tonic inhibitory control in vivo and that this control may only in part be accounted for by catecholamines.

Uteri from immature New Zealand rabbits (typically six rabbits per experiment) were pooled, the endometrium removed and myometrial strips divided equally into two groups. Myometrium in the first group was used immediately in isometric contraction studies or was homogenized and a particulate fraction prepared for binding studies, while myometrium in the second group was cultured. After 24 h in organ culture, strips were selected for isometric contraction studies or prepared for binding studies. 3H-dihydroergocryptine (DHE) was used to identify myometrial α -adrenergic receptors as described by Roberts et al.4 with several modifications (see Table 1). Binding of DHE to rabbit myometrium had been shown previously to meet the criteria for binding to the α -adrenergic receptor⁶. ³H-dihydroalprenolol (DHA) binding was found to be compatible with interaction with the β -adrenergic receptor.

Particulate fractions prepared from immature rabbit myometrium demonstrated a single class of binding site with low capacity and high affinity for DHE. After 24 h in organ culture, the concentration of DHE binding sites was significantly (P < 0.005) increased but the dissociation constant was unchanged (Table 1). The differences in the concentration of DHE binding sites in the particulate fractions prepared from cultured myometrium and myometrium that had not been cultured could not be attributed to either differences in numbers of cells as determined by DNA content⁷ of the homogenized strips (control, $5.06 \pm 0.50 \mu g$ DNA per mg wet weight; 24 h culture, 6.21 ± 0.67) or differential recoveries of protein⁸ (control, $1.6 \pm$ 0.2 mg per g wet weight; 24 h culture, 1.4 ± 0.4) or plasma membrane as determined by 5'-nucleotidase activity (control, $2.83 \pm 0.48 \mu g P_i$ per mg protein per min; 24 h culture, $2.24 \pm$ 0.41). 5'-Nucleotidase activity of the particulate fractions was determined by the method of Solymon and Trams⁹ at a protein of 0.16 ± 0.20 mg ml⁻¹ for 20 min. concentration Free inorganic phosphate was determined by the method of Chen et al. 10

DHE binding to the particulate fractions prepared from cultured myometrium retained the characteristics of α adrenergic receptor interactions. The DHE binding site was stereoselective; the (-)-steroisomer of adrenaline (K = 0.2)μM) was 20 times more potent than the (+)-stereoisomer $(K_i = 4.0 \mu M)$ in competing for DHE binding. The order of

^{*} Present address: Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, Arkansas 72205, USA.

Table 1 Dissociation constants and concentration of binding sites for DHE and DHA in rabbit myometrium

	DHE bin	C .	DHA binding (n = 4)		
	Concentration of binding sites (fmol per mg protein)	Dissociation constant (nM)	Concentration of binding sites (fmol per mg protein)	Dissociation constant (mM)	
Control 24 h organ culture	91 ± 10 $250 \pm 6*$	2.6 ± 0.3 2.9 ± 0.6	53 ± 6 46 ± 4	0.5 ± 0.1 0.3 ± 0.1	

Myometrium was cut into pieces not larger than 3×10 mm and washed in Ham's F-10 culture medium at room temperature. Myometrial strips (two to three per well) were cultured in 3 ml of serum-free Ham's F-10 culture medium with penicillin at 100 U ml^{-1} and streptomycin at $100 \ \mu g \ ml^{-1}$ in a humidified 95% air/5% CO₂ atmosphere at 37 °C. Particulate fractions were prepared as previously described except that the 10,000g centrifugation step was deleted from the protocol and dithiothreitol was omitted from the buffer used in the final pellet suspension. The DHE radioligand binding assay was used as previously described except that dithiothreitol was omitted from the assay tubes and instead ascorbic acid at a final concentration of 1.0 mM was used as an antioxidant, and the wash buffer used was $20 \ \text{mM K}_2\text{HPO}_4$, $1 \ \text{mM MgSO}_4$ at pH 8.0. Final concentrations of DHE were 1–16 nM. The DHA radioligand binding assay was identical to the DHE assay except that the temperature of the wash buffer was $4 \ \text{C}$ and the wash volume was $25 \ \text{ml}$. Final concentrations of DHA were 0.1– $16 \ \text{nM}$.

*Significantly (P < 0.005) different from control as determined by use of unpaired Student's t-test.

potency of agonist competition for binding to particulate fractions prepared from cultured myometrium—adrenaline $(K_i = 0.6 \pm 0.2 \ \mu\text{M}) \ge \text{noradrenaline} \ (K_i = 1.8 \pm 0.7 \ \mu\text{M}) \gg \text{isoprenaline} \ (K_i > 10 \ \mu\text{M})$ —is identical to the order of potency for these agonists in stimulating contraction of rabbit myometrium². Inhibitory constants (K_i) were calculated from the relationship $K_i = \text{ED}_{50}/(1 + [\text{DHE}]/K_d)$, described by Cheng and Prusoff¹¹ and are presented in Table 1 as the $\bar{X} \pm \text{s.e.m.}$ from three experiments using particulate fractions from different rabbits.

The increased concentration of DHE binding sites in myometrium after 24 h in culture was accompanied by a significant increase in sensitivity to contraction induced by noradrenaline (Fig. 1a) and methoxamine, an α -adrenergic agonist subject to neither nerve re-uptake nor metabolism by catechol-O-methyltransferase (Fig. 1b). However, there was no change in sensitivity of cultured myometrium to acetylcholine (Fig. 1c).

The leftward shifts of the dose-response curves for noradrenaline- and methoxamine-induced contraction indicate that myometrial sensitivity to adrenergic stimulation increases during organ culture. The known examples of increased sensitivity or 'supersensitivity' have been divided into two major types¹². 'Deviation' supersensitivity, or prejunctional supersensitivity¹², is characterized by changes in the amount of agonist that is available to interact with receptors. Supersensitivity of this type would arise in our system if either decreased adrenergic neuronal uptake or decreased metabolism of amines

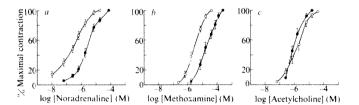


Fig. 1 Contractile response of myometrial strips that had not been cultured (●) and myometrial strips that had been cultured for 24 h (○) to cumulative doses of noradrenaline (a), methoxamine (b) and acetylcholine (c). Calculated ED₅₀ (X̄±s.e.m.) from individual experiments (n = 10) were as follows: a, ●, 2.8±0.6 μM; ○, 0.6±0.2 μM (P<0.05); b, ●, 22.2±4.0 μM; ○, 3.9±1.0 μM (P<0.05); c, ●, 3.8±1.7 μM; ○, 1.4±0.5 μM (not significant). Statistical significance was determined by use of unpaired Student's t-test. Isometric contraction studies were carried out in isolated baths maintained at 37 °C with Krebs-Ringer bicarbonate buffer (1.8 mM Ca²+, glucose at 1 mg ml⁻¹, pH 7.4) and aerated with 95% O₂/5% CO₂. Myometrial strips were suspended from a Grass FT03 strain-gauge transducer at an initial tension of 1 g for 1 h during which time the Krebs-Ringer solution was replaced at 15-min intervals. After 1 h, the tension was reduced to 0.25 g and the response recorded with a Grass physiograph. Response was calculated by integrating the area under the tracing for a standardized period of time.

was present in the cultured myometrium. As cultured myometrium was also more sensitive to methoxamine-induced contraction it is unlikely that 'deviation' supersensitivity is the underlying mechanism.

'Nondeviation' supersensitivity, or postjunctional supersensivity, as reviewed by Fleming et al.¹³, develops as a result of suppression of contact between neurotransmitter and its effector cell. Several different mechanisms have been proposed for postjunctional supersensitivity, including increased receptor concentration¹⁴. For example, in the rat depletion of neurotransmitter with reserpine leads to a leftward shift in the doseresponse curve for noradrenaline- and carbachol-stimulated potassium release of submaxillary gland slices 15 and this supersensitivity has been correlated with an increase in both α - and β -adrenergic receptor concentration¹⁶. In smooth muscle, postjunctional supersensitivity has been characterized by a nonspecific increase in sensitivity to various agonists acting on their specific receptors, and the response to changes in extracellular potassium and calcium ion concentration is altered^{14,17}. Our results show that cultured myometrium did not exhibit an increased sensitivity to acetylcholine, indicating that the observed increase in sensitivity is specific for α -adrenergically mediated contraction.

Table 2 Effect of the addition of (-)noradrenaline to the culture medium on the concentration of DHE binding sites

Treatment	DHE binding site concentration (% of control)
Control	100
8 h culture	145 ± 18
0.1 mM ascorbate	
8 h culture	101 ± 9
0.1 mM ascorbate	
1.0 μM (-)noradrenaline	

Myometrial strips were cultured as described in the text except that the culture medium was changed at 2-h intervals and after 8 h the strips were homogenized and particulate fractions prepared. In a preliminary experiment, Scatchard analysis indicated similar dissociation constants for DHE binding to particulate fractions prepared from myometrial strips cultured in the presence of either ascorbate or ascorbate and noradrenaline. Thus, it was unlikely that the particulate fractions prepared from noradrenaline-treated myometrium contained a higher concentration of noradrenaline which competed with DHE in the binding assay thereby producing an apparent decrease in DHE binding sites. To conserve tissue, the concentration of DHE binding sites was estimated by using a saturating concentration (~ 12 nM) of DHE and 10 μ M phentolamine to define nonspecific binding and was expressed as a percentage of DHE binding sites in myometrium from the same animals that had not been cultured (n=4).

* Significantly different from strips cultured for 8 h with 0.1 mM ascorbate as determined by use of paired Student's t-test.

Table 3 Effect of hexamethonium on myometrial DHE and DHA binding sites

Treatment	DHE binding sites (fmol per mg protein)	DHA binding sites (fmol per mg protein)
Control 10 mg per kg hexamethonium	80 ± 14 $159 \pm 20*$	52 ± 13 $72 \pm 5 \dagger$

Immature rabbits received five subcutaneous injections hexamethonium (10 mg per kg) dissolved in 0.9% NaCl at 6-h intervals, were killed within 1 h of the last injection and their uteri removed. Preparation of myometrial particulate fractions and binding assays, including radioligand concentrations, were performed as described in Table 1 legend. Data are reported as the $\bar{X} \pm s.e.m.$

* Significantly (P < 0.01) different from control; unpaired Student's

† Significantly (P < 0.05) different from control; unpaired Student's t-test.

The observed increase of α -adrenergic receptors in cultured myometrium suggested that tonic inhibitory influences are present in vivo. The addition of noradrenaline (1.0 µM) to the culture medium prevented the increase in α -adrenergic receptors (Table 2). However, the results from this experiment alone do not indicate a regulatory role of catecholamines in vivo. To examine this possibility further, immature rabbits were treated with hexamethonium, a ganglionic blocking agent¹⁸, resulting in a significant increase in DHE binding sites (P < 0.01) and in DHA binding sites (P < 0.05).

The finding that noradrenaline prevented the in vitro increase in the concentration of myometrial α -adrenergic receptors whereas in vivo treatment of rabbits with hexamethonium resulted in an increase in the concentration of these receptors suggests that catecholamines may tonically down-regulate the α -adrenergic receptor in vivo. However, this may not be the only process involved, because the magnitude of the increase (99%) in α -adrenergic receptors with 24 h hexamethonium treatment was less than that (260%) observed during the organ culture over the same period of time. This difference could reflect an incomplete ganglionic blockade with the dose of hexamethonium used. However, there was no further increase in myometrial DHE binding sites $(150 \pm 14 \text{ fmol per mg protein})$; n = 3) of rabbits in which the dose of hexamethonium was increased to 20 mg per kg.

Thus, our studies suggest that in addition to regulation by sex steroids³⁻⁵, the myometrial α -adrenergic receptor of the immature rabbit seems to be under inhibitory control in vivo by endogenous catecholamines and an unidentified mechanism. The techniques described for organ culture of rabbit myometrium should facilitate investigation of the cellular mechanisms involved in the control of adrenergic receptors and their relationship to physiological response in this tissue.

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- Catt, K. J., Harwood, S. P., Aguilera, G. & Dufau, M. L. Nature 280, 109-116 (1979).
 Ahlquist, R. P. Am. J. Physiol. 153, 586-600 (1948).

- Millians, L. T. & Lefkowitz, R. J. J. clin. Invest. 60, 815-818 (1977).
 Roberts, J. M., Insel, P. A., Goldfien, R. D. & Goldfien, A. Nature 270, 624-625 (1977).
 Roberts, J. M., Goldfien, R. D., Goldfien, A. & Insel, P. A. in Catecholamines: Basic and Clinical Frontiers Vol. 1 (eds Usdin, E., Kopin, I. J. & Barchas, J.) 441-443 (Pergamon,
- Williams, L. T., Mullikin, D. & Lefkowitz, R. J. J. biol. Chem. 251, 6951-6923 (1979).
- Giles, K. W. & Myers, A. Nature **206**, 93 (1965). Bradford, M. M. Analyt. Biochem. **72**, 248–254 (1976)
- Brautord, M. M. Analyl. Biochem. 72, 240–234 (1976).
 Solymon, A. & Trams, E. G. Enzyme 13, 329–372 (1972).
 Chen, P. S., Toribora, T. Y. & Warner, H. Analyl. Chem. 28, 1753–1758 (1956).
 Cheng, Y. & Prusoff, W. H. Biochem. Pharmac. 22, 3099–3108 (1973).
 Fleming, W. W. Fedn Proc. 34, 1969–1970 (1975).

- Fleming, W. W., McPhillips, J. J. & Westfall, D. P. Ergebn. Physiol. 68, 55-119. (1973)
 Fleming, W. W. Rev. Neurosci. 2, 43-90 (1976).
- Martinez, J. R. & Quisell, D. O. J. Pharmac. exp. Ther. 201, 206-217 (1977).
 Pointon, S. E. & Barnerjee, S. P. Biochim. biophys. Acta 548, 231-241 (1979)
- Carrier, O. & Jurevics, H. A. J. Pharmac. exp. Ther. 184, 81-94 (1973).
 Taylor, P. in Pharmacologic Basis of Therapeutics (eds Gilman, A. G., Goodman, L. S. &
- Gilman, A.) 211-219 (Macmillan, New York, 1980).

Monosynaptic muscarinic activation of K⁺ conductance underlies the slow inhibitory postsynaptic potential in sympathetic ganglia

John P. Horn & Jane Dodd

Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02114, USA

Slow muscarinic inhibition, which lasts seconds or longer, is probably an important modulator of synaptic interactions in the central and peripheral nervous systems. Neither the cellular location of muscarinic receptors nor the ionic mechanism underlying the inhibition is well understood. In parasympathetic neurones of the cardiac ganglion in the mudpuppy, activation of muscarinic receptors leads to an inhibitory postsynaptic potential (i.p.s.p.) produced by an increase in membrane conductance to K⁺ (ref. 1). At other sites, including sympathetic ganglia, however, the situation is less clear. In the 9th and 10th paravertebral sympathetic ganglia of the bullfrog stimulation of synaptic inputs to C neurones produces a slow muscarinic i.p.s.p.2. From extracellular recordings, it was suggested3 that this slow i.p.s.p. is mediated through muscarinic excitation of an inhibitory, catecholamine-releasing interneurone. However, using similar methods, Weight et al. 4.5 reported that muscarinic receptors are located on C neurones themselves. Further controversy exists over the ionic basis of the i.p.s.p.; stimulation of an electrogenic ion pump^{6,7} and reduction of membrane Na⁺ conductance⁸ have both been proposed but remain unsubstantiated. We now present evidence, from intracellular recordings, that acetylcholine (ACh) produces a monosynaptic activation of muscarinic receptors located on sympathetic C neurones, and the i.p.s.p. is accompanied by an increase in membrane K conductance.

Sympathetic chains, including ganglia 7-10, were isolated from 4-6-inch bullfrogs (Rana catesbiana) and maintained in vitro. Spinal nerves 7 and 8, the sympathetic chain above ganglion 7 and the sciatic nerve were each fitted with suction electrodes to permit orthodromic and antidromic stimulation of ganglion cells. Cell bodies were impaled with microelectrodes made from 1.0-mm o.d. wall glass (Haer, WPI) and filled with 3 M KCl (resistances = $70-120 \text{ M}\Omega$). A bridge circuit was used to estimate membrane potential while passing current through the microelectrode. Iontophoretic pipettes were filled with 1 M ACh (resistance 100 MΩ, backing current 1-4 nA). Micropipettes were positioned under visual control with Zeiss Nomarski optics (×40 water immersion objective). The preparation was superfused with Ringer's solution composed of 115 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM HEPES, pH 7.2. Dihydro- β -erythroidine HBr (DH β E, gift of Merck), d-tubocurarine Cl (dTC, Sigma) and atropine (Sigma) were applied by superfusion in the Ringer's.

Two classes of neurone are found in the 9th and 10th ganglia. They are identified by the origin and latency of the input producing a fast, nicotinic excitatory postsynaptic potential (e.p.s.p.). In agreement with others^{9,10}, we found that C neurones received their innervation from spinal nerves 7 and 8, whereas B neurones received theirs from higher spinal segments through the sympathetic chain. The latencies of nicotinic e.p.s.ps were 5-10 times greater in C neurones than in B neurones. In a given preparation, C neurones were generally much smaller than B neurones and cell size was used as a guide to impale C neurones preferentially. I.p.s.ps were never observed in a B neurone (see also ref. 2).

A single orthodromic shock, or a short train at 10 Hz, to an impaled C neurone in drug-free Ringer's elicited suprathreshold fast e.p.s.ps followed by a hyperpolarization of 5-25 mV, lasting 1-4 s (Fig. 1a). Superfusion with 100 nM atropine blocked the hyperpolarization which then recovered when atropine was

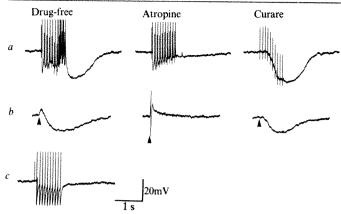


Fig. 1 Pharmacology of the i.p.s.p. a, Response of a C neurone to 10 stimuli at 10 Hz to nerves 7 and 8. b, After each synaptic trial a single iontophoretic pulse (15 nA, 5 ms) of ACh was applied (arrow). c, A train of antidromic action potentials elicited by 10 stimuli to the sciatic nerve. The tops of the action potentials in drug-free Ringer's and in 100 nM atropine have been cropped. Shock artefacts are evident in a and c. Atropine blockade was maximal by 10 min and was reversed by washing in drug-free Ringer's for 45 min. During atropine blockade, the nicotinic response to ACh became suprathreshold. After 20 min in 100 µM dTC, the fast e.p.s.p. and ACh-evoked depolarization were blocked, revealing pure hyperpolarizing Temperature = 27 °C.

washed out. The orthodromic hyperpolarization was distinctly larger than the summated afterpotential following a comparable train of antidromic action potentials (Fig. 1c). When the fast e.p.s.p. and consequent spike were blocked with either $4 \mu M D H \beta E$ or $100 \mu M d T C$, a pure, atropine-sensitive, hyperpolarizing i.p.s.p. remained. These synaptic responses were mimicked by iontophoretically applied ACh (Fig. 1b). In normal Ringer's, a single pulse of ACh produced a biphasic response consisting of an early, depolarizing phase lasting $200 \mu M c$ ms, followed by a larger, hyperpolarizing phase lasting $200 \mu M c$ selectively abolished the hyperpolarization while curariform drugs blocked the depolarization. Thus the muscarinic i.p.s.p. is present in drug-free C neurones and can be mimicked by the application of ACh.

The possibility that an interneurone is involved in the generation of the i.p.s.p. was tested by measuring the effect of blocking synaptic release on the response to iontophoretically applied ACh in a curarized preparation (Fig. 2). The iontophoretic current was adjusted so that the ACh response closely mimicked the amplitude and time course of the i.p.s.p. Then the preparation was superfused with 0.18 mM Ca²⁺, 8 mM Mg²⁺ Ringer's, which abolished the nerve-evoked slow i.p.s.p. but left the iontophoretic response unchanged. The synaptic response returned when normal Ringer's was reintroduced and both the i.p.s.p. and the ACh responses were then blocked completely with atropine. This demonstrates that the muscarinic action of ACh is independent of Ca²⁺-sensitive release and is probably a direct effect on C cells.

The mechanism underlying the i.p.s.p. was examined by varying the membrane potential. In 20 cells, in which 10 stimuli at 10 Hz elicited an average i.p.s.p. of $12\pm1.8\,\mathrm{mV}$ (s.e.m.) at resting potential $-51\pm1.5\,\mathrm{mV}$ (s.e.m.), polarizing current produced the effects illustrated in Fig. 3a. Depolarizing current reduced the amplitude and duration of the i.p.s.p. With progressive hyperpolarization, i.p.s.p. amplitude increased to a peak value, then became smaller and reversed. The i.p.s.p. reversed at a membrane potential near that at which the antidromic action potential afterpotential reverses. From recordings made with a balanced bridge circuit we estimated that the i.p.s.p. reverses at $-100\pm4.2\,\mathrm{mV}$ (s.e.m.), n=4 and the spike afterpotential reverses at $-88\pm2.4\,\mathrm{mV}$ (s.e.m.), n=5 (for example Fig. 3c,d).

Interpretation of the voltage sensitivity of the i.p.s.p. requires information about the current-voltage (I-V) relationship of C

cells. Figure 3b is an I-V plot obtained by passing a slow current ramp through a balanced electrode. It is representative of data from five cells in which the average slope resistance increased sharply from $93\pm14~\mathrm{M}\Omega$ (s.e.m.) to $380\pm59~\mathrm{M}\Omega$ (s.e.m.) during hyperpolarization from rest. Over the linear, high-resistance range of the I-V curve, i.p.s.p. amplitude was a linear function of membrane potential and underwent reversal, suggesting a synaptically activated increase in membrane conductance. The curved portion of the I-V curve coincided with the range in which i.p.s.p. amplitude and spike afterpotential amplitude deviated from a linear dependence on membrane potential (compare Fig. 3b with c and d). In other words, both the i.p.s.p. and the afterpotential appeared to be shunted by a decrease in membrane input resistance at depolarized potentials.

The muscarinic conductance change was also investigated by passing 0.5-s current pulses across the membrane before and during the i.p.s.p. (not shown). As expected, there was always a decrease in membrane resistance during the i.p.s.p. over the linear, high-resistance part of the I-V relationship. In the region of transition from low- to high-input resistance we sometimes observed a resistance increase during the i.p.s.p. (see also refs 4, 8). However, this increase was consistently smaller than that produced by simply hyperpolarizing the membrane with injected current in the absence of an i.p.s.p. Thus despite the apparent decrease, the i.p.s.p. was always accompanied by an increase in membrane conductance. In three cells the conductance increase during the i.p.s.p. ranged from 1.6 to 3.9 nS. These estimates were made over the linear portion of the I-V plot and represent approximately a doubling of resting membrane conductance $(1/380 \text{ M}\Omega = 2.6 \text{ nS})$.

The preceding experiments imply that K^+ and/or $C1^-$ carry synaptic current. Such possibilities are usually distinguished by altering ion concentration gradients. The fact that use of KCl-filled microelectrodes had no discernible effect on the i.p.s.p. reversal potential suggests that $C1^-$ is not involved. In contrast, when extracellular K^+ was increased from 2 to 10 mM, the reversal potential shifted to a substantially depolarized value (Fig. 4), indicating that K^+ carries synaptic current.

Taken together, these experiments indicate a similarity between the muscarinic i.p.s.ps of bullfrog sympathetic neurones and mudpuppy parasympathetic neurones: each involves a monosynaptic process dominated by an increase in K⁺ conductance. Furthermore, the inhibitory nature of this mechanism has been demonstrated. In some conditions (Fig. 3a, 0 pA and +40 pA) C neurones fired repetitively in response to a single antidromic shock and the i.p.s.p. blocked this firing. The

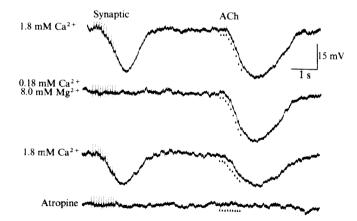
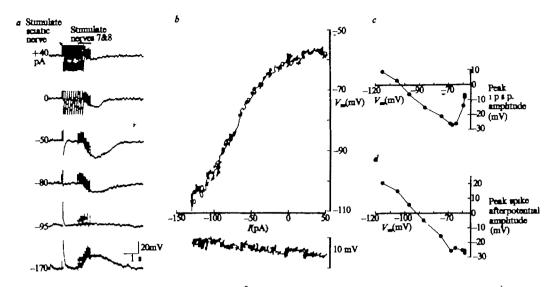


Fig. 2 Blocking synaptic release does not alter the muscarinic action of ACh on a C neurone. In normal Ringer's, containing $100 \,\mu\text{M}$ dTC, the synaptic response (10 stimuli at 10 Hz to nerves 7 and 8) was mimicked by iontophoretically applied ACh (nine pulses of 20 nA, 4 ms, marked by arrows). After 10 min in 0.18 mM Ca²⁺, 8 mM Mg²⁺ Ringer's, the synaptic response was selectively blocked. After 20 min of washing in normal Ringer's, the synaptic response returned. Both the synaptic and the AChevoked responses were completely blocked by 100 nM atropine. Temperature = 22 °C.

Fig. 3 Voltage sensitivity of the slow i p.s.p. spike afterpotential and input registance of a C neurone bathed in 100 µM dTC. a, Intracellular recording of responses to a angle antidromic shock followed by a short orthodromic train at different lovols polarizing current (left). At 0pA and +40pA the antidromic shock produced repetitive firing which was stopped by the i.p.s.p. (Control trials showed that the i.p.s.p magnitude was unaffected by the repetitive firing.) b. Top, after balancing the bridge circuit of the microelectrode amphifier, a slow current ramp (such that $dV/dt < 4 \text{ mV s}^{-1}$) was passed into the cell



and used to construct an I-V curve by playing the data into an x-y plotter. Bottom, the electrode was then withdrawn from the cell and the same current ramp was passed through the electrode alone. Membrane potentials were estimated in the range over which the unbalanced electrode resistance was <10% of the total measured intracellularly. The unbalanced electrode resistance was -20 MΩ. Subtracting this from the top plot gives alope resistances of 135 MΩ at 0 pA and 505 MΩ. at -100 pA c, A graph of peak: p.a.p. amplitude versus membrane potential (Vm). d, Graph of peak action afterpotential versus membrane potential Note that the bend in the cell's F-V relationship coincides with the potential at which the relationships in c and d deviate from linearity. Temperature = 22 °C.

powerful influence of the i.p.s.p. on membrane properties suggests how monosynaptic inhibition might contribute to the integrative function of autonomic neurones.

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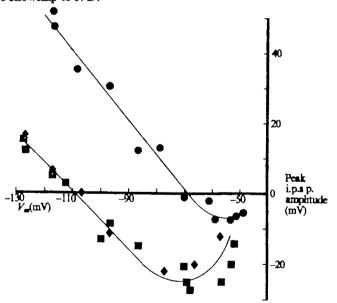


Fig. 4 K⁺ dependence of the i.p.s.p. reversal potential. A plot of peak i.p.s.p. amplitude versus membrane potential. ◆, In 2 mM K Ringer's, the i.p.s.p. reversed at −110 mV. ♠, After 20 min. washing in 10 mM K⁺ Ringer's, the i.p.s.p. reversed at −68 mV. ■, After 20 min washing in 2 mM K+ Ringer's, the reversal returned to -110 mV. Lines were drawn by eye. Assuming intracellular K concentration to be constant, the shift in K+ equilibrium potential, calculated from the Nernst equation, would be 41 mV. Altering extracellular K+ concentration produced no obvious change in either the resting potential (-51 mV) or the I-V relationship of this cell. At the i.p.s.p. reversal potential, the input resistance of the cell was 247 MΩ, aftf [bial (-51 mV) or the I-V relationship of this cell. At the i.p.s.p. reversal potential, the input resistance of the cell was 247 M Ω , after correcting for 24 M Ω of unbalanced electrode resistance. Temperature = 23 °C.

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- Hartzell, H. C., Kuffler, S. W., Stickgold, R. & Yoshikama, D. J. Physiol., Lond. 271, 817-846 (1977)L

- Tonka, T., Chichiou, S. & Libet, B. J. Neurophyssel. 31, 596-409 (1968).
 Libet, B. & Kobayasia, H. J. Neurophyssel. 37, 805-814 (1974).
 Weight, F. F. & Padjen, A. Bram Res. 85, 225-228 (1973).
 Weight, F. F. & Smith, P. A. m. Hissocherustry and Cell Busings of Autonomic N. Cells and Paironaeuvers (ach Brainto et al.) 159-171 (Raven, New York, 1980).
 Nisha, S. & Kohotsu, K. Life Sci. 6, 2049-2055 (1967).
- Nutsi, S & Kokotsu, K. J. Nesrophyssol. 31, 717-728 (1968) Weight, F. F. & Padyon, A. Bratis Rev. 55, 219-224 (1978)
- Nish, S., Soods, H. & Kohster, K. J. cell comp. Physiol. 66, 19–32 (1965)
 Libet, B., Chichbu, S. & Tosaka, T. J. Nesrophysiol. 31, 383–395 (1968)

Recognition of H-2 domains by cytotoxic T lymphocytes

C. Weyand, G. J. Hämmerling* & J. Goronzy

Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, Heidelberg, 6900 Heidelberg. Im Neuenheimer Feld 280, FRG

The polymorphic major histocompatibility antigens (H-2) have a crucial role in the activation of antigen-specific T lymphocytes. Thus, H-2 antigens are not only recognized by allogeneic lymphocytes leading to generation of cytotoxic T lymphocytes (CTLs), but it has also been demonstrated that in syngeneic systems most T cells are only able to recognize foreign antigens in conjunction with their own MHC (major histocompatibility complex) antigens. This phenomenon, termed H-2 restriction¹ may be the key to our understanding of the biological function of MHC antigens. It is not clear whether recognition by T cells of H-2 on a molecular level is confined to particular domains on the H-2 molecule, nor whether the same polymorphic H-2 sites, which are characterized by antibodies, are recognized by allogeneic as well as by H-2-restricted syngeneic CILs. Previous findings² indicate the existence of at least two major poly-morphic domains on the H–2K^k molecule as defined by antibodies. Here we show the existence of CTLs with specifity for these polymorphic domains, and the preferential recognition of a particular domain by both alloreactive as well as H-2-restricted CTLs.

Recent competitive antibody-binding studies using six monoclonal antibodies against different determinants on the

^{*} To whom correspondence should be addressed

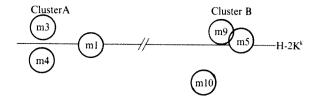


Fig. 1 Antigenic determinants on the H-2K^k molecule are concentrated in two clusters. This schematic diagram of the spatial relationship of H-2K^k determinants was obtained by antibody competition studies in which the binding of six different radiolabelled monoclonal antibodies to H-2K^k-bearing cells was competed by addition of various cold antibodies in all possible permutations. The 50% inhibition values have been used as a very rough measure of the relative distance between determinants on the H-2K^k molecule. Antibodies within one cluster cross-block each other to varying degrees but do not interfere with binding to the other cluster, as described in more detail elsewhere². Biochemical evidence indicates that all six determinants are located on the same molecule.

H-2Kk molecule³ have provided information on the steric relationship of individual determinants². The six H-2K^k determinants defined by monoclonal antibodies are concentrated on the H-2K^k molecule in two spatially separated polymorphic domains, clusters A and B, which are shown schematically in Fig. 1. We have used these monoclonal antibodies to block the target antigen for allogeneic as well as H-2-restricted CTLs to assess which H-2 sites are target epitopes for CTLs. H-2Kkspecific CTLs were produced in vitro by activation of DBA/2 splenocytes with irradiated A/J stimulator cells. Figure 2 shows the ability of different concentrations of monoclonal anti-H-2K^k antibodies to block target cell lysis. None of the monoclonal antibodies completely inhibited cytolysis, whereas a mixture of these plus a polyvalent anti-H-2Kk alloantiserum almost completely blocked target cell lysis. These findings indicate that single monoclonal antibody bound to a particular determinant on the H-2Kk molecule prevents the interaction of only some, but not all, CTL clones with the target molecule, suggesting the existence of different CTL clones with specificity for distinct H-2 determinants. This conclusion has been supported by target inhibition of alloreactive as well as H-2-restricted CTL clones generated in a limiting dilution system which showed that individual monoclonal antibodies block only 10-50% of CTL clones (our unpublished data).

Figure 2 clearly shows that the monoclonal antibodies fall into two distinct groups which differ with regard to their inhibitory capacity. Antibodies belonging to cluster A (see Fig. 2a, centre panel) reduce 51 Cr release from 80% to \sim 40% of the plateau level while those of cluster B reduce cytolysis to \sim 20%. The same difference is observed when the monoclonal antibodies are used at maximal inhibitory concentration (1:50 or 1:100 dilution), the ratio of killer cells to targets being varied (see Fig. 2b). Again monoclonal antibodies to cluster B are much more efficient in target inhibition, suggesting that more CTL clones are reactive against determinants belonging to cluster B than against those belonging to cluster A.

Next, we analysed an H-2-restricted CTL system in which CTLs from H-2^k mice recognize the hapten trinitrophenyl (TNP) linked to the H-2K^k molecule⁴. CBA splenocytes were sensitized in vitro with TNP-conjugated CBA spleen cells and cytolytic activity was determined after 6 days of culture on TNP-conjugated H-2^k target cells. Control experiments established that the TNP-specific CTLs were predominantly restricted by the K end (data not shown). Inhibition of the TNP-specific CTLs by anti-K^k antibodies showed again that each individual antibody blocks only a fraction of the CTL effector population while a mixture of monoclonal antibodies or a polyvalent antiserum against K^k almost completely prevents lysis (see Fig. 3). These findings indicate that CTLs can recognize TNP in conjunction with different H-2 determinants, or in other

words, that one $H-2K^k$ molecule carries more than one restriction site. As was also observed for allogeneic anti- K^k CTLs (Fig. 2), it was found for the TNP system that antibodies to cluster B were much more effective in preventing target cell lysis than antibodies to cluster A. The same conclusion was reached when the antibody concentration was constant at maximal inhibitory concentration (1:50-1:100 dilution) and the killer cells titrated (data not shown).

Calculation of lytic units provides an estimate of the relative number of CTLs in a population⁵. These data (see Table 1) show that in the presence of monoclonal antibodies to cluster B, on average three times fewer lytic units are measured than in the presence of monoclonal antibodies to cluster A. Taken together, these data suggest that about three times as many alloreactive and self-restricted CTLs are present which react with determinants of cluster B.

Target inhibition by monoclonal antibodies has also been described by others^{6,7} but our report provides additional information on the correlation of CTL specificity with the steric arrangement of H-2 determinants. The data show that both alloreactive as well as TNP-specific H-2-restricted populations are heterogeneous with regard to which H-2 epitopes on a given molecule are recognized, and that both CTL populations show preference for a particular domain on the H-2K^k molecule which is characterized by antibodies to cluster B. It is not clear from our results whether CTLs recognize exactly the same determinants as monoclonal antibodies or whether the latter block spatially related determinants only by steric hindrance. Another possibility is that monoclonal antibodies induce con-

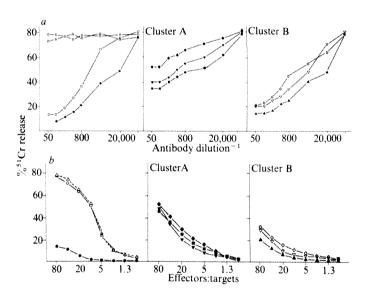


Fig. 2 Target inhibition of H-2Kk-specific alloreactive CTLs by monoclonal anti-H-2K antibodies. 10×10^6 DBA/2 (d/d) spleen cells were stimulated with 12×10^6 A/J (k/d) irradiated spleen cells as described elsewhere. After 5 days, CTL activity was measured against 51 Cr-labelled L929 (H-2k) target cells for 4 h in the presence of anti-Kk antibodies in a total volume of 200 µl using methods described previously⁶. These CTLs were shown to be specific for K^k (results not shown). In some experiments B10.D2 responders and B10.A stimulators were used. The figure shows a typical experiment which was repeated several times with the same results. a, Effect of different concentrations of monoclonal antibodies ascites on cytolysis at an effector to target ratio of 50:1. Left frame: \bigcirc , medium control; \triangle , control ascites 13/18; \square , hyperimmune BALB/c anti-A/J alloantiserum; , mixture of six monoclonal anti-K^k antibodies. Middle frame, anti-K^k antibody describing cluster A: ∇ , m3 = H100-5R28; \blacksquare , m4 = H100-27R55; ◆, m1 = H116-22R7. Right frame, monoclonal anti-K^k antibody describing cluster B: \diamondsuit , m5 = H100-30R23; \triangledown , m9 = H142-23/3; \triangle , m10 = H142-45/2. b, CTL titration in the presence of a constant amount of blocking antibodies (1:50 dilution of ascites). Same symbols as for a.

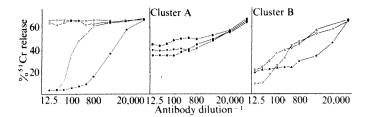


Fig. 3 Target inhibition of TNP-specific $H-2K^k$ -restricted CTLs from CBA $(H-2^k)$ mice by monoclonal anti- K^k antibody. TNPspecific CTLs were produced in vitro by activation of CBA splenocytes against TNP-conjugated CBA spleen as described elsewhere⁴. CTL activity was measured at an effector to target ratio of 50:1 against TNP-conjugated ⁵¹Cr-labelled L929 (H-2^k) target cells in the presence of various concentrations of blocking antibodies. By using different targets as well as cold target inhibition it was established that most CTLs were restricted by Kk. From other studies it is known that the antibodies used here mediate their blocking effect by inhibition of the target determinant and not when they are directed against the effector cells⁶. Symbols are the same as for Fig. 2. Left frame: ○, medium control; △, control ascites 13/18; , BALB/c anti-A/J alloantiserum; , mixture of six monoclonal anti-K^k antibodies. Middle frame, ascites of monoclonal antibody to cluster A (m1, m3, m4); right frame, monoclonal antibody to cluster B (m5, m9, m10).

formational changes at distal parts of the H-2 molecule which influence the interaction with CTLs. However, we consider it more likely that both CTLs and antibodies recognize basically the same polymorphic domains. The different blocking capacity of monoclonal antibodies to clusters A and B is not likely to be due to distinct physical properties of the monoclonal antibodies because those used here have similar affinities (ref. 8 and H. Lemke, personal communication) and belong to similar immunoglobulin classes (IgG2a and IgG2b, respectively). An exception is H145-45/2, which after recent re-evaluation was found to be IgM (unpublished data); the fact that it is pentameric may explain its very strong inhibitory capacity.

A possible explanation for the striking preference of alloreactive and H-2-restricted CTLs for cluster B is that both H-2^d (DBA/2 or B10.D2) and H-2^k mice use a similar or related pool of T-cell receptors for recognition of the Kk molecule, whether an alloantigen (in the case of DBA/2) or a self antigen (in the case of CBA) is recognized. Such a hypothesis would be compatible with dual recognition models, one binding site being for TNP and the other for H-2 determinants. Arguments in favour of single recognition models assume that neodeterminants formed by TNP modification of either H-2 or other surface components are predominantly located in the

Table 1 Lytic units of alloreactive and self-restricted CTL effector cells in the presence of blocking antibody against target determinants

Anti-H-2 antibody		Source DBA ā	of CTLs CBA ā
added to effector phase	H-2 cluster recognized	A/J (LU per	CBA-TNP 10 ⁶ cells)
No antibody	~~~	10.0	14.1
13/18 (Ia.7)	-	10.0	13.2
H100-5R28 (m3)	Α	1.03	2.6
H100-27R55 (m4)	Α	0.84	1.9
H116-22R7 (m1)	A.	1.33	4.35
H142-23/3 (m9)	В	0.35	0.54
H100-30R23 (m5)	В	0.35	0.82
H142-45/2 (m10)	В	0.12	0.72
Mixed monoclonal antibodies	A+B	0.02	< 0.01

Lytic units (LU) were calculated according to Cerottini et al.5 on the basis of CTL effector titrations as shown in Fig. 2b for alloreactive CTLs. The corresponding titration curves for TNP-specific CTLs are not shown. One lytic unit is defined as the number of effector cells required to lyse 50% of the target cells.

direct vicinity of polymorphic H-2 domains. Target inhibition of restricted CTLs by anti-H-2 would then be caused by steric hindrance. Note that preference of different CTL populations for the same cluster could be merely coincidental as it is possible that an H-2 molecule carries only few regions which, for steric reasons, are accessible to T cells. Further analysis of Kk-restricted CTL systems will clarify this question.

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- 1. Zinkernagel, R. M. & Doherty, P. C. Nature 248, 7@1-702 (1974).
- 2. Lemke, H. & Hämmerling, G. J. in Monoclonal Antibodies and T Cell Hybridomas (eds Hämmerling, G. J., Hämmerling, U. & Kearney, J. F.) (Elsevier, Amsterdam, in the
- Lemke, H., Hämmerling, G. J. & Hämmerling, U. Enmun. Rev. 47, 175-206 (1979).
 Shearer, G. M., Lozner, E. C., Rehn, T. G. & Schmitt-Verhulst, A-M. J. exp. Med. 141,
- Cerottini, J. C., Engers, H., Robson, H., MacDonald, H. R. & Brunner, K. T. J. exp. Med. 140, 703-717 (1974).
- Fischer-Lindahl, K. & Lemke, H. Eur. I. Immun. 9, 526-536 (1979). Epstein, S. L., Ozato, K. & Sachs, D. H. J. Immun. 125, 129-135 (1980)
- 8. Liberti, P. A., Hackett, C. J. & Askonas, B. A. Eur. J. Immun. 9, 751–757 (1979). 9. Nabholz, M. et al. Eur. J. Immun. 4, 378–387 (1974).

Light chain isotypes selectively associate with heavy chain idiotypes in T-dependent and T-independent dextran-specific precursors

Ronald E. Ward*, John F. Kearney† & Heinz Köhler*

- * La Rabida-University of Chicago Institute and the Departments of Microbiology and Pathology, The University of Chicago, Chicago, Illinois 60649, USA
- † Department of Microbiology, University of Alabama, Birmingham, Alabama 35294, USA

In murine immunoglobulins, the κ chain is the major light chain isotype, as exemplified by the small amount of λ immunoglobulins present in normal sera, the rare occurrence of λ plasmacytomas and the preponderance of κ light chains in induced antibodies. Notable exceptions to this are the response to $\alpha(1\rightarrow 3)$ dextran¹ in BALB/c mice and the heteroclytic antibodies raised against the nitrophenol hapten in C57BL/6 mice². In the former response, the expression of idiotypes in the hypervariable regions of the heavy chain³ is characteristically restricted. We have now analysed the B-cell precursors for the $\alpha(1 \rightarrow 3)$ linkage-specific response induced by T-dependent and T-independent dextran antigens with respect to light chain isotype and heavy chain idiotype expression. Our findings clearly demonstrate that the two forms of dextran antigen trigger different B-cell precursor subpopulations. Furthermore, each individual animal appears to possess a different repertoire of idiotype-committed precursors. This contrasts with the response of BALB/c mice to phosphorylcholine (PC) where most anti-PC antibodies and precursors respond with the dominant expression of the T15 idiotype family, regardless of whether T-dependent or T-independent PC antigens are used*.

BALB/c mice immunized with the T-independent antigen dextran B1355s respond with the expression of unique idiotypes (IdI) J558 and M104E, and a cross-reacting IdX idiotype⁵. Schilling and co-workers⁶ have shown that the unique IdI specificities are generated during the V-D-J joining process of heavy chain genes. While the expression of λ chains in the T-independent anti-dextran BALB/c response is consistently >90%, the amount of J558 and M104E IdI idiotypes varies between individual animals^{7,8}. This finding led us to speculate that the variable expression of heavy chain idiotypes might be caused by individually different, idiotype-specific regulation of B-cell precursors. Alternatively, during the development of the dextran-specific repertoire individually different exposures to environmental antigens could influence precursor maturation. According to the latter hypothesis, serum expression of light

Table 1 Co-expression of idiotypes and light chain isotypes by individual dextran-specific precursors

Group	Co-expression* of	By T-independent clones† (%)	Expected precursor doublets‡	By T-dependent clones (%)	Expected precursor doublets
1	λ and IdX	73 (41/56)	******	24 (12/51)	systematic
2	λ and J558 IdI	11 (14/129)	-	5 (9/182)	and more
3	λ and M104E IdI	7 (9/129)	*****	2 (3/182)	
4	k and IdX	Not found (0/56)		Not found $(0/51)$	
4	k and J558 IdI	Not found (0/56)		Not found $(0/51)$	****
2	k and M104E IdI	Not found (0/56)		Not found $(0/51)$	-
6	λ. J558 IdI and IdX	5 (3/56)	0.5/56	6 (3/51)	0.1/51
/		Not found $(0/56)$	0.3/56	Not found (0/51)	0.03/51
8 9	λ, M104E IdI and IdX λ, M104E and J558 IdI	0.77 (1/129)	0.1/129	Not found $(0/182)$	0.02/182

* Dextran-specific precursors were analysed for the co-expression of the given light chain isotype and the heavy chain idiotype.

‡ The statistical probability that the two given idiotypes will be co-expressed, based on the relative frequency of each idiotype alone.

chain isotypes and heavy chain idiotypes should directly reflect the differently established primary B-cell repertoire in individual animals.

Spleen cells from normal BALB/c mice were transferred in limiting cell numbers to 1,400 rad-irradiated normal or haemocyanin-primed syngeneic recipients. Twenty hours later, splenic fragment cultures were prepared according to the technique of Klinman⁹, modified for the analysis of T-dependent and T-independent B-cell precursors⁴. Fragment cultures were immunized with either dextran B1355s or dextran coupled to haemocyanin as described previously¹⁰. Culture supernatants were collected at 3-day intervals beginning on day 6 of culture and assayed for light chain isotype, heavy chain idiotypes and total anti-dextran antibody production, using enzyme-linked assays or radioimmunoassays (RIPs). Monoclonal antibodies were used to detect the cross-reacting IdX idiotype and the J558 and M104E IdI idiotypes¹¹. Figure 1 shows the percentage of heavy chain idiotypes and light chain isotypes calculated from data on 182 T-dependent and 129 T-independent dextran-

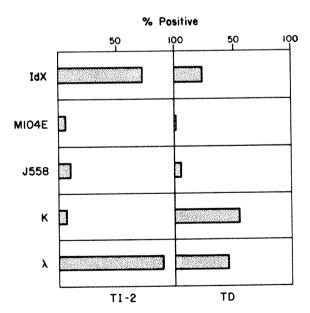


Fig. 1 The per cent positive dextran-specific precursors is plotted against the heavy chain isotype and the light chain idiotypes. Splenic fragments were immunized with either dextran B1355s (T-independent (TI)-2) or dextran-haemocyanin (T-dependent, TD). Supernatants were collected every 3-4 days and analysed for total anti-dextran antibodies, light chain isotype and heavy chain idiotype by RIA and enzyme-linked immunosorbent assay using monoclonal anti-J558 EB3-7-2. γ1k, raised in A/J mice, monoclonal anti-M104E SJL 18-1, μk, raised in SJL mice, and monoclonal anti-IdX CD3-2, γ1λ, raised in A/J mice.

specific precursors. Precursor clones responding to the $\alpha(1 \rightarrow 6)$ linkage, present in dextran B1355s, were excluded by testing for inhibition with dextran B512 $[\alpha(1\rightarrow 6)]$ in the anti-dextran RIA¹⁰. The data show that the IdI-producing clones in the T-dependent and T-independent anti-dextran precursors are infrequent; most T-independent precursors express the crossreacting IdX heavy chain idiotope while T-dependent precursors express the IdX idiotype in <25% of dextran-specific clones. Furthermore, T-dependent precursors express the k chain more frequently than do T-independent precursors. The ability of T-dependent precursors to express both λ and κ light chains could explain why the frequency of dextran precursors responding to the T-dependent antigen is higher than that of T-independent precursors¹⁰. Although the relative fraction of IdX precursors is smaller in the T-dependent than in the Tindependent response, the absolute number of IdX precursors does not appear to differ much for T-dependent and Tindependent dextran antigens. Thus the selection of heavy and light chains is dictated by the type of dextran antigen.

The question of whether heavy chain idiotypes can freely combine with λ and κ light chains or whether their association is restricted cannot be answered from the collective precursor analysis data shown in Fig. 1. Therefore, individual clones were grouped together according to their co-expression of light chain isotypes with heavy chain idiotypes. The relative frequencies of clones co-expressing a certain light chain with a certain idiotype were calculated from the total number of T-independent and T-dependent dextran-specific clones. The data are shown in Table 1. The most frequent combination is the co-expression of λ and IdX in T-independent and T-dependent precursors (group 1). No precursors were found which co-expressed κ and any of the heavy chain idiotypes. A small number of Tindependent and T-dependent precursors were positive for J558 IdI and IdX; none was positive for M104E IdI and IdX. These data demonstrate that the J558-M104E IdI idiotype site is different from the IdX site, in agreement with the assignment of IdI and IdX sites in hybridoma anti-dextran antibodies made by Schilling et al.6. The failure to find M104E IdI- and IdX-positive clones is not surprising considering the rare occurrence of M104E IdI precursors. However, the failure to detect known heavy chain idiotypes, that is, M104E, J558 and IdX, in precursors expressing the k light chain is striking.

It is important for the evaluation of fragment cultures secreting more than one idiotypic antibody to determine the probability of two different clones being present in one fragment. The expected frequency of clonal precursor doublets was calculated from the number of single idiotype-secreting cultures. The finding of a clone expressing J558 and M104E is unexpected, and unlikely to result from the presence of two different clones present in a single fragment culture as the likelihood of a clonal doublet at the given experimental limiting cell dose is >1 in 1,000 (see group 9 in Table 1; 0.1 in 129 T-independent clones).

[†] Splenic fragments were immunized with either dextran B1355s (T-independent-2) or dextran-haemocyanin (T-dependent). Supernatants were collected every 3-4 days and analysed for total anti-dextran antibodies. Positive wells were selected and analysed for the co-expression of the appropriate light chain isotype and heavy chain idiotype. The numbers in parentheses represent the number of clones which co-express the isotype and idiotype out of the total number of positives examined.

Table 2 Analysis of dextran-specific precursors in individual BALB/c mice

Animal no.	Precursor per 10 ⁵ B cells		% J558 IdI		% M104E IdI		% IdX	
	TI	TD	TI	TD	TI	TD	TI	TD
1	4.50	4.36	<4(0/25)	23(3/13)	20(5/25)	< 8(0/13)	ND	ND
ž	2.00	7.41	25(3/12)	5(1/21)	< 8(0/12)	< 5(0/21)	ND	ND
3	0.80	1.60	<20(0/5)	<20(0/5)	< 20(0/5)	<20(0/5)	ND	ND
4	0.80	5.49	<20(1/5)	<6(0/16)	20(1/5)	< 6(0/16)	ND	ND
· 5	1.82	6.99	10(1/10)	<5(0/19)	20(2/10)	< 5(0/19)	ND	ND
6	1.39	5.66	<6(0/17)	<3(0/32)	<6(0/17)	6(2/32)	86(12/14)	19(3/16)
7	1.48	3.65	<4(0/25)	5(1/22)	<4(0/25)	5(1/22)	95(20/21)	24(5/21)
8	3.51	ND	38(3/8)	ND	<13(0/8)	ND	38(3/8)	ND
9	ND	6.82	ND	4(1/22)	ND	<4(0/22)	ND	16(4/25)
10	0.32	7.81	50(1/2)	9(3/22)	< 50(0/2)	< 5(0/22)	ND	ND
11	0.97	1.60	<17(0/6)	<20(0/5)	<17(0/6)	<20(0/5)	ND	ND

Normal or haemocyanin-primed BALB/c mice were irradiated with 1,400 rad and used as recipients of individual adult syngeneic cells. Splenic fragments were immunized with dextran B1355s or dextran-haemocyanin and supernatants were collected every 3-4 days and analysed for total anti-dextran antibodies. Dextran-specific precursors were analysed for J558 IdI, M104E IdI and IdX idiotype as described in Fig. 1 legend.

It would be attractive to assume for the double IdI-positive clone that both chromosomes have made a productive but different DNA rearrangement resulting in two active genes producing J558 and M104E messages.

We have previously^{7,8} observed variation of the amount of heavy chain IdI idiotypes in individual mice immunized with dextran B1355s. For example, the amount of J558 IdI in four randomly selected BALB/c mice 7 days after immunization with dextran B1355s varied from 7 to 53% of the total antidextran reponse. To determine whether this individually different idiotype expression is the result of idiotype-specific regulation, induced by antigenic stimulation, or is predetermined by the number of idiotype-committed precursors, precursor analysis of individual BALB/c mice was performed. Table 2 shows that individual mice indeed express the IdI idiotypes in significantly different amounts for T-dependent and T-independent responses. The relative J558 IdI frequency for T-independent precursors in individual animals was 4-50% and for T-dependent J558 IdI 3-23% (see Table 2). The overall (averaged) expression of J558- and M104E-positive precursors, as well as the number of T-dependent and T-independent responding precursors, is in good agreement with the data from analysis on precursors from pooled cell donors.

The present findings lead to three conclusions important for the understanding of mechanisms controlling idiotype expression. (1) Certain heavy chain idiotypes seem to depend on the combination with one kind of light chain. (2) Different antigenic forms of the same antigenic determinant can trigger different precursors: T-dependent dextran antigen triggers significantly more κ precursors than the T-independent dextran B1355s. Conceivably, T cells, which participate in the T-dependent response, help to recruit κ chain B-cell precursors. (3) Individual mice possess variable frequencies of precursors expressing different idiotypic specificities. Although the individually variable expression of idiotypes after immunization^{7,8} has not been formally linked to variable idiotype precursor frequencies, we could infer that individual idiotype expression stems directly from the presence of variable precursor compositions. Accordingly, individual variation of precursor frequencies must be generated during the maturation of the B-cell repertoire in the fetal-neonatal period¹². Idiotype-specific factors might be important in controlling the development of B-cell repertoires¹³. In addition, isotypic network interactions control the expression of idiotypes in the adult animal during primary or established immune responses 14-17. Comparison of the immature with the mature idiotype repertoire will help to define the role of regulatory mechanisms in establishing clonal immune profiles18

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- Carson, D. & Weigert, M. Proc. nam. Acad. Sci. U.S.A. 76, 235 (1973). Kelsoe, K., Reth, M. & Rajewsky, K. Immun. Rev. 52, 75-88 (1980). Blomberg, B., Geckler, W. R. & Weigert, M. Science 177, 178 (1972). Fung, J. & Kohler, H. J. Immun. 125, 640-646 (1980).

- Fung, J. & Kohler, H. J. Immun. 125, 640-646 (1980).
 Hansburg, D., Briles, D. E. & Davie, J. M. Immunology 119, 1406-1412 (1977).
 Schilling, J., Clevinger, B., Davie, J. M. & Hood, L. Nature 283, 35-40 (1980).
 Fung, J., Gleason, K., Ward, R. & Kohler, H. in Membranes, Receptors and the Immune Reponse (eds Cohen, E. P. & Kohler, H.) 203-214 (Liss, New York, 1980).
 Ward, R. & Kohler, H. Cell. Immun. 58, 286 (1981).
 Klinman, N. R. J. exp. Med. 136, 241 (1972).
 Ward, R. & Kohler, H. J. Immun. 126, 146-149 (1981).
 Kearney, J. K. Fedn Am. Soc. exp. Biol. 38, 1421 (1979).
 Sigal, N. H., Pickard, A. R., Metcalf, E. S., Gearbart, P. J. & Klinman, N. R. J. exp. Med.

- Sigal, N. H., Pickard, A. R., Metcalf, E. S., Gearhart, P. J. & Klinman, N. R. J. exp. Med. 146, 933 (1977).
- Fung, J. & Kohler, H. J. exp. Med. 153, 1262-1273 (1980).
 Kluskens, L. & Kohler, H. Proc. natn. Acad. Sci. U.S.A. 71, 5083-5087 (1974).
 Cosenza, H. Eur. J. Immun. 6, 114 (1976).
- Rowley, D. A., Kohler, H. Schreiber, H., Kay, S. T. & Lorbach, I. J. exp. Med. 144, 946-959
- 17. Goidl, E. A., Schrater, A. F., Siskind, G. W. & Thorbecke, G. J. J. exp. Med. 150, 154
- 18. Kohler, H. & Fung, J. in B Lymphocytes in the Immune Response (eds Klinman, N., Mosier, D. E., Scher, I. & Vitteta, E. S.) 69 (Elsevier, Amsterdam, 1981).

Independent control of immunoglobulin heavy and light chain expression in a murine pre-B-cell line

Christopher J. Paige*, Paul W. Kincade† & Peter Ralph†

- † Basal Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basal, Switzerland
- † Laboratory of Haematopoietic Development, Sloan-Kettering Institute, Rye, New York 10580, USA

B lymphocytes differentiate from multipotential stem cells by an unknown number of intermediate stages. Although the earliest stages of this sequence remain obscure, cells, which appear to be intermediate in this process, have been described1-7. Large, proliferating, rapidly sedimenting cells which synthesize immunoglobulin chains that can be easily detected in the cytoplasm (clg) but are either absent or less stable on the cell surface (sIg) have been found in both fetal liver and adult bone marrow. Smaller clg⁺ slg⁻ cells have also been described^{2,5,7}. These cells are designated pre-B cells and may comprise a functionally heterogeneous group of progenitors. Several cell lines, which share many properties with these postulated precursors, have been useful in establishing an asynchronous onset of immunoglobulin expression characterized by the appearance of μ heavy chains in the absence of light chain expression 10-12. We confirm here that the mitogen-responsive pre-B-like cell line, 70Z/3, in normal growth conditions, only expresses heavy chain, although it has a fully rearranged light chain gene 13. 70Z/3 cells can also display the μ chain on the cell surface in the absence of light chain induction, most notably after exposure to dextran sulphate.

The 70Z/3 cell line synthesizes immunoglobulin detectable only intracellularly, but on exposure to lipopolysaccharide (LPS) the cIg⁺ sIg⁻ cell type rapidly acquires sIg⁵. Concomitant with the induction of sIg is the appearance of κ light chains¹⁴. There is, however, no evidence for secretion⁹. The increase in κ chain synthesis is related to induction of κ mRNA¹⁵.

The initial hypothesis, that light chain synthesis is causally related to surface acquisition of immunoglobulin, is not necessarily the case. This was demonstrated using a second B-cell mitogen, dextran sulphate (DxS). As shown in Fig. 1, exposure to LPS led to the acquisition of both μ and κ , whereas exposure to DxS resulted in a similar level of surface μ expression without expression of κ chains. Furthermore, the kinetics of μ expression differ after exposure to these two mitogens. Figure 2 shows that the detection of μ after DxS stimulation preceded that of both the μ and κ induced after LPS stimulation although ultimately similar levels were achieved. DxS does not, however, inhibit κ induction by LPS (data not shown).

Exposure to these mitogens neither altered the rate of proliferation, nor caused cell death and, although the kinetics of induction were variable, in most experiments > 90% of the cells were sIg⁺ within 24 h (two cell cycles). It thus seems unlikely that different subpopulations of cells account for these results. Furthermore, most clones of 70Z/3 cells also respond to both DxS and LPS (see below), and the relative sensitivity of the reagents used to detect μ and κ chains was found to be similar.

We observed that whereas the μ and κ found on the membrane of LPS-stimulated cells was distributed over most of the cell surface, the μ chains detected on DxS-stimulated cells were usually found in tight caps. These tight caps were partially inhibited (95% capped reduced to 50%) by prior treatment with

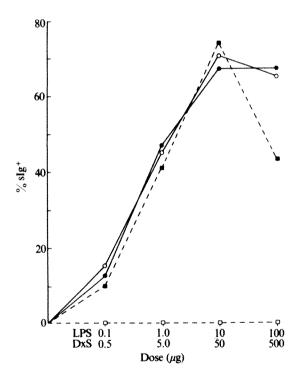


Fig. 1 Increase in surface immunoglobulin expression in 70Z/3 cells exposed to various concentrations of LPS (Salmonella typhosa WO 901, Difco) or DxS (Pharmacia) for 36 h. Surface expression of either μ or κ chains was assessed using affinity-purified, rhodamine-conjugated goat antibodies specific for either μ or κ which were prepared and used as previously described. LPS, μ ; \bigcirc , LPS, κ ; \bigcirc , DxS, μ ; \bigcirc , DxS, κ ; 70Z/3 cells were grown in RPMI-1640 containing 10% FCS (Associated Biomedic Systems) and supplemented with 5 mM glutamate, 10^5 U⁻¹ 1 penicillin, 0.1 g⁻¹1 steptomycin and 5×10^{-5} M 2-mercaptoethanol. Cell cultures were initiated at 2.5×10^5 mi⁻¹ grown in 25-cm³ tissue culture flasks (Corning) and kept at 37 °C in a humid atmosphere containing 7% CO₂.

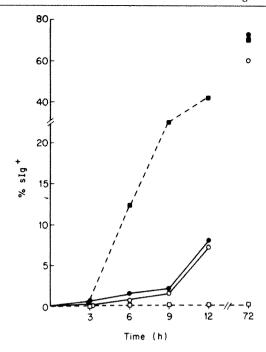


Fig. 2 Difference in time of appearance of cell-surface immunoglobulin chains after stimulation with LPS (10 μg ml⁻¹) or DxS (50 μg ml⁻¹). ♠, LPS, μ (10 μg ml⁻¹); ○, LPS, κ (10 μg ml⁻¹); ■, DxS, μ (50 μg ml⁻¹); □, DxS, κ (50 μg ml⁻¹). Cell cultures were initiated at 2.5 × 10⁵ ml⁻¹ in 25-cm² tissue culture flasks.

cytochalasin B (30 min, 37 °C, 10 μg ml⁻¹) but not by staining in the presence of 1% NaN₃.

Possible explanations for the different cell surface distribution of μ chains after exposure to LPS or DxS include that: (1) the μ appearing after DxS stimulation differs from the μ found after LPS stimulation; (2) the presence of κ chains, although not necessary for the appearance of μ on the surface, is required for the stability of the sIg; or (3) a cell-surface alteration unrelated to immunoglobulin molecules may be responsible. It is also possible that the uninduced cells possess a small but undetectable amount of surface μ which undergoes a conformational change on exposure to DxS, leading to enhanced expression. Biochemical evaluation of the DxS- and LPS- induced μ is underway and may resolve these issues.

Additional differences in the responses to these two mitogens was found by analysing clones of 70Z/3 obtained by growth in soft agar. Although 19 of these clones (70Z/3.2-70Ž/3.20) had similar characteristics to the parent line and could respond to both LPS and DxS, one clone, 70Z/3.1, lost the ability to respond to LPS, although retaining DxS responsiveness (Table 1). This clone was refractory to even 100 times the concentration of LPS normally used. Furthermore, the parent line and LPS-responsive clones, if grown in the presence of serum-free medium, failed to respond to LPS although the DxS response was normal (Table 1). After an initial period of adaptation, growth in this medium was similar to growth in serumcontaining medium and LPS responsiveness was restored by addition of 2% fetal calf serum (FCS). This may indicate that serum contains components which condition the cells for responsiveness to LPS. Previously we found that different batches of either FCS or newborn serum themselves could cause background levels of sIg+ cells to vary from 1 to 99% (ref. 9, and unpublished data). It should be noted that in almost all these cases of elevated background expression of μ , light chains were not detectable. Thus, DxS is not obligatory for the detection of the 'u only' surface phenotype. Furthermore, recent data suggest that the surface μ is indeed membrane μ and not secretory. First, similar to our previous report with LPS-induced cells, the supernatants of 70Z/3 cells exposed to DxS contained no detectable immunoglobulin (C. Sidman and C. J. P., in preparation). Second, biochemical analysis of the μ proteins of 70Z/3 cells reveal (1) a cell-surface form, labelled by lactoperoxidase-catalysed iodination, which corresponds in size to membrane μ characteristic of small resting B cells, and (2) biosynthetically labelled intracellular forms which correspond to the smaller precursors of both the secretory and membrane pathways (ref 16, and C. Sidman and C. J. P., in preparation). Analysis using tunicamycin of the non-glycosylated products further revealed a precursor pattern previously described for both membrane and secretory μ^{16} . In this respect 70Z/3 cells are similar to other B cells containing the precursors for both pathways but the final product of only the membrane form¹⁶.

The detection of cIg⁺ sIg⁻ cells which acquire sIg confirms the circumstantial evidence which suggests that this transition normally occurs during B-cell differentiation 7,17,18. Our findings are also consistent with reports that the onset of heavy and light chain synthesis is asynchronous^{12,19}. It is not clear whether the expression of μ chains has a regulatory role in B-cell development before light chain appearance. One report suggests that some pre-B cells may be capable of secreting μ chains¹⁹. Our findings, although different in that 70Z/3 cells do not secrete their μ chains, also suggests the possibility of interaction with the external environment through the variable region of the μ chain. This interaction might occur before synthesis of complete antibody molecules and thus be relevant to theories of clonal abortion, tolerance induction and network formation. Maki et al. have recently shown that whereas μ only fetal liver hybridomas have unrearranged light chain genes, the κ gene of ' μ only' 70Z/3 has already undergone rearrangement¹³. Thus several stages of pre-B cells may exist based on both DNA molecular arrangement and the expression of rearranged gene products.

We do not yet know the incidence of precursors in fetal and adult tissues with properties corresponding to 70Z/3 cells. These cells may represent a transient stage of B-cell maturation which is subject to regulation by factors such as those present in certain serum lots and by mitogens.

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Note added in proof: A human lymphoma has recently been described which also expresses μ chain in the absence of light chains; "A human B-cell lymphoma synthesizing and expressing surface μ chain in the absence of detectable light chain" (Gordon, J., Hamblin, T. J., Smith, J. C., Stevenson, F. K. & Stevenson, G. T. Blood (in the press).)

Table 1 Differential responsiveness of 70Z/3 and 70Z/3.1 to LPS and DxS

	% surface μ^+				
Stimulus	70Z/3	70Z/3-SF	70Z/3.1		
	2	0	0		
LPS(1 μ g ml ⁻¹)	82	0	0		
DxS (50 μg ml ⁻¹)	76	73	76		

Cell cultures were initiated at 2.5×10⁵ ml⁻¹ and exposed to either LPS or DxS for 24 h. Surface μ expression was determined using rhodamine-conjugated [F(ab')2] fragments of goat antibodies. For 70Z/3-SF cells, 70Z/3 cells were grown in serum-free Iscove's modified Dulbecco's medium (Gibco, No. 430-220) supplemented with lecithin/cholesterol, human transferrin (Hoechst) and delipidated bovine serum albumin (Behring, no. TR05) as previously described²⁰. The cells had been growing serum free for 6 weeks at the time of this experiment. For 70Z/3.1 cells, clones of 70Z/3 cells were obtained by initiating growth in RPMI medium (supplemented as described in Fig. 1 legend) containing 0.3% agar (Difco). Approximately 60-75% of the cells formed discrete colonies in these conditions. Clones were picked at day 6, transferred to microwells containing 0.2 ml medium and subsequently maintained in tissue culture flasks.

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- 1. Lafleur, L., Underdown, B. J., Miller, R. G. & Phillips, R. A. Ser, Haemat, 5, 50-63 (1972).
- Osmond, D. G. J. reticuloendothel. Soc. 17, 99-114 (1975).
- Melchers, F., von Boehmer, H. & Phillips, R. A. Transplant, Rev. 25, 27-56 (1975).
- 4. Cooper, M. D., Kearney, J. F., Lydyard, P. M., Grossi, C. E. & Lawton, A. R. Cold Spring
- Harb. Symp. quant. Biol. 41, 139-145 (1976). 5. Owen, J. J. T., Wright, D. E., Habu, S., Raff, M. C. & Cooper, M. D. J. Immun. 118, 2067-2072 (1977)
- Raff, M. C., Megson, M., Owen, J. J. T. & Cooper, M. D. Nature 259, 224-226 (1976)
- 7. Burrows, P. D., Kearney, J. F., Lawton, A. R. & Cooper, M. D. J. Immun. 120, 1526-1531
- Paige, C. J., Kincade, P. W., Moore, M. A. S. & Lee, G. J. exp. Med. 150, 548-563 (1979).
 Paige, C. J., Kincade, P. W. & Ralph, P. J. Immun. 121, 641-647 (1978).
- Siden, F. L., Baltimore, D., Clark, D. & Rosenberg, N. E. Cell 16, 389-396 (1979).
 Boss, M., Greaves, M. & Teich, N. Nature 278, 551-553 (1979).

- Boss, M., Greaves, M. & Teich, N. Nature 218, 351-353 (1979).
 Burrows, M., Lejeune M. & Kearney, J. F. Nature 280, 838-841 (1979).
 Maki, R., Kearney, J., Paige, C. & Tonegawa, S. Science 209, 1366-1369 (1980).
 Ralph, P., Paige, C. J. & Nakoinz, I. in T and B Lymphocytes: Recognition and Function (eds Bach, F. H., Bonavida, B., Vitetta, E. S. & Fox, C. F.) 143-154 (Academic, New York, 1972).
- 15. Perry, R. P. & Kelly, D. E. Cell 18, 1133-1339 (1979).
- Sidman, C. Cell 23, 379-389 (1981).
 Osmond, D. G. & Nossal, G. V. Cell. Immun. 13, 132-145 (1974).
- Ryser, J. & Vasalli, P. J. Immun. 113, 719-728 (1974)
 Levitt, D. & Cooper, M. D. Cell 19, 617-625 (1980).
- 20. Iscove, N. N. & Melchers, F. J. Expl Med 147, 923-933 (1978).

Hormonal regulation of synthesis of volk proteins and a larval serum protein (LSP2) in *Drosophila*

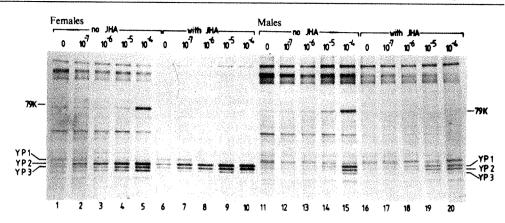
T. Jowett* & J. H. Postlethwait†

- Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK
- † Department of Biology, University of Oregon, Eugene, Oregon 97403, USA

Juvenile hormone (JH) and 20-hydroxyecdysone (20-HE) have a wide range of roles in insects. They interact during development to determine the quality of the moult1-4 and both hormones have been implicated in control of yolk protein synthesis and ovarian development. However, their functions vary in different insects⁵⁻⁷. In adult Drosophila, they have specific effects on the haemolymph proteins. Both JH analogue (JHA) and 20-HE stimulate the synthesis and secretion of three yolk polypeptides (YPs) into the haemolymph of females^{8,9}. However, whereas JHA stimulates synthesis of YPs in both the fat body and the ovary, 20-HE stimulates the synthesis only in the fat body 10 the fat body¹⁰, and JH, but not 20-HE, induces YP deposition in oocytes^{9,11,12}. 20-HE, but not JHA, evokes YP synthesis in Drosophila males (which do not normally produce these proteins¹³), and 20-HE treatment of isolated female abdomens stimulates incorporation of ³⁵S-methionine into a protein of molecular weight 79,000 (designated 79K). We have now compared the responses of the YPs and 79K to combinations of JHA and 20-HE given to both male and female Drosophila and show that 79K is a larval serum protein (LSP2) normally secreted by the fat body of third instar larvae. JHA and 20-HE have additive effects on YP synthesis, but JHA antagonizes 20-HE stimulation of LSP2 synthesis. This effect is probably caused by JHA induction of fat body cell death.

In an initial experiment, animals were ligated soon after eclosion, aged for 24 h to allow endogenous levels of hormone to decay and then treated in groups of 16 with increasing amounts of 20-HE in the presence or absence of JHA. After 6 h the haemolymph proteins were labelled by injecting 35S-methionine and finally separated on SDS-polyacrylamide gel electrophoresis (PAGE). This experiment revealed three different effects of the hormone treatments. (1) Incorporation of label into YPs was stimulated additively by 20-HE and JHA in isolated female abdomens (Fig. 1, lanes 1-10, and Fig. 2), confirming earlier reports^{9,10}. (2) Secretion of newly synthesized YPs was also stimulated by 20-HE in isolated male abdomens

Fig. 1 JHA inhibits the 20-HEstimulated label incorporation into 79K but augments that into the YPs. Isolated abdomens were prepared from newly eclosed flies by ligation with nylon thread between the thorax and abdomen, and removal of the head and thorax with scissors. After ageing for 24 h to allow the endogenous hormone evels to decline, abdomens were treated in groups of 16 with different concentrations of 20-HF in 1% ethanol/Ringer's solution. A second set of abdomens was treated with the same concentrations of 20-HE in the presence of a juvenile hormone analogue, ZR-515 (ref. 22) (0.3 µl of an



acetone solution of JHA was added topically to each abdomen giving a final dose of 1.6 ng ZR-515 per abdomen). After a 6-h incubation, the newly synthesized haemolymph proteins were labelled by injecting $1 \mu \text{Ci}^{35}\text{S}$ -methionine per abdomen in *Drosophila* Ringer²³. After a further 2-h incubation, the haemolymph was collected and mixed with sample buffer²⁴. One-quarter of each sample electrophoresed on an SDS-containing 9-15% polyacrylamide gel²⁵; the Coomassie blue-stained gel was dried and autoradiographed. The figure is a photograph of the resulting autoradiograph. Lanes 1-10 and 11-20 are from female and male isolated abdomens respectively. Lanes 1-5 and 11-15 are from abdomens treated with increasing doses of 20-HE alone, while lanes 6-10 and 16-20 are from abdomens treated with increasing doses of 20-HE in the presence of JHA. 20-HE-stimulated label incorporation into YP1, YP2 and YP3 and the 79K polypeptide. Treatment with both hormones together stimulated label incorporation into YPs but not 79K.

(Fig. 1, lanes 11–15), demonstrating that the anterior of the male fly does not mediate the action of 20-HE. JHA alone had no effect in male abdomens (Fig. 1, lanes 11 and 16). Whereas the response to the hormones was approximately additive in females (Fig. 1, lanes 2–5 and 7–10), in males JHA made no consistent alteration to the 20-HE response (Fig. 1, lanes 12–15 and 17–20). (3) 20-HE stimulated synthesis of 79K in both males (Fig. 1, lanes 11–15) and females (Fig. 1, lanes 1–5), but this response was abolished in both sexes by the presence of JHA (Fig. 1, lanes 16–20 and 6–10, and Fig. 2). Thus in young adult haemolymph, the labelling of two simultaneously occurring groups of polypeptides is stimulated by 20-HE, and JHA adds to the 20-HE stimulation for one group of polypeptides but abolishes it for the other.

Because the nature and the site of synthesis of 79K were unknown, experiments were performed to identify the 79K polypeptide. During our work with YPs, we had noted that 79K was always seen in Coomassie blue-stained gels of haemolymph from newly eclosed males and females, but that it gradually disappeared from haemolymph of older animals (see Fig. 1 in ref. 10). This development programme for 79K coincides with that for the larval fat body. About 1,000 larval fat body cells survive metamorphosis but then histolyse in the first few days of adult life under the influence of JH^{14,15}. We thus suspected that 79K might be synthesized and secreted by the larval fat body cells that remain after eclosion. The major proteins synthesized and secreted by the larval fat body are the larval serum proteins (LSPs)¹⁶—LSP1, a hexamer containing combinations of three different polypeptides^{17,18}, and LSP2, a homohexamer¹⁹. As the four constituent polypeptides have estimated molecular weights of 75,000-83,000, it seemed reasonable that 79K seen in adult haemolymph at eclosion was a polypeptide constituent of LSP1 and/or LSP2 which was synthesized and secreted by the surviving larval fat body cells.

To test this hypothesis, ³⁵S-methionine-labelled haemolymph samples from abdomens which had been injected with either Ringer's solution or 20-HE were subjected to immunoelectrophoresis with antisera raised against purified LSP1 and LSP2 and compared with labelled haemolymph from mid-third instar larvae (Fig. 3). Larval haemolymph forms two major precipitin arcs, one corresponding to LSP2 and the other containing the three subunits of LSP1. Immunoelectrophoresis of the adult haemolymph showed that no label was incorporated into LSP1 in isolated abdomens irrespective of treatment with 20-HE. In contrast, LSP2 was heavily labelled in the haemolymph from the 20-HE-treated isolated abdomens and was weakly labelled in haemolymph from control abdomens.

To confirm that the arc formed with anti-LSP2 antisera indeed represented the 79K polypeptide identified by SDS-PAGE, a section of each precipitin arc was cut out of the agar plate and the sample run on SDS-PAGE with some of the original haemolymph samples. The labelled LSP1 precipitin arc from larval haemolymph separated as three distinct polypeptides but each of these was absent from the isolated abdomen haemolymph samples. The LSP2 precipitin arc was labelled in

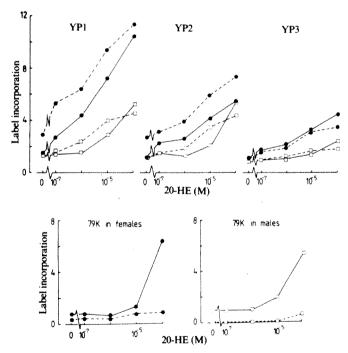


Fig. 2 Quantification of the response to hormone. Autoradiographs of the experiment in Fig. 1 were scanned using a Joyce–Loebl microdensitometer and the label incorporation estimated by measuring the peak areas of the 79K and YP bands. Each point represents the mean of two experimental replicates. Top panel, YPs; bottom panel, 79K. ♠, Female abdomens; □, male abdomens. For both panels a broken line indicates JHA treatment with different concentrations of 20-HE; a solid line indicates 20-HE treatment in the absence of JHA. In females the two hormones stimulated label incorporation into the YPs additively, whereas JHA had no effect on the labelling of YPs in males. In both males and females 20-HE stimulated labelling of the 79K polypeptide but this effect was abolished by simultaneous treatment with JHA.

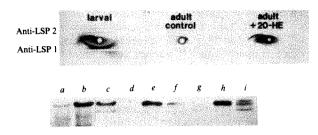


Fig. 3 79K is immunoprecipitated by antisera against LSP2. Labelled larval haemolymph was obtained by injecting mid-third instar larvae with S-methionine dissolved in Ringer and collecting the haemolymph 2 h later. The adult haemolymph was obtained from abdomens which had been ligated at eclosion and allowed to age for 24 h. Controls were injected with Ringer and after 8 h injected with 35S-methionine and haemolymph collected after a further 2 h. The 20-HE-treated abdomens were injected with 10⁻⁴ M 20-HE in 1% ethanol/Ringer 8 h before treatment with label. All haemolymph samples were mixed with unlabelled larval haemolymph, to act as carrier, and immediately subjected to immunoelectrophoresis. They were run in agar on microscope slides with 0.1 M sodium barbitone buffer (pH 8.4) at 80 V for 45 min at 4 °C. After electrophoresis each slot was filled with either anti-LSP1 or anti-LSP2 antisera, raised in rabbits from the purified proteins. After precipitation overnight the white precipitin arcs could be seen, and the agar was washed thoroughly in phosphate-buffered saline and stained with amido-Schwartz, dried and autoradiographed. The resulting autoradiograph is shown in the top panel. Larval haemolymph produced two main precipitin arcs corresponding to each of the LSPs. The adult haemolymph did not show any label incorporation into the LSP1 precipitin arc. A small amount of label was incorporated into the LSP2 arc of the control abdomens while considerably more was found in the haemolymph of those treated with 20-HE. Identical precipitin arcs were cut out of the agar without staining and mixed with SDS buffer before running on an SDS-containing 9% polyacrylamide gel. The gel was dried and autoradiographed. The 79K region of the resulting autoradiograph is shown in the lower panel: a, haemolymph from the control abdomens; b, haemolymph from abdomens treated with 20-HE; c, labelled mid-third instar larval haemolymph; d, LSP1 precipitin arc from 20-HE-treated abdomens; e, LSP2 precipitin arc from 20-HEtreated abdomens; f, LSP2 precipitin arc from control abdomens; g, LSP1 precipitin arc from control abdomens; h, LSP2 precipitin arc from larval haemolymph; and i, LSP1 precipitin arc from larval haemolymph. The LSP1 precipitin arc from larval haemolymph separates into the three LSP1 subunits but label is absent from this protein in the adult abdomens. The LSP2 precipitin arc is present in the haemolymph from the abdomens and label incorporation is stimulated by the treatment with 20-HE.

both adult haemolymph samples and co-migrated with LSP2 from larval haemolymph. In addition, the LSP2 from 20-HEtreated abdomens contained considerably more label than that from abdomens injected with Ringer alone.

These experiments have shown that both JHA and 20-HE act additively on YP synthesis. They also provide the first evidence that the appearance of LSP2 is also hormonally regulated, and that, in contrast with the YPs, its synthesis is stimulated by 20-HE only and abolished by JHA.

It follows from our observations that the larval fat body persisting in the newly eclosed adult is still capable of synthesizing LSP2. It also seems that the absence of LSP2 synthesis in the intact early adult is primarily because of the endogenous secretion of JH since merely ligating the abdomen causes a partial stimulation in synthesis of LSP2 and addition of JHA abolishes both this and the response to 20-HE. Once the source of JH has been removed by isolation of the abdomen, the larval fat body is able to synthesize LSP2 in response to 20-HE. Thus, although the effect has only been demonstrated at a developmental stage when the LSP2 gene is no longer active because of the presence of JH, it nevertheless suggests that the initial appearance of LSP2 may also be ecdysone induced. This is consistent with the observed ecdysone pulse at the second larval moult shortly before the appearance of LSP2 at the beginning of the third instar²⁰

If 20-HE is indeed the hormonal trigger for LSP2 synthesis at the onset of the third larval instar, then either the titres of JH must be low or the larval fat body at that stage refractory to any naturally occurring JH. The latter is quite likely as larval fat body transplanted from third instar larvae into adults in contrast to larval fat body which has been through metamorphosis 15,21 not susceptible to JH-induced histolysis. As the susceptibility of the larval fat body to JH changes during pupation, the inhibition of the response to 20-HE could provide a means of monitoring the transition. The simplest explanation for the inhibitory action of JHA on the stimulation of LSP2 by 20-HE is that JH triggers cellular responses that lead to death of the remaining larval fat body15. However, this action of JH at the molecular level may be complex because the JH-induced death of the larval fat body is blocked by inhibitors of protein synthesis15

Study of the appearance of LSP2 associated with the changes in the susceptibility of the larval fat body to these two major hormones should elucidate their actions on specific gene expression during development.

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- Willis, J. H. A. Rev. Ent. 19, 97-115 (1974)
- Slama, K. J. Insect Physiol. 21, 921-955 (1975)
- Wigglesworth, V. B. Insect Hormones (Oliver & Boyd, Edinburgh, 1970). Mitsui, T. & Riddiford, L. M. Devl Biol. 62, 193–205 (1978).
- Mundail, E. C., Tobe, S. S. & Staye, B. Nature 282, 97 (1979) Engelmann, F. Adv. Insect Physiol. 14, 49–108 (1979).
- Hagedorn, H. H. & Kunkel, J. G. A. Rev. Ent. 24, 475-505 (1979) Wigglesworth, V. B. Nature 174, 556 (1954).
- Postlethwait, J. H. & Handler, A. M. J. Insect Physiol. 25, 455-460 (1979).
- Jowett, T. & Postlethwait, J. H. Devi Biol. 80, 225-234 (1980)

- Jowett, T. & Postlethwait, J. H. Devi Biol. 80, 225-234 (1980).
 Postlethwait, J. H. & Weiser, K. Nature new Biol. 244, 284-285 (1973).
 Gavin, J. & Williamson, J. J. Insect Physiol. 22, 1737-1742 (1976).
 Postlethwait, J. H., Bownes, M. & Jowett, T. Devi Biol. 79, 379-387 (1980).
 Butterworth, F. M. & La Tendresse, B. L. J. Insect Physiol. 19, 1487-1499 (1973).
- Butterworth, F. M. & La Tendresse, B. L. J. Insect Physiol. 19, 1487-1499 (197 Postlethwait, J. H. & Jones, G. J. J. exp. Zool. 203, 207-214 (1978). Roberts, D. B., Wolfe, J. & Akam, M. E. J. Insect Physiol. 23, 871-878 (1977). Wolfe, J., Akam, M. E. & Roberts, D. B. Eur. J. Biochem. 79, 47-53 (1977). Roberts, D. B. & Evans-Roberts, S. Nature 280, 691-692 (1979).
- 16.

- Akam, M. E., Roberts, D. B. & Wolfe, J. Biochem. Genet. 16, 691-692 (1978). Hodgetts, R., Sage, B. & O'Connor, J. D. Devl Biol. 60, 310-317 (1977).
- Butterworth, F. M. Wilhelm Roux's Arch. dev. Biol. 172, 263-270 (1973). Postlethwait, J. H. Biol. Bull. 147, 119-135 (1974).
- Chan, L. & Gehring, W. Proc. natn. Acad. Sci. U.S.A. 68, 2217–2221 (1971). Hames, B. D. & Bownes, M. Insect Biochem. 8, 319–928 (1978).
- O'Farrell, P. H. J. biol. Chem. 250, 4007-4021 (1975).

A homoeotic mutation transforming leg to antenna in Drosophila

Gary Struhl

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Insects are thought to have evolved from millipede-like ancestors composed largely of a series of identical, leg-bearing segments. This view of insect evolution is supported by the existence of homoeotic mutations which transform particular abdominal and head segments into thoracic segments 1-3. Such transformations are described as atavistic3, because they return specialized segments to a more primitive condition. In Drosophila, several dominant mutations of the homoeotic locus Antennapedia (Antp) lead to a transformation of the antenna to the second leg⁴⁻⁸. Here, I describe the isolation and characterization of apparent null alleles of the Antp locus. These mutations lead to a homoeotic phenotype which is the reverse of the dominant Antennapedia phenotype, namely, they result in the transformation of the second leg into an antenna but do not alter the development of the normal antenna itself. This result indicates that (1) the product of the wild-type Antp gene is normally active in the mesothorax where it promotes a mesothoracic pathway of development instead of an antennal pathway, (2) the Antp⁺ gene product is normally absent or inactive in the antenna, and (3) dominant mutations of the locus result in the inappropriate activity of the wild-type gene product in the antenna, and hence in the transformation of antenna to leg. Thus, unlike most other homoeotic gene products, the product of the Antp+ gene seems to promote, not to repress or modify, an atavistic condition.

Table 1 Homozygous Antp^{Ns+RC3} clones in the eye-antenna and mesothorax

Compartment	No. of blastoderm clones*	No. of first instar clones*	Phenotype†
Eye-antenna $(A + P)$	18	43	Normal
Leg 2 (A)	16(24)	18(24)	Antennal
Leg 2 (P)	14(15)	8(8)	Antennal
Notum + wing (A)	1(2)	9(16)	Abnormal

Marked clones of cells lacking the Antennapedia $^+$ gene were obtained as follows. Embryos or larvae of the genotype $Ant^{Ns+RC3}\,e^{11}/Ki\,Sb^{63b}$ $M(3)w^{124}$ were irradiated at appropriate times after egg laying $(3\pm$ 0.7 = blastoderm stage; 24-48 h = first instar) (see Fig. 1 for description of mutations). All the mutations are located on the right arm of the third chromosome; because Ki is positioned closest to the centromere, mitotic recombinations which occur between Ki and the centromere can give rise to homozygous Ki^+ cells which must also be homozygous for $Antp^{Ns+RC3}$, Sb^+ , e^{11} and $M(3)w^+$. 2,128 flies were screened following irradiation with 500 rad at the blastoderm stage, and 959 flies were screened following irradiation with 1,000 rad during the first larval instar. Clones lacking the Antp+ gene were identified under the dissecting microscope by the appearance of marked bristles and because they frequently disrupted the normal morphology. (Clones which mark, but do not transform, the distal portions of leg are difficult to identify under the dissecting microscope, and hence may have been missed.) All clones were mounted and inspected under the compound microscope. A comparable analysis of first instar clones of a second revertant allele, , gave similar results.

*Number of clones in which some of the cells showed the normal, antennal or abnormal phenotype indicated in the last column (in those compartments in which some, but not all, of the clones were normal, the total number of clones is indicated in parentheses).

†Clones in the anterior compartment of the second leg were classified as antennal only when they formed antennal tissue. Clones in the posterior compartment of the second leg were classified as showing the antennal phenotype if they removed portions of the compartment—even when antennal tissue was not found (see text). The abnormal phenotype observed in notum clones is described in the text.

Mutations which eliminate most, or all, of the activity of the Antp⁺ gene were obtained using the general procedure of selecting phenotypic reversions of a dominant mutation9. This approach has been used by others 10,11 to isolate apparent null alleles of the Antp locus. Homozygous Antp No males were treated with the mutagen ethyl methane sulphonate (EMS)12 and outcrossed to appropriately marked females. From among ~10,000 F₁ progeny, 35 phenotypic revertants were found; 12 of these progeny were found to carry 'revertant' alleles of the $Antp^{Ns}$ mutation (designated $Antp^{Ns+RC1, 2, 3, \dots, 12}$). Three lines of evidence suggest that these revertant alleles eliminate most, or all, of the wild-type activity of the Antp locus. First, they arose with the expected frequency of null mutations in a single gene following a standard EMS mutagenesis¹³. Second, all these mutations are lethal over a deficiency of the Antp locus (Df(3R)4Scb; G. Jürgens, personal communication), as well as over other recessive lethal mutations of the locus (for example, Antp^B, Antp^{73b}, Antp^{Scx}). Third, animals which are homozygous or hemizygous for any of these reversions die as first instar larvae and show the characteristic terminal phenotype caused by loss of the wild-type Antp gene¹⁴ (but not those phenotypes associated with the loss of neighbouring genes, such as Scr, R14)¹⁴. It is possible that reversions of the $Antp^{Ns}$ mutation may eliminate more than one genetic function; however, in the absence of substantiative evidence that the Antp gene is itself complex, it is simplest to treat these reversions as mutations in a single gene.

The consequences of removing the wild-type Antp gene from particular cells during development have been studied by generating clones of cells homozygous for $Antp^{Ns+RC}$ mutations in animals otherwise heterozygous for the mutation (see Fig. 1, Table 1). Cells belonging to these clones were independently marked using the bristle mutations Kinked, Stubble, $Minute(3)w^{124}$ and ebony (Fig. 1, Table 1; see refs 15 and 16 for descriptions of mutations). In addition, the Minute technique of

clonal analysis¹⁷ was used, so that clones of homozygous, $Antp^{Ns+RC}$ $M(3)w^+$ cells grew faster than surrounding, $Antp^{Ns+RC}/M(3)w^{124}$ cells, and hence formed large portions of the adult compartments^{18,19}. Clones were induced at the blastoderm stage as well as at later times during larval development. The results are shown in Fig. 1 and Table 1 and are described below.

First, clones in the eye-antennal segment were normal even when they were large enough to form most of the antenna, head capsule and maxillary palp (Fig. 1b, c). Similarly, clones in the proboscis, as well as at least the first six abdominal segments, were normal.

Second, clones in the anterior compartment of the second leg frequently transformed some parts of the leg into corresponding antennal structures (for example, distal portions of the leg, such as the tarsus, were transformed into tissue characteristic of the distal antenna, such as the third antennal segment (Fig. 1d-f)). However, only some of the marked cells showed the homoeotic phenotype while the remaining marked cells formed normal second leg structures (Fig. 1d, e).

Third, clones in the posterior compartment of the second leg were generally associated with the absence of parts of the posterior compartment; in a few cases, vesicles of antennal tissue were also found associated with the clone (Fig. 1g). Note that the posterior compartment of the antenna is relatively small (Fig. 1c)²⁰. Consequently, a transformation of the posterior leg compartment into the posterior antenna compartment is expected to replace the leg compartment with only a small amount of antennal tissue.

Fourth, following irradiation at the blastoderm stage, the number of clones obtained in the anterior wing and notum (the dorsal derivatives of the mesothorax) were disproportionally small compared with the number of clones obtained in other appendages (Table 1). This result suggests that most blastoderm clones induced in the wing and notum primordium were lost during development or prevented the emergence of the flies bearing them. Wing and notum clones induced during the first larval instar and later, were found with similar frequencies to clones induced at the same time in other appendages. However, most of these clones showed abnormal patterns of notum bristles and in a few cases, atypical bristles were also observed.

Finally, clones induced during the blastoderm stage in the first and third legs were abnormal in about half the cases. Generally, such abnormal clones were associated with the absence of portions of the leg and with atypical cuticular patterns which could not be identified.

The principal result is that the phenotype of apparent null mutations of the *Antennapedia* locus (second leg transformed into antenna) is the reverse of that of dominant mutations of the

Table 2 Control of antennal versus second leg development by the $Antp^+$ gene product

The state of the s	Ante	nna	Second leg		
Genotype	Antp ⁺ gene product	Structure formed	Antp ⁺ gene product	Structure formed	
Wild type $Antp^{Ns}/+Antp^{-}/Antp^{-}$	Absent (Present) Absent	Antenna (Leg 2) Antenna	Present Present Absent	Leg 2 Leg 2 Antenna	

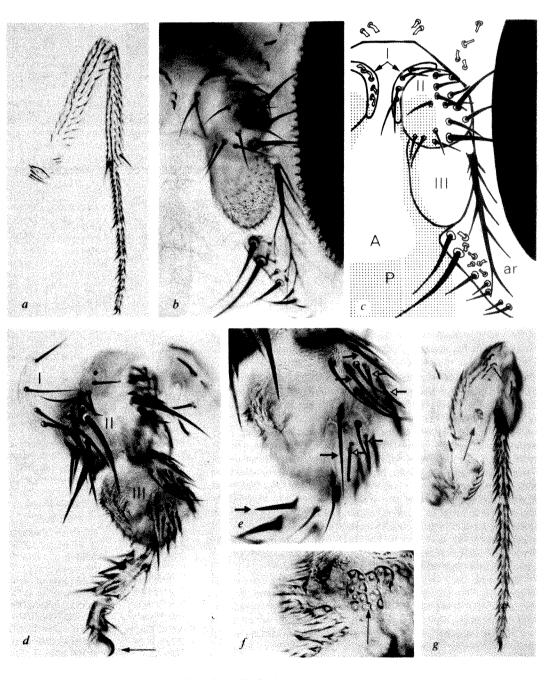
Model for the control of antennal versus second leg development by the $Antp^+$ gene product. In both the antennal and second leg, presence (and activity) of the $Antp^+$ gene product leads to leg development whereas absence of the active gene product leads to antennal development. Normally, the $Antp^+$ gene product is absent (or inactive) in the antennal primordium, but present (and active) in the leg primordium. Mutations conferring the dominant Antennapedia phenotype result in the presence of active gene product in the antenna leading to an antennal to leg transformation (because this transformation is variable and incomplete it is likely that the gene product is only partially expressed or partially active in the antenna). Apparent null alleles of the $Antp^+$ gene result in the absence of active $Antp^+$ gene product in both the antenna and second leg, leading to a transformation of the leg into antenna.

locus (antenna transformed into second leg). This situation parallels that of dominant and recessive mutations of several of the genes of the bithorax complex1, and leads to the following conclusions: (1) in wild-type flies, the Antp+ gene product is normally active in the mesothorax where it promotes mesothoracic as opposed to antennal development, but absent or inactive in the eye-antennal segment, and (2) mutations of the locus which confer a dominant Antennapedia phenotype result in inappropriate activity of the Antp⁺ gene product in the antenna, thereby transforming the antenna into a second leg (Table 2). These conclusions must be considered in the light of the homoeotic phenotypes of embryos lacking the Antp⁺ gene. Wakimoto and Kaufman¹⁴ have described this phenotype as a partial transformation of the meta- and mesothorax into prothoracic segments, and concluded that the Antp+ gene is normally required in the meso- and metathorax for promoting a normal as opposed to a prothoracic pathway of development. If their interpretation of the embryonic phenotype is correct, the results presented here would indicate that the $Antp^+$ gene product controls one developmental alternative (mesothorax instead of prothorax) in embryonic cells giving rise to the larval cuticle, and another developmental alternative (mesothorax instead of eye-antenna) in imaginal cells giving rise to the adult cuticle.

A second result of interest is that the leg-to-antenna transformation observed in clones lacking the $Antp^+$ gene is shown by some, but not all, of the cells belonging to the clone, the remaining cells forming normal second leg structures. Although there are many possible explanations for this apparent non-autonomy, the simplest is that the wild-type Antp gene product can pass between cells, thereby allowing heterozygous cells to rescue neighbouring mutant cells. This interpretation is supported by the observations that large clones generally formed antennal structures whereas small clones did not (data not

Fig. 1 Phenotype of somatic clones lacking the Antennapedia+ gene in the antenna and second leg. a, Wild type, second leg $\times 56$, b, c, Fly's head bearing a marked clone induced at the blastoderm stage which forms a normal antenna and part of the head capsule. Cells of the clone are marked by the recessive mutation $ebony^{11} (e^{11})$ which darkens the normal colour of cuticular structures, particularly bristles; cells outside the clone are marked by the dominant mutations Kinked (Ki), Stubble 63b (Sb 63b) and Minute (3) w^{124} (M(3) w^{124}) which together result in short, stunted bristles (see refs 15, 16 for descriptions of mutations). In c, bristles belonging to the clone are blacked in. whereas bristles outside the clone are drawn in outline. The three antennal segments (I, II and III), the arista (ar) and the anterior unshaded) and posterior (P; shaded) compartments of the eye-antenna are indicated inc. ×186. d. Blastoderm clone which forms almost the entire anterior compartment of the second leg, transforming it into corresponding portions of the antenna. Note the of structures presence characteristic of the first (I), second (II) and third (III) antennal segments. Note also that marked cells situated at the margins of the clone form normal leg tissue (in this case, a claw (arrow), and bristles characteristic of the distal The untransformed posterior compartment lies beneath the transformed anterior compartment and hence is out of the plane of focus. ×224. e, Detail of d showing apparent of the leg-toautonomy antenna transformation. Marked cells at the periphery of the clone form bracted bristles characteristic of the

(filled



indicate bristles formed by the clone, outlined arrows indicate bristles formed by cells outside the clone; bracts can be seen as small spikes at the base of most leg bristles. ×352. f, Detail of the antennal tissue in e showing an antennal sense organ, the sacculus²³ (arrow), formed inside the homoeotic antenna. ×520. g, Second leg bearing a blastoderm clone which forms virtually the entire posterior compartment. Although the most proximal and most distal portions of the posterior compartment are not transformed (even though marked by the clone), the middle portions of the compartment are absent, giving the leg a truncated appearance (compare with a). Note also a vesicle of antennal tissue within the leg (arrow). ×96.

shown), and that mutant cells which were untransformed were generally positioned at the periphery of mutant clones, next to heterozygous cells (for example, Fig. 1e). In this context, it is interesting that regions of the leg such as the distal tarsus and claw which were never transformed (Fig. 1d) are situated next to the anteroposterior compartment boundary21, and hence, are always adjacent to heterozygous cells which may rescue the mutant phenotype. The possibility that the wild-type Antp gene product can pass between cells also provides a plausible explanation for the results of Kauffman and Ling²², who found that wild-type (+/+) clones in the antennae of $Antp^{Ns}/+$ flies often formed leg tissue.

Studies of genes of the bithorax complex have provided support for the supposition that a series of thoracic-like segments was progressively modified during evolution to form the sequence of specialized segments of the insect abdomen and thorax1. If a similar process modified more anterior, thoracic-

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- Lewis, E. B. Am. Zool. 3, 33-56 (1963); Nature 276, 565-570 (1978).
 Tazima, Y. The Genetics of the Silkworm (Logos, London, 1964).
- Garcia-Bellido, A. Am. Zool. 17, 613-629 (1977). Yu, S. thesis, California Inst. Technol. (1949).
- Le Calvez, J. Bull. Biol. Fr. Belg. 82, 97-113 (1948). Lewis, E. B. Drosoph. Inf. Serv. 30, 76-77 (1956).
- Gehring, W. Arch. Klaus-Stift. Vererb.-Forsch. 41, 44-54 (1966). Kaufman, T. C., Lewis, R. & Wakimoto, B. Genetics 94, 115-133 (1980).

- Lifschytz, E. & Falk, R. Genetics 62, 353-358 (1969).
 Denell, R. E. Mutat. Res. 15, 221-223 (1972).
 Duncan, I. W. & Kaufman, T. C. Genetics 80, 733-752 (1975).

like segments into the specialized segments of the head, it is possible that a small number of control genes may be responsible for specifying the development of the head segments. Clearly, the products of such head promoting genes should not function in the thoracic segments, otherwise they also would develop into head segments. It is possible, therefore, that the product of the Antp⁺ gene normally acts in the thorax to repress the function of head promoting genes, but is absent (or inactive) in the head segments where the functions of these genes are required for normal development.

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- 12. Lewis, E. B. & Bacher, F. Drosoph, Inf. Serv. 43, 193 (1968).
- Lewis, E. B. & Bacner, F. Drosopn. Inj. Serv. 43, 173 (1700).
 O'Brian, S. J. & MacIntyre, R. J. in Genetics and Biology of Drosophila Vol. 2 (eds Ashburner, M. & Wright, T. R. F.) 395-551 (Academic, London, 1978).
- Wakimoto, B. T. & Kaufman, T. C. Devl Biol. 81, 51-64
- Lindsley, D. L. & Grell, E. L. Camegie Inst. Wash. Publ. 627 (1968).
 Ferrus, A. Genetics 79, 589-599 (1975).
 Morata, G. & Ripoll, P. Devl Biol. 42, 211-221 (1975).

- Garcia-Bellido, A., Ripoll, P. & Morata, G. Nature new Biol. 245, 251-253 (1973); Devl Biol. 48, 132-147 (1976).
- 19. Crick, F. H. C. & Lawrence, P. A. Science 189, 340-347 (1975)
- Morata, G. & Lawrence, P. A. Nature 274, 473-474 (1978); Devi Biol. 70, 355-371 (1979).
 Lawrence, P. A., Struhl, G. & Morata, G. J. Embryol. exp. Morph. 51, 195-208 (1979).
 Kauffman, S. A. & Ling, E. Wilhelm Roux Arch. EntwMech. Org. 189, 147-153 (1980).
- 23. Ferris, G. F. in Biology of Drosophila (ed. Demerec, M.) 369-419 (Wiley, New York, 1950).

Intervening sequences in ribosomal RNA genes and bobbed phenotype in Drosophila hydei

Gerald Franz & Werner Kunz

Institut für Genetik, Universität Düsseldorf, Universitätsstrasse 1, D-4000 Düsseldorf, FRG

The 'bobbed' (bb) mutation in Drosophila is represented phenotypically by shortened and abnormally thin scutellar bristles and by delayed development. There is a direct correlation between bristle size and ribosomal RNA (rRNA) synthesis1, and the bb mutation was at first explained as a deficiency of rRNA genes (rDNA)2. However, the bb phenotype can occur in Drosophila melanogaster and Drosophila hydei³ with high rDNA content, while phenotypically wild-type flies are known with few rRNA genes, suggesting that what matters is not the number of rRNA genes but their transcriptional activity. In D. melanogaster, it has recently emerged that rRNA genes interrupted by an intervening sequence are not transcribed4. We now report that in D. hydei, the length of the scutellar bristle is directly proportional to the number of rRNA genes without this intervening sequence.

D. hydei has three clusters of rRNA genes (nucleolar organizers, NOs), one on the X and two on the Y chromosome^{5,6}. We have previously described flies containing only Y-chromosomal NOs which were not bobbed in spite of a low rDNA content. In contrast, flies with only X-chromosomal NOs, containing significantly more rDNA, were bobbed3. Clearly in D. hydei, the NOs on the Y and X chromosomes do not have a comparable influence on the bobbed phenotype.

Many X-chromosomal rRNA genes in D. hydei contain an intervening sequence 6 kilobases (kb) long (ivs + genes)7, but in several genotypes with only Y-chromosomal NOs, no ivs+ rRNA genes have been detected. As in D. melanogaster, ivs+

genes in D. hydei do not seem to be significantly transcribed8. Consequently, the X-chromosomal NO, in contrast with the Y-NO, does not seem to be completely functional with respect to rRNA transcription.

Total rRNA gene numbers were determined by filter saturation hybridization experiments between ¹⁴C-DNA from whole adult flies and 28S ³H-rRNA. To eliminate variation of bb expression within each of our laboratory strains, each investigated strain was newly started from a single pair mating, and flies from the F₂ or F₃ generation used. Among 20 genotypes investigated, 11 had either the wild-type scutellar bristle length of 0.57 mm or very close to it, in spite of the fact that their rDNA content varied from 210 to 1,113 genes per two chromosome sets (Fig. 1, Table 1). Two genotypes showed slightly reduced bristle lengths (0.50 and 0.52 mm), although their rRNA gene number was very high (780 and 1,067). Flies from seven other genotypes were phenotypically clearly bobbed, with bristle sizes

Bristle length and number of ivs and ivs rRNA genes per two chromosome sets from bb and wild-type flies

	Daiotle leveth	rRNA gen	e number:	ŧs.e.m.
Genotype	Bristle length \pm s.e.m. (n)	ivs -	ivs +	F; G
$X^{bb1}/O \ X^{bb1}/X^{bb1}$	0.212 ± 0.004 (33)	28 ± 4	189 ± 8	18; 3
$X^{bb\ 1}/X^{bb\ 1}$	$0.316 \pm 0.002(31)$	55 ± 6	365 ± 14	18; 3
X^{bb2}/O	0.306 ± 0.004 (10)	70 ± 7	456 ± 13	40; 4
X^{bb3}/O	0.240 ± 0.002 (21)	52 ± 6	388 ± 13	14; 3
$X^{bb 4}/O$	0.305 ± 0.002 (69)	80 ± 6	341 ± 13	23;4
X^{bb4}/X^{bb4}	0.495 ± 0.006 (25)	135 ± 9	645 ± 20	19; 3
X^{bb5}/O	$0.354 \pm 0.006 (10)$	94 ± 5	422 ± 15	30; 4
$\frac{w^{m1}}{w^{m1}}/O$	0.518 ± 0.004 (30)	142 ± 14	925 ± 30	30; 3
w^{m1}/O	$0.335 \pm 0.002 (100)$	70 ± 6	851 ± 31	28; 3
X/O	$0.568 \pm 0.002 (39)$	220 ± 17	151 ± 16	27; 3
X/X	$0.554 \pm 0.003 (20)$	428±15	276 ± 12	23; 3

n, Number of flies of which the two posterior scutellar bristles have been measured. F, number of filters which have been measured per genotype to determine the rDNA percentage. G, number of gels used for Southern transfer, hybridization and determination of the ivs "/ivs rRNA gene relationship.

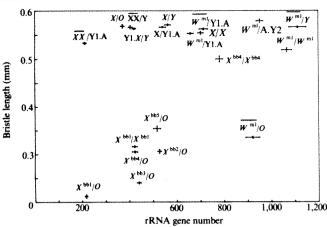


Fig. 1 Plot of rRNA gene numbers per two chromosome sets of different genotypes of D. hydei relative to the length of the two posterior scutellar bristles. The bristle length of 10-100 individuals of each genotype was measured under a dissecting microscope. DNA from several hundred flies of each genotype was extracted, nick-translated with deoxy-14C-ATP, and 2-µg samples were loaded on to nitrocellulose filters, essentially following the methods described previously 13. To determine the per cent rDNA in the different genotypes, the DNA was hybridized to 28S 3HrRNA from cultured cells of D. melanogaster. Hybridization was in the saturation range at Rot values between 0.4 and 0.5 mol RNA s I⁻¹. The measured d.p.m. values have been corrected for non-specific background binding and for DNA loss during the hybridization procedure. The gene numbers have been calculated on the basis of a diploid genome size of 0.5 pg DNA and on the estimation that the X chromosome includes 17% and the Y chromosome 9% of the DNA of a diploid D. hydei genome²⁰ Chromosome symbols: \overline{XX} , attached \overline{X} , a chromosome without rDNA; w^{m1} , \overline{X} chromosome with two NOs²¹; w^{m1} , attached w^{m1} chromosome, carrying two NOs; Y1·X, Y1·A and A·Y2, Y-X and Y-autosome translocation chromosomes, each with only one Y-chromosomal NO.

between 0.21 and 0.35 mm. However, their rRNA gene number was of the same order of magnitude as that of wild-type flies: between 217 and 921. Nine genotypes of phenotypically wild-type flies had either the complete Y chromosome with two NOs or a Y-chromosomal fragment with one NO; two genotypes with wild-type bristles had only X-chromosomal NOs. The seven genotypes whose flies were distinctly bobbed all contained only X-chromosomal NOs. The fact that six of them had more rDNA than the wild-type X/O male demonstrates that not only Y-chromosomal but also X-chromosomal rRNA gene number is not directly correlated with the bobbed phenotype.

To determine the relative amounts of ivs+ and ivs genes, the DNA from bobbed flies and from X/O or X/X wild-type flies was double-digested with EcoRI and PstI. The fragments were separated on 1% agarose gels, transferred to nitrocellulose filters according to Southern⁹ and hybridized with a ³²P-rDNA fragment (pDh2-A4) cloned from D. hydei 10 (Fig. 2). This fragment, which consists mainly of the 28S coding region, hybridizes to an EcoRI-PstI fragment of 5.4 kb with ivs genes. With ivs + genes it hybridizes to a slightly smaller 4.8-kb band (Figs 2 and 3). The use of such similar-sized fragments for quantification of DNA after Southern transfer reduces the danger of an unequal transfer to nitrocellulose of DNA molecules with widely differing molecular weights. The two bands were quantified by scanning the autoradiograms on a Joyce-Loebl Chromoscan 200. The absolute ivs and ivs gene numbers were calculated using the values from Fig. 1, giving the results shown in Table 1. All bobbed genotypes possess few ivs genes compared with the wild type.

When only the *ivs* gene number of each genotype is plotted relative to the bristle length, a direct proportionality between the gene number and phenotypic *bobbed* expression is evident in the range between 30 and $\sim 150~ivs$ genes (Fig. 4). The correlation coefficient in this range is 0.95. The *bobbed* phenotype is

Table 2 ivs rRNA gene number per two chromosome sets in salivary gland DNA

	ivs rRNA gene	
Genotype	number \pm s.e.m.	F; G
X/X	$149 \pm 8 (13)$	21; 3
X/O	143 ± 5	5; 2
X^{bb4}/X^{bb4}	104 ± 7	9; 3
X^{bb} $^4/O$	48 ± 5	10; 3

X/O and X/X constitutions are compared in the wild-type and the bb4 stock. The total rDNA percentage was measured in DNA from hand-dissected salivary glands from third instar larvae by filter saturation hybridization (for methods, see ref. 13). The ivs rRNA gene content was determined photometrically after EcoRI-PstI digestion, gel electrophoresis, Southern transfer, hybridization and autoradiography as described for DNA from whole flies. The values have been corrected for non-specific background binding, for DNA loss during the hybridization procedure and for differences in genome size. As only the euchromatic portion of the genome is involved in polytenization to chromosome sets in a polyploid nucleus are 0.37 pg DNA in an X/X genotype of D. hydei and 0.33 pg DNA in an X/O genotype (values calculated from the data of Mulder et al. 20). F; G, for explanation, see Table 1 legend.

no longer expressed when $\sim 200 \, ivs^-$ genes are reached. A further increase in gene number $(X/O:220, X/X:428 \, ivs^-$ genes) does not result in larger scutellar bristles (Table 1).

Gene numbers have been measured on DNA extracted from whole flies, which contain both diploid and polyploid tissue. However, rDNA percentages measured in the DNA from whole flies differ from those in polyploid tissue as during polytenization¹¹ and polyploidization¹² in D. hydei, the rDNA is under-replicated. Furthermore, in particular genotypes the rDNA undergoes disproportionate replication: 'gene compensation' increases the rDNA content in some diploid organs relative to polyploid organs¹³ and 'independent rDNA polyploidization' raises the rDNA percentage in polyploid cells relative to diploid cells^{13,14}. As the bristle-forming cells in Drosophila are polyploid¹⁵, the rRNA gene number in polyploid tissue rather than the average number in whole flies should directly influence the bristle length. However, in genotypes without gene compensation or independent polyploidization, the amount of rDNA in whole flies is proportional to that in polyploid tissue. Thus, in such genotypes measurements on whole flies are sufficient.

Because gene compensation in D. hydei has hitherto been only observed with Y-chromosomal $NOs^{13,16}$, we selected genotypes with only X-NOs for our investigations. It is less easy to exclude independent rDNA polyploidization, because this process occurs with both Y- and X-chromosomal NOs^{13} . For example, the ivs^- rDNA percentage in whole X/O males is almost exactly half that in X/X females (Table 1), but in salivary glands the ivs^- rRNA gene numbers of both genotypes were the

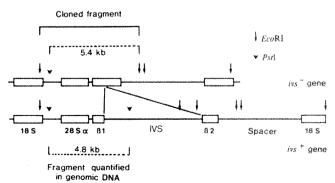


Fig. 2 Schematic diagram showing the two types of rRNA gene with their *EcoRI* and *PstI* restriction sites¹⁰. The indicated cloned fragment (pDh2-A4) was used for hybridization (see Fig. 3). For quantification of the two fragments in genomic DNA, it was taken into consideration that the 4.8-kb fragment hybridizes only partially to the cloned fragment. IVS, intervening sequence.

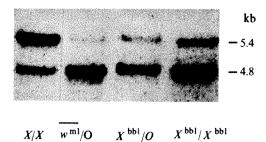


Fig. 3 Autoradiogram of EcoRI-Pst I-digested DNA hybridized with the cloned fragment pDh2-A4 (ref. 10) (see Fig. 2), demonstrating different ivs //ivs f gene relationships in four genotypes. In this experiment, 1 µg of nuclear DNA from whole adult flies of each genotype was loaded on 1% agarose gels, transferred to a filter and hybridized. The hybridization conditions used were: (1 μ g cloned DNA in 10 ml 2×SSC (1×SSC=0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 0.2% SDS, 1× Denhardt's solution; 40 h. The measured relationship of the two fragments within the genotype was independent of the amount of input DNA. For tracing on the Joyce-Loebl Chromoscan, autoradiograms were used only where the film density was linear with increasing exposure time.

same (Table 2). If such independent rDNA polyploidization also occurs in genotypes with bobbed X chromosomes, the difference in bristle length between X/O and X/X constitutions measured in bobbed-1 and bobbed-4 stocks (Fig. 4) is difficult to understand.

As it is very difficult to determine gene numbers directly on bristle-forming cells, we used a representative polyploid tissue, salivary glands, to determine the ivs rRNA gene numbers in our bobbed-4 stock. We found that X^{bb4}/X^{bb4} females have twice as many ivs rRNA genes per chromosome set as X^{bb4}/O males (Table 2). Thus the bobbed-4 X chromosome differs from the wild-type X chromosome by being replicated proportionally during polyploidization with respect to its ivs rRNA genes. The bobbed chromosomes in the other stocks probably behave similarly, so that bobbed flies with low ivs rRNA gene numbers also have low numbers in their polyploid tissue.

Note that none of our investigated genotypes has a low number of both types of rRNA gene (Table 1). Genotypes with low ivs gene numbers have relatively high percentages of ivs genes (81-92%), whereas the wild type has only 40% ivs genes. It is possible that the two types of rRNA gene change their degree of multiplicity in a dependent manner; distinct loss in ivs genes (resulting in the bobbed phenotype) seems to be accompanied by an expansion of ivs + genes. Further experiments are being done to investigate this possible correlation in stocks where spontaneous bobbed mutations occur. Such a correlation would explain the observation¹⁷ that the bobbed mutation in D. hydei is almost exclusively associated with the

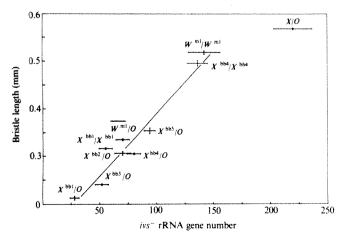


Fig. 4 Direct correlation between ivs rRNA gene number per two chromosome sets and the length of the two posterior scutellar bristles of the same genotypes as in Table 1.

X-chromosomal NO. Only two bobbed mutants of the Y chromosome have hitherto been recorded18. In contrast, in D. melanogaster bobbed Y chromosomes are frequent². In this species, ivs+ genes also occur in the Y-chromosomal NO, whereas in all genotypes hitherto investigated in D. hydei, the Y-NOs seem to be completely free of ivs + rRNA genes.

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- Shermoen, A. W. & Kiefer, B. I. Cell 4, 275-280 (1975). Ritossa, F. M., Atwood, K. C. & Spiegelman, S. Genetics 54, 819-834 (1966). Schäfer, U. & Kunz, W. Heredity 37, 351-355 (1976). Long, E. O. & Dawid, I. B. Cell 18, 1185-1196 (1979).

- Hennig, W., Link, B. & Leoncini, O. Chromosoma (Berl.) 51, 57-63 (1975). Schäfer, U. & Kunz, W. Molec. gen. Genet. 137, 365-368 (1975). Kunz, W., Petersen, G., Renkawitz-Pohl, R., Glätzer, K. H. & Schäfer, M. Chromosoma (Berl.) (in the press).
- Glätzer, K. H. Chromosoma (Berl.) 75, 161-175 (1979). Southern, E. M. J. molec. Biol. 98, 503-517 (1975).
- Renkawitz-Pohl, R., Glätzer, K. H. & Kunz, W. Nucleic Acids Res. 8, 4593-4611 (1980).
 Hennig, W. & Meer, B. Nature new Biol. 233, 70-72 (1971).
- Renkawitz, R. & Kunz, W. Chromosoma (Berl.) 53, 131-140 (1975)
 Grimm, C. & Kunz, W. Molec. gen. Genet. 180, 23-26 (1980).

- Spear, B. B. & Gall, J. G. Proc. natn. Acad. Sci. U.S.A. 70, 1359-1363 (1973).Overton, J. J. Morph. 122, 367-380 (1967).
- Kunz, W. & Schäfer, U. Genetics 82, 25-34 (1976). Spencer, W. P. Genetics 29, 520-536 (1944).
- Beck, H. Drosoph. Inf. Serv. 50, 94–95 (1973).
 Gall, J. G., Cohen, E. H. & Polan, M. L. Chromosoma (Berl.) 22, 319–344 (1971).
- Mulder, M. P., van Duijn, P. & Gloor, H. J. Genetica 39, 385-428 (1968)
 van Breugel, F. M. A. Genetica 41, 589-625 (1970).

Multiple copies of iso-insertion sequences of IS1 in Shigella dysenteriae chromosome

Hisako Ohtsubo, Kate Nyman, Włodzimierz Doroszkiewicz & Eiichi Ohtsubo

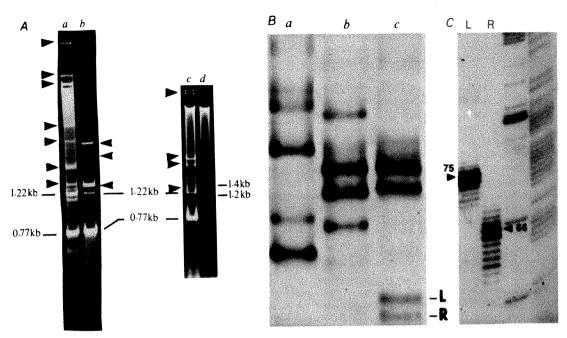
Department of Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794, USA

The insertion sequence, IS1, which is 768 base pairs(bp) long1, is the smallest active translocatable DNA element in bacteria (for review see ref. 2), and appears as a repeated DNA sequence in some Enterobacteriaceae species³. In particular, most Shigella species contain more than 40 copies of a sequence that hybridizes to $IS1^3$. Here, we report the isolation of various repeated sequences from the Shigella dysenteriae chromosome and the DNA sequence analysis of the class, which is abut 770 bp long. The results demonstrate that there are two major types of sequence in this class; one is identical to IS1 except for 10-bp substitutions and is present in about 50 copies in the chromosome; the second sequence, 766 bp long, is repeated more than 150 times and has ~55% homology to IS1 on the nucleotide sequence level. Because of this high degree of homology, we call these 'iso-insertion sequences' of IS1. Further examination of the nucleotide sequence reveals common coding frames between these sequences, suggesting the existence of two genes that are required for multiplication of these sequences.

Examination of Sh. dysenteriae DNA by electron microscopy after denaturation and renaturation revealed many singlestranded loop structures with duplex stems, indicative of the presence of inverted repeat sequences4. Of the 70 duplex stems examined, 44 formed a distinguishable peak at 0.77 ± 0.06 kilobases (kb) in the histogram of length versus number of molecules. This size category of inverted repeat sequence comprised 3.94% of the total DNA strands measured. Assuming that the Sh. dysenteriae chromosome is the same size as that of Escherichia coli, namely 2.5×10^9 molecular weight (M_r) or 4×10^6 kb (ref. 5), the estimated number of copies of these inverted repeat sequences is ~200 per chromosome.

We then used an S₁ nuclease digestion technique to purify the inverted repeat sequences on the chromosomes (see Fig. 1 legend). Figure 1A shows the repeated sequences purified from

Fig. 1 A, 1.4% agarose gels, showing bands of duplex DNA isolated from Sh. sonnei (a), Sh. dysenteriae (h. c) and F. coli K-12 (W3110) (d) chromosomes. The duplex DNAs were prepared as follows. The chromosomal DNA first isolated by the method described by Saito and Miura⁶, dissolved in 10 ml of TE buffer containing 0.01 M Tris (pH 7.2) and 0.001 M EDTA, titrated to pH 12.3 with 2 M NaOH at room temperature further incubated for 15 min: The solution was then neutralized with 2 M HCl to pH 7.0. The Na+ concentration in the DNA solution was adjusted to 0.3 M with 3 M NaCl and the solution incubated at 65 °C for 1 min for renaturation of inverted DNA sequences on the DNA strands. The singlestranded DNA in the solution was digested at 37°C for 1 h with 50 U per μg DNA of S. nuclease in a



buffer containing 40 mM sodium acetate (pH 4.6) and 4.5 mM ZnCl₂. After extraction of the solution with phenol saturated with TE buffer, the DNA was precipitated with ethanol and then suspended in 1 ml of TE buffer. 7 ml of 0.12 M sodium-potassium phosphate buffer (Na-P buffer, pH 6.8) containing 2 ml of hydroxyapatite (HAP) suspension (Bio-Rad) was added to the DNA solution and incubated at 65 °C for 30 min. The solution was occasionally stirred with a glass rod to enhance the efficiency of adsorbing double-stranded DNA. The HAP-DNA complex was precipitated by centrifugation and resuspended in the Na-P buffer and the adsorption step above was repeated three times. Double-stranded DNA was then eluted from the HAP in 5 ml of 0.4 M Na-P buffer (pH 6.8) by incubation at 65 °C for 30 min. The elution step was repeated and a total of 10 ml of eluate saved. The solution was concentrated to 2 ml in dialysis tubing which was embedded in polyethylene glycol powder (Carbowax 6000, Bio-Rad), followed by dialysis against TE buffer. After precipitation of the DNA with ethanol, the DNA was resuspended in 200 µl of TE buffer. 10 µl of the solution was used for gel electrophoresis. The above procedure also extracts the plasmid duplex DNAs, present in the bacterial cells. The bands corresponding to plasmid DNA linear, open and closed circular configurations are shown by solid triangles. B, autoradiograms of 6% polyacrylamide gels showing 5'-3'P-labelled fragments of purified inverted repeat DNA sequences of the Sh. dysenteriae chromosome which were cleaved with HaeII (a), HpaII (b) and AluI (c), respectively. C, autoradiograms of 10% polyacrylamide-7 M urea gel containing the two small 5'-3'P-labelled AluI fragments, R and L, which were denatured with alkali and heated at 95 °C after elution of DNA bands shown in Bc. The ladders of bands (differing by one nucleotide in size) suggest that the 5' ends of these fragments are actually heterogeneous. (Ladders seen in two other lanes on the right are size markers that were obtained

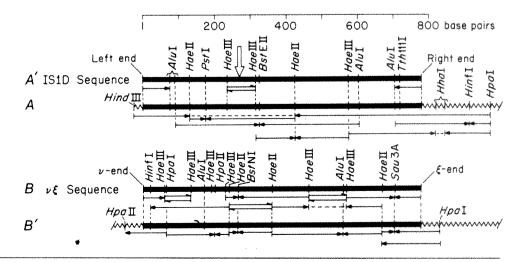
Sh. dysenteriae, Shigella sonnei and E. coli K-12 chromosomal DNA after gel electrophoresis. It is clear that all produce a number of gel bands similar in size to known insertion sequences such as IS1, IS2 and IS3. Note, however, that Sh. dysenteriae (also Sh. sonnei) produces an extremely dense band of DNA about 770 bp long.

We purified the 770-bp sequences of the Sh. dysenteriae chromosome, labelled the 5' ends of the DNA with ³²P and cleaved with various restriction endonucleases. As shown in Fig. 1B, electrophoresis and autoradiography showed two pairs of bands, suggesting that DNA from the 770-bp region is composed of two major sequences that yield different cleavage patterns for the enzymes used. Ethidium bromide staining of these digests revealed two major groups of gel bands with

different intensities (data not shown). The fragments from each group had a sum molecular length of about 770 bp. The ratio of the two components was estimated as 1:3 from the autoradiogram.

Restriction mapping revealed only a single difference between the minor 770-bp sequence and the IS1 sequenced by H.O. and E.O.¹. A HaeIII site absent in the minor fragment (we named this IS1D) was present in IS1 (ref. 1) (we now call this IS1R) (see Fig. 2A). However, the map of the major 770-bp component (which we call $\nu\xi$ in order to assign an orientation to this sequence) was completely different (see Fig. 2B). The same results were obtained using restriction fragments of the Sh. dysenteriae chromosomal DNA which had been cloned into the plasmid pBR322 (see Fig. 2).

Fig. 2 A and A' show cleavage maps of IS1D purified by S nuclease and of IS1D present in a HindIII fragment cloned into pBR322, respectively. In A', the Sh. dysenteriae unique sequence is shown by sawtooth lines. Note that HaeIII site at the position indicated by a vertical open arrow is present in IS1R but is absent in IS 1D. B and B' show cleavage maps of the $\nu\xi$ sequence purified by S_1 nuclease and of that isolated as a BamHI fragment cloned into pBR322, respectively. In B' the sawtooth lines represent the unique sequence of the Sh. dysenteriae The chromosome. numerous horizontal arrows indicate the strategy used for determining the nucleotide sequence of IS1D and vE.



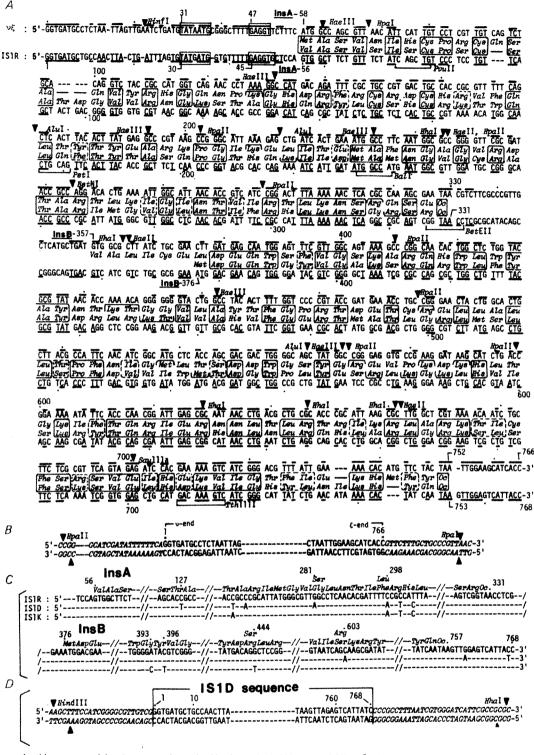


Fig. 3 A, the entire nucleotide sequence of the $\nu\xi$ sequence determined by the method of Maxam and Gilbert?. The sentence is aligned with the nucleotide sequence of ISIR, which has previously been determined, to indicate the sequence homology between them (see lines on the common nucleotides between them). The two sequences encode two common frames, insA and insB, coding for hypothetical amino acid sequences as shown. Homologous amino acids are boxed. B, a part of $\nu\xi$ and unique chromosomal sequence (italic letters) in the fragment cloned into pBR322. C, critical parts of the ISIR sequences together with the amino acid sequences of InsA and InsB. Nucleotide sequences of ISID and ISIR differ from ISIR as shown. Some base substitutions cause amino acid changes as indicated. D, a part of the ISID sequence as well as a unique chromosomal sequence (italic letters) of the Sh. dysenteriae chromosome which has been cloned into pBR322.

Different regions of the $\nu\xi$ sequence were labelled with ^{32}P and used as probes to identify $\nu\xi$ in the chromosomes of various Shigella species and the E. coli K-12 strain, JE5519, using the Southern blotting and hybridization techniques that we have previously used³. The $\nu\xi$ probes hybridized with many EcoRI restriction fragments of Sh. dysenteriae DNA, but the pattern of these fragments was completely different from that previously seen when an IS1R probe was used. Also, the $\nu\xi$ probe hybridized with only one EcoRI restriction fragment from Shigella flexneri and Shigella boydii and none from Sh. sonnei or JE5519

(data not shown), although several restriction fragments of these four species hybridized to IS1 R^3 . These results suggest that $\nu\xi$ does not cross-hybridize with sequences which hybridize to IS1 probes.

We next used the Maxam and Gilbert method⁷ to determine the entire nucleotide sequence of $\nu\xi$ and IS1D isolated by the S₁ nuclease technique and by cloning into pBR322. The strategy used for sequencing is shown in Fig. 2. Sequencing the ends of the fragments purified using S₁ nuclease presented some difficulty, because the 5' ends were found to be heterogeneous

(see Fig. 1C and its legend). Figure 3A shows the entire nucleotide sequence of $\nu\xi$ purified by the S_1 nuclease technique. The nucleotide sequence of the cloned copy of $\nu\xi$ gave the same result, suggesting that the repeated sequences of this kind in the Sh. dysenteriae chromosome are homogeneous. Although the cleavage map of the $\nu\xi$ sequence was completely different from that of IS1R (and IS1D), the nucleotide sequence of $\nu\xi$ showed 55% homology with that of IS1R as represented in Fig. 3A. We also found that a 31-base sequence at the ν end of the $\nu\xi$ sequence appeared in an inverted orientation at the ξ end, although there are 6 base mismatches between them. Small inverted repeat sequences are also present in IS1 $R^{1.8-10}$ and are quite homologous to those at the ends of $\nu \xi$.

When the $\nu\xi$ and IS1R sequences were searched for possible coding frames, two common frames, which we call insA and insB, were present (Fig. 4). They code for polypeptides of very similar size (InsA, $M_r \sim 10,000$, and InsB, $\sim 15,000$) and their amino acid sequences are remarkably similar (see boxed region in Fig. 3A). If pairs of related amino acids (for example, Arg-Lys) are considered to be equal, the two polypeptides from $\nu\xi$ show about 56% homology to those from IS1R.

The nucleotide sequence analysis of IS1D showed only 10 base pair changes between IS1R and IS1D. As shown in Fig. 3C, only two of these substitutions in insA and one in insB cause amino acid changes.

It has been reported that the nucleotide sequence of an IS1 present in the E. coli K-12 chromosome shows seven base pair changes when compared with that of IS1R8. In this IS1, which we refer to as IS1K, one of the base changes causes an amino acid change in polypeptide InsA, another changes an amino acid in InsB (Fig. 3C).

We postulate that the two coding frames, insA and insB, and the inverted repeat sequences seen at the ends of the $\nu\xi$ and IS1D sequences are necessary for translocation of IS1 and its iso-insertion sequences. In fact, genetic studies in our laboratory show that deletion mutations in either of these two coding frames or in the two ends of IS1R cause loss of translocation activity 11,12

In the region preceding the insA coding frame, but not the insB coding frame, we identified both a ribosome binding (Shine-Dalgarno) sequence¹³ (nucleotides 47-51 in $\nu\xi$ and 45-50 in IS1R) and a -10 homology region (or Pribnow box)^{14,15} (nucleotides 31-37 in $\nu\xi$ and 30-36 in IS1R) as indicated in Fig. 3A. This suggests that the insA message is probably transcribed and translated efficiently. On the other hand, the lack of obvious promoter sequence in the region preceding insB suggests either that insB may be transcribed very inefficiently or that it is transcribed as a part of a polycistronic message beginning at the insA promoter. The fact that the region preceding insB also lacks a Shine-Dalgarno sequence may suggest, however, that insB is very poorly translated even when the polycistronic message is transcribed efficiently. The presumed inefficient translation of InsB may be related to control of the rate at which IS1 multiplies in the chromosome.

The nucleotide sequences of unique chromosomal DNA adjacent to both ends of the $\nu \xi$ sequence and IS 1D in the cloned fragments are shown in Fig. 3B and D. Although it is generally accepted that insertion sequences and transposable elements generate directly repeated duplications of several bases at the target site on translocation (for review see ref. 2), no such direct repeat sequences are present at the junctions of $\nu \xi$ and IS1D.

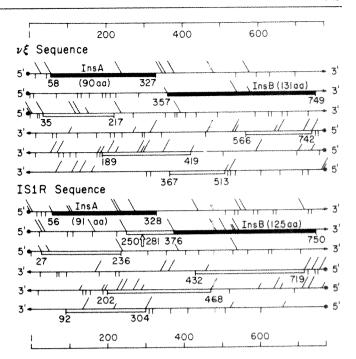


Fig. 4 The possible initiation and termination codons formed within v & and ISIR. All three reading frames of both strands were analysed as shown. Long angled lines represent AUG start codons; short-angled lines represent GUG start codons. Vertical lines indicate the presence of a UGA, UAA or UAG termination codon. All open frames for possible polypeptides are indicated in positions with numbers (see Fig. 3). The two open frames common in the two sequences are labelled InsA and InsB as shown. Note that the base change in ISID at position 281 (which eliminates a HaeIII site present in IS1R) introduces a termination codon into an open frame which precedes insB of IS1R (see open arrow), indicating that the open frame from 250 to 376 is not essential for translocation of IS1R or IS1D.

The absence of direct repeat sequences could be explained in two ways. First, deletions which occurred at the precise ends of the insertion sequences after the translocation of these sequences would result in loss of the part of the chromosome that includes the duplicated sequence. Alternatively, two copies of these repeated sequences flanking a unique chromosomal sequence could become a transposable element [as has been observed for transposable elements such as Tn9 and Tn1681 (refs 16-18)] and move into other sites on the chromosome. In these cases, the individual IS1 components of the transposable element are not flanked by direct repeats 1,19

IS1 is not thought to be essential for the survival of bacterial cells. Nevertheless, it can multiply within a bacterial host, resulting in large numbers of IS1 sequences within the host chromosome. Also, the various types of base changes that we have observed in iso-insertion sequences suggest that IS1 can evolve independently within a bacterial cell. In this respect, IS1 may be an intracellular parasite even more primitive than bacteriophages or bacterial plasmids.

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- Ohtsubo, H. & Ohtsubo, E. Proc. natn. Acad. Sci. U.S.A. 75, 615-619 (1978).
 Calos, M. P. & Miller, J. H. Cell 20, 579-595 (1980).

- Nyman, K., Nakamura, K., Ohtsubo, H. & Ohtsubo, E. Nature 289, 609-612 (1981). Ohtsubo, H. & Ohtsubo, E. in DNA Insertion Elements, Plasmids and Episomes (eds Bukhari, A. I., Shapiro, J. A. & Adhya, S. L.) 49-63 (Cold Spring Harbor Laboratory
- Brenner, D. J., Fanning, G. R., Skerman, F. J. & Falkow, S. J. Bact. 109, 953-965 (1972)
- Saito, H. & Miura, K. Biochim. biophys. Acta 72, 619-629 (1963).

 Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560-564 (1977)
- Johnsrud, L. Molec, gen. Genet. 169, 213–218 (1979).
 Calos, M. P., Johnsrud, L. & Miller, J. H. Cell 13, 411–418 (1978).
 Grindley, N. D. F. Cell 13, 419–426 (1978).

- Ohtsubo, E. et al. Cold Spring Harb. Symp. quant. Biol. 45, 283–295 (1981).
 Machida, Y., Machida, C., Ohtsubo, H. & Ohtsubo, E. Broc. natn. Acad. Sci. U.S.A. (in the
- 13. Shine, J. & Dalgarno, L. Proc. natn. Acad. Sci. U.S.A. 71, 1342-1346 (1974)
- Gilbert, W. in RNA Polymerase (eds Losick, R. & Chamberlin, M.) 193-205 (Cold Spring Harbor Laboratory, New York, 1976).
- Rosenberg, M. & Court, D. A. A. Rev. Genet. 13, 319-353 (1979).
 MacHattie, L. A. & Jackowski, J. in DNA Insertion Elements, Plasmids and Episomes (eds.)
- Bukhari, A. L., Shapiro, J. A. & Adhya, S. L.) 219-228 (Cold Spring Harbor Laboratory, New York, 1977).
- So, M., Heffron, F. & McCarthy, B. Nature 277, 453-456 (1979)
- So, M. & McCarthy, B. Proc. natn. Acad. Sci. U.S.A. 77, 4011-4015 (1980). 19. Johnsrud, L., Calos, M. P. & Miller, J. H. Cell 15, 1209-1219 (1978)

Non-parallel evolution of metabolic and growth-promoting functions of insulin

George L. King & C. Ronald Kahn

Section on Cellular and Molecular Physiology, Diabetes Branch, NIAMDD, National Institutes of Health; Bethesda, Maryland 20205, USA

Insulin has both metabolic and growth-promoting activities¹⁻³. From extensive studies on the relative potencies of naturally occurring and chemically modified insulins, a region of the insulin molecule that is essential for its metabolic effects has been proposed⁴. Here, we have compared the effects of 23 different insulins and insulin analogues on growth (thymidine incorporation into DNA) and metabolic activities (glucose oxidation). For many insulins striking differences in potency in these two assays were observed. Most exhibited greater relative activity in growth-promoting than in the metabolic assay, suggesting that growth and metabolic activities of the insulin molecule have evolved in a non-parallel fashion and may involve separate functional domains of the molecule.

Insulin effects at the cellular level are diverse. Exposure of cells to low concentrations of insulin results in a rapid stimulation of a variety of enzymes and transport systems involved in cellular metabolism⁵⁻⁷. At higher concentrations and after longer times, insulin also exerts growth-promoting effects with stimulation of macromolecular synthesis^{3,8,9}. Using antibodies to the insulin receptor, we have recently shown that the metabolic activity of insulin is mediated by the insulin receptor, whereas the growth-promoting effects are probably mediated through one of the receptors for the insulin-like growth factors (IGFs)10. The latter are a family of polypeptides that are structurally homologous to insulin and share many common biological activities, but are immunologically distinct9. The IGFs include IGF-I, IGF-II, somatomedins A and C and multiplication stimulating activity (MSA). In addition, IGFs have their own cell-surface receptors⁹⁻¹². Whereas the biologically active site of insulin for metabolic activity and insulin receptor binding has been extensively mapped^{4,13}, little information exists on the structural requirements for its growth-promoting activity. To this end we have compared the effects of a wide variety of insulins and IGFs in two bioassays.

Dose-response curves of insulins for stimulation of glucose oxidation in dispersed rat epididymal adipocytes demonstrated the wide range of potencies of insulin analogues for metabolic effects (Fig. 1a). For turkey, pork and bonito insulins, proinsulin and desoctapeptide insulin, the relative potencies (%) in this assay were 240, 100, 42, 2 and 0.4, respectively. In contrast, dose-response curves for the stimulation of thymidine incorporation into DNA of cultured human fibroblasts by these same analogues showed both different relative potencies and a

Table 1 Comparison of insulins and IGFs in metabolic and growth assays

	$(ED_{50} \text{ pork/}ED_{50} $ analogue) × 100			
Insulin	Stimulation of glucose oxidation (A)	,	B/A	
Pork (monocomponent)	100	100	1.0	
Beef	100	98	1.0	
Human (synthetic)	94	120	1.3	
Mouse	82	130	1.6	
Sheep	75	100	1.3	
Horse	87	112	1.3	
Proinsulin (pork)	2	51	26	
Proinsulin (beef)	3	63	21	
Turkey	240	350	1.5	
Bonito (fish)	42	160	3.8	
Hagfish	7	8	1.1	
Guinea pig*	3	12	4	
Porcupine*	3	110	37	
Coypu*	1	190	190	
Casiragua*	2	43	22	
Desoctapeptide (DOP) insulin	0.4	30	75	
Des-Ala des-Asp insulin	5	10	2	
Des-Gly A1 des-Phe B1 insulin	0.8	14	18	
Butoxycarbonyl A1 insulin	50	80	1.6	
A1-B29 adipoyl insulin	6	98	16	
Insulin-like growth factor I (IGF-I)	0.2	860	4,300	
Insulin-like growth factor II (IGF-II)	1.0	430	430	
Multiplication stimulating activity (MSA)	0.2	120	600	

Stimulation of glucose oxidation was measured in dispersed epididymal adipocytes of Sprague–Dawley rats (100–140 g) as the conversion of $^{14}\mathrm{C}$ -glucose to $^{14}\mathrm{CO}_2$ as described by Rodbell et al. 5 . For measuring incorporation of $^3\mathrm{H}$ -thymidine into DNA of cultured human fibroblasts 8 , cells used were from the 5th to 15th passages. After each passage, the cells were allowed to reach confluency with media containing 20% fetal bovine serum (Flow Labs 29101420), then maintained in serum-free Eagle's minimal essential media for an additional 5–7 days before use. The results shown are the average of quadruplicate assays. A control dose–response curve with porcine insulin was done with each experiment. Inter- and intra-assay variations were less than 25% and 20%, respectively.

 $^*\mathrm{ED}_{50}$ values for the hystricomorphs were derived by taking the concentration of insulin needed to stimulate thymidine incorporation that corresponds to the half-maximum of pork insulin's dose-response curve.

different order (Fig. 1b). Turkey (350%) and bonito insulins (160%) were more potent than pork insulin. Porcine proinsulin and desoctapeptide insulin remained less potent than pork insulin, but both were considerably more potent in the thymidine incorporation assay than in glucose oxidation. These differences in potency were not due to a change in the rate of degradation (data not shown). Furthermore, the differences are unlikely to be due to contamination with IGFs, because fully

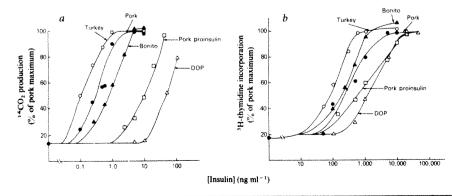


Fig. 1 Dose-response curves of some naturally occurring and chemically modified insulins for the stimulation of glucose oxidation in rat adipocytes (a) and thymidine incorporation into DNA in human fibroblasts (b). The methods are described in Table 1 legend.

100,000

120 120 *CO, production (% of pork maximum) IGF-H IGF-I iGF-II 100 100 80 (% of pork maximum) 60 1,000 10 100 0.1 [Protein] (ng ml - 1) 14CO₂ production (% of pork maximum) H-thymidine incorporation (% of pork maximum)

Fig. 2 a, b, Dose-response curves of pork insulin and various IGFs for the stimulation of glucose oxidation in rat adipocytes (a) and thymidine incorporation into DNA in human fibroblasts (b). c, d, Dose-response curves of pork insulin and various hystricomorph insulins for the stimulation of glucose oxidation in rat adipocytes (c) and thymidine incorporation into DNA in human fibroblasts (d). The methods are as described in Table 1 legend.

synthetic human insulin is equally potent with pork insulin in both bioassays (Table 1 and ref. 14).

0.1

10

1.0

100

[Insulin] (ng ml-1)

The divergence between insulin's metabolic and growth effects is further substantiated by data with other insulin analogues (Table 1). With the exception of hagfish insulin, all the naturally occurring insulins were at least 50% as active as pork insulin in the thymidine incorporation assay, suggesting that insulin's growth effects have withstood evolutionary changes in the molecule better than its metabolic effects. This observation is strengthened by the results with chemically modified insulins which have retained their growth activity to a greater degree than their potencies in glucose oxidation.

The most extreme examples of divergence in the two assays are shown by IGF-I, IGF-II and MSA (Fig. 2a, b). All these growth factors have <1% of pork insulin's metabolic potential, with insulin » IGF-II > MSA > IGF-I. However, when comparing their growth potentials, the order of potencies is completely reversed, with IGF-I being the most potent and insulin the least.

At high concentrations almost all the insulins and IGFs produced the same maximal response in the bioassays. Furthermore, insulin's growth effect on the human fibroblast was not additive to that of the IGFs (ref. 11 and data not shown), suggesting that these peptides have a common rate limiting step either at the receptor or beyond.

However, insulins from the family of mammals referred to as hystricomorphs (guinea pig, porcupine, coypu and casiragua) did not conform to this finding. These insulins differ from other mammalian insulins in that they are unable to bind zinc, and exhibit low degrees of dimerization and low potency for metabolic effects 4.15.16 (Fig. 2c). In contrast, when these insulins were studied in the thymidine incorporation assay, two points of interest emerged (Fig. 2d). First, like many other insulins, at low concentrations the hystricomorph insulins exhibited a greater relative potency in the growth assay than in their metabolic effects. More importantly, at higher concentrations these insulins stimulated thymidine incorporation into DNA to a greater maximum than any other insulin, insulin-like growth factors or combination of the two tested (Fig. 2d). In fact, at high concentrations guinea pig insulin approached the activity of

20% fetal calf serum, the most potent stimulus known in this assay.

10

From these data, some of the properties of the insulin molecule that are responsible for growth can be deduced. First, the growth-promoting action of insulin can tolerate a greater change in structure than the metabolic effects. Specific substitution of histidine in the A8 (A chain, residue 8) residue of insulin (for example, turkey insulin) enhances both its metabolic¹⁷ and growth effects. The B22-B30 region of insulin, which is vital for insulin's metabolic activities4, is not crucial for insulin growth effects. This is clearly demonstrated by the results of desoctapeptide insulin, and is consistent with the model for the structure of insulin-like growth factor I proposed by Blundell et al. 18 in which this portion of the molecule is covered. Finally, the unexpected finding that the hystricomorph insulins gave a higher maximum response than other insulins in the growth-promoting assay raises the possibility that they are closer functionally and evolutionarily to the IGFs than other mammalian insulins. Four areas of similarities between the sequences of hystricomorph insulins and IGFs may be important: B10 (Asn, Gln and Glu in the hystricomorphs and IGF; His in other insulins), B13 (Asp in IGF and hystricomorphs; Glu in others), B26 (Tyr in coypu, casiragua and IGFs, Phe in others) and A4 (Asp in hystricomorphs, Glu in others).

The present data thus indicate that the growth-promoting activity of the insulin molecule is distinct from its metabolic activity and is less affected by amino acid substitutions. Presumably, this reflects differences in the specificity of the receptors for the IGFs and insulin. The insulins from the hystricomorph family seem to be unique in their ability to stimulate DNA synthesis. Preliminary studies in our laboratory suggest that this superagonist property can be explained by a receptor which uniquely recognizes the hystricomorph insulins, indicating that the hystricomorph insulins may represent another class of 'insulin-like growth factors'.

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butoxycarbonyl A1, and A1-B29 adipoyl insulins), R. E. Humbel (IGF-I and IGF-II), and M. M. Rechler and S. P. Nissley (MSA) for various insulins noted in Table 1. All other insulins were from Novo. We also thank Dr Jesse Roth for his guidance, Dr T. B. Blundell for suggestions in the project and Mrs Nancy Conley for secretarial assistance.

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- 1. Cahill, G. F. Jr Diabetes 20, 780-797 (1971)
- Czech, M. P. A. Rev. Biochem. 46, 354-384 (1977) Kahn, C. R. Trends biochem. Sci. 263, 4-7 (1979).
- Blundell, T. L., Dodson, G. C., Hodgkin, D. C. & Mercola, D. A. Adv. Protein Chem. 26, 279-402 (1972).

- 279-402 (1972).
 Rodbell, M. J. biol. Chem. 239, 375-380 (1966).
 Olefsky, J. M. J. clin. Invest. 60, 1094 (1976).
 Gliemann, J. & Witsell, R. R. Diabetologia 13, 396 (1977).
 Rechler, M. M., Podskalny, J. M., Goldfine, J. D. & Wells, C. A. J. clin. Endocr. Metab. 39, 512-521 (1974).
- Zanf, J., Rinderknecht R. E. & Froesch F. R. Metaholism. 27, 1803-1827 (1978).
- King, G. L., Kahn, C. R., Rechler, M. M. & Nissley, P. S. J. clin. Invest. 66, 130–140 (1980).
 Rechler, M. M., Nissley, S. P., Podskalny, J. M., Moses, A. C. & Fryklund, L. J. clin. Endocr.
- Metab. 44, 820-831 (1978).
- Zapf, J., Schoenle, E. & Froesch, E. R. Eur. J. Biochem. 87, 285-296 (1978)
- 13. DeMeyts, P., VanObberghen, E., Roth, J., Wollmer, A. & Brandenburg, D. Nature 273, 304-509 (1978).
- Petrides, P. E. & Böhlen, P. Biochem. biophys. Res. Commun. 95, 1138-1144 (1980).
- Zimmerman, A. E., Moule, M. L. & Yip, C. C. J. biol. Chem. 249, 4026-4029 (1974). Horuk, R. et al. Nature 279, 439-440 (1979).
- Piron, M. A., DeMeyts, P., Pitts, J., Simon, J. & Rittel, W. Diabetes 29, Suppl. 2, 193
- 18. Blundell, J. L., Bedarkar, S., Rinderknecht, E. & Humbel, R. E. Proc. natn. Acad. Sci. U.S.A. 75, 180-184 (1978)

Is the similarity of monozygotic twins due to genetic factors alone?

Klaus Gärtner & Eva Baunack

Laboratory Animal Science Unit, Medical School Hannover. 3000 Hannover 61, FRG

It is generally assumed that the identity of the genotype explains why there is less difference between monozygotic than between dizygotic twins. But monozygotic twins are also exposed to identical non-genetic influences, which act on the common zygote until the time of separation into the two twin partners. We have studied such influences by comparing genetically identical monozygotic and dizygotic mouse twins transplanted into foster mothers at the 8-cell stage. We show that monozygotic twins have a greater degree of similarity than genetically identical dizygotic twins. This suggests that the differences between dizygotic twins may be due in part to non-genetic influences on the zygote as far as the third cleavage stage.

Differences between monozygotic (MZ) twins derived from a single zygote of an inbred line were compared with differences between dizygotic (DZ) twins from two ova of one mother of the same line. Body weight and onset of signs of somatic maturation (first appearance of hair, eye and ear opening) were determined. The high degree of isogenicity of inbred mice has been discussed elsewhere 1-4. Our experiments were carried out on inbred AKR/NHan mice derived from brother-sister mating over more than 150 generations. Isogenicity in these inbred strains was continuously verified by testing different immunogenic and biochemical genetic markers, and by measuring morphometrical traits of the mandibula. Over 100 gene loci have been tested by the breeding units at Zentralinstitut für Versuchstiere, FRG; the results have been reported elsewhere4.

MZ twins were produced as follows: ova were collected by flushing the tuba uteri on day $3\frac{1}{2}$ post coitum (p.c.) (vaginal plug = day 1) after spontaneous ovulation. The flushing fluid⁵ contained phosphate-buffered saline (PBS) supplemented with 20% heat-inactivated (30 min at 56 °C) fetal calf serum (FCS). The zona pellucida was removed by incubation with 0.5% pronase⁶ and the denuded ova were cultured in the flushing medium at 37 °C for 3 h in an atmosphere of 95% air, 5% CO₂ (refs 7, 8). The 8-cell stages, in a microdrop of the flushing medium, were mechanically separated under liquid paraffin using an extended glass capillary tube, and both halves were incubated for 3 h as described above. The two halves, together with five to seven denuded 8-cell stages of C57BL/6JHan were then transferred to the uterus of $2\frac{1}{2}$ -day pseudopregnant, uniparous C57BL/6JHan recipients^{6,9}. DZ twins were produced in the same way except that, instead of two halves of one 8-cellstage zygote, two undivided, denuded 8-cell-stage zygotes derived from one mother were transferred.

Animals were born at \sim day 20 p.c. The litters of 4–8 offspring each were suckled by their uterine foster mothers, weaned at 25 days of age, and the males and females of each litter placed in separate cages. All animals were examined daily for health and signs of maturation, and weighed every 10 days from 31 to 101 days old. MZ and DZ AKR twins were identified by their white hair among their black C57BL/6JHan littermates. In ~200 transfer experiments, 10 litters had living MZ twins. In the same period, 13 pairs of DZ twins were produced in ~50 attempts. For each of the measured characteristics mean and standard deviation, and 'within pairs' (MS_w) and 'between pairs' (MS_b) mean squares were calculated. Analysis of variance model II (ref. 10) was used to calculate the component of variance (s_b^2) for the variation between the pairs in each group of MZ and DZ. that of male and female twin pairs separately, using the formula: $s_b^2 = 0.5$ (MS_b-MS_w). A typical calculation is given in Table 1.

Table 1 Body weights of pairs of 51-day old male MZ and DZ and estimation of mean squares and components of variance analysis model II (random effects)

				Body weig	ght (g)			
		MZ	•			DZ		
		23.0	21.5			28.0	21.5	
		23.0	25.5			27.0	26.5	
		30.0	31.0			26.0	24.0	
		29.0	27.0			21.0	25.0	
		27.0	27.0			24.5	22.5	
		26.0	25.5					
		28.0	28.0			24.6 ±	2.37	
		26.5	24.5					
		26.2 ±	2.66	manus .				
	d.f.	Sum of squares	Mean square (MS)	Components of variance (s^2)	d.f.	Sum of squares	MS	Components of variance (s^2)
Total	15	99.6	6.64		9	50.4	5.60	
Between	7	90.7	12.97	5.93	4	17.2	4.3	1.18
Within	8	8.9	1.11	1.11	5	33.3	6.65	6.65
** 1613111	9	3.7		55/1.11 = 5.99				
				< 0.005				

Table 2 Mean ± s.d., MS_w and s_b² estimated for age of MZ and DZ at first appearance of hair, and eye and ear opening of males and females combined, and for body weight of males and females of different age

Character	Mean ± s.d.			Component of variance			
	3.77	DZ		MZ	DZ	F	P
	MZ 6.00 ± 1.65	6.35 ± 1.32	8 b	2.89	0.91	3.18	< 0.05
Appearance of hair (days)		(26)	MS _w	< 0.001	0.88	380.00	< 0.001
	(20)	13.65 ± 2.65	s _b ²	5.96	6.17	1.04	NS
Eye opening (days)	14.20 ± 2.37	(26)	MŠ,	< 0.001	1.11	1,110.00	< 0.001
	(20)	13.50 ± 2.86	s _b ²	9.60	7.22	1.33	NS
Ear opening (days)	13.60 ± 3.01	(26)	MŠ _w	< 0.001	1.20	1,270.00	< 0.001
	(20) 17.50 ± 3.53	15.80 ± 2.79	s _b ²	11.19	4.15	2.70	NS
Body weight (g), ♂		(10)	MS _w	2.06	4.10	1.99	NS
day 31	(16)	15.41±3.35	5 6	18.00	3.12	5.77	NS
Body weight (g), ♀	13.50 ± 3.46	(16)	MŠ.	< 0.00005	8.33	13,900.00	< 0.001
day 31	(4)	$20.70 \pm 2.20^{\circ *}$	s _b ²	9.05	1.80	7.67	< 0.05
Body weight (g), ざ	23.34 ± 3.01	(10)	MS.	0.61	3.25	5.33	< 0.05
day 41	(16)	19.50 ± 3.54	5 _b	3.12	3.32	1.06	NS
Body weight (g), ♀	20.75 ± 1.44	19.30 ± 3.34 (16)	MS,	< 0.00005	9.47	189,400.00	< 0.001
day 41	(4)	24.60 ± 2.37	5 5 6	5.93	1.18	5.03	NS
Body weight (g), ♂	26.41 ± 2.58	24.60 ± 2.57 (10)	MS _w	1.11	6.65	5,99	< 0.05
day 51	(16)	22.56±3.81	s _b ²	2.53	6.06	2.40	NS
Body weight (g), ♀	23.87 ± 1.31	(16)	MŠ,	0.06	8.87	147.83	< 0.01
day 51	(4)		5 b	5.21	2.49	2.09	NS
Body weight (g), ರ	28.78 ± 2.27	27.20 ± 2.29 (10)	MS,	0.26	7.50	28.85	< 0.01
day 61	(16)	26.22±4.12	νιο _* ₅ ²	1.06	3.83	3.61	NS
Body weight (g), ♀	25.5 ± 0.91		MS _w	0.12	13.39	111.06	< 0.01
day 61	(4)	(16)	s_b^2	7.07	1.94	3.64	NS
Body weight (g), ♂	30.34 ± 2.64	28.95 ± 2.69	MS.	0.39	5.52	14.15	< 0.01
day 71	(16)	(10)	s_b^2	7.70	7.64	1.01	NS
Body weight (g), d days 81, 91, 101	32.60 ± 2.94 (16)	29.68 ± 4.11 (10)	MS _w	1.13	9.49	8.40	< 0.01

Mean ± s.d., MS_w and the between pairs component of variance (s_b²) estimated in groups of MZ and DZ twins for the day of first appearance of hair, and eye and ear opening of male and females combined, and for the body weights (g) of males and females of different age. Numbers in parentheses are number of animals. NS, not significant.

Means are significantly different (P < 0.05).

The F-test was used to compare s_b² and MS_w between the MZ and DZ twin groups; the results are shown in Table 2. The mean age at first appearance of hair and of eye and ear openings did not differ (P>0.5) between MZ and DZ groups. In addition, the mean body weights of MZ and DZ did not differ (P > 0.1) in 15 out of 16 measurements made between 31 and 101 days old. The mice stopped growing at ~70 days old; the mean body weight at 81, 91 and 101 days remained constant, therefore the results of these ages were combined.

A comparison of the total variance between MZ and DZ with respect to the measured characteristics reveals no detectable trend. MS_w and s_b² for the pairs of twins are significantly different in the two groups (see Table 2, columns 4-8). In monozygotic twins MS, is extremely small, most of the variance being due to s_b^2 . In dizygotic twins MS_w was greater than s_b^2 for 9 of the 13 measurements. MS, for the monozygotic twins was significantly smaller than that of the dizygotic twins, for the observed ages of maturation and body weights in males and females up to the time of maturation (61 days). This difference remained significant in males even after maturation, but it disappeared in adult females. From repeated weight estimation for each MZ and DZ adult male at 81, 91 and 101 days of age the intra-individual component of variance was calculated to be 2.392 g. This component of variance is approximately equal to the MS, of the MZ but is smaller (P < 0.05) than that in DZ.

Thus in spite of the fact that both MZ and DZ were isogeneic and developed in identical pre- and postnatal environments, the MZ pairs showed fewer differences than the DZ pairs. A possible explanation for these findings is residual heterozygosity in the AKR inbred strain, but there is no support for such a hypothesis. We are following a divergent breeding programme in our AKR/NHan strain that is selective for the characteristics under study, to provide further evidence for the homogeneity of the gene loci involved. An alternative explanation for the larger differences observed in the DZ twins is that experimental manipulation differed between the MZ and DZ groups; whereas the 8-cell embryos of the MZ group were divided, those of the DZ group were not. However, it is difficult to see how experimental manipulation of this kind could create greater similarity. To investigate this further, control studies are being done in which, for example, two embryos at the 8-cell stage are divided and one 4-cell entity from each is transferred to a common foster mother.

From the available evidence, we conclude that each zygote is modified by a non-genetic mechanism before the third cell division in such a way that postnatal maturation times and body weights and possibly other characteristics are considerably different between individuals. If a zygote is divided after the third cell division, the modification is decisive for both monozygotic partners in the same way. It has long been known that there is a non-genetical component of variance of many quantitative characteristics which could not be accounted for by environmental influence, that is, by circumstances external to the individual. This component was called 'intangible variance'11,12 and was attributed also to 'accidents' of development. We believe that the quantitative component of variance described here is a manifestation of such intangible variance.

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- Deol, M. S. & Grüneberg, H. Genet. Res. 1, 50-88 (1968)
- Hedrich, H. J., Rapp, K., Zschege, C. Z. Versuchstierk. 17, 263–274 (1975).
 Festing, M. Inbred Strains in Biomedical Research (Macmillan, London, 1979).
 Hedrich, H. J. in The Mouse in Biomedical Research Vol. 1. (eds Foster, H. L., Small, J. D. & -176 (Academic, New York, 1981)
- 5. Nimierko, A. & Opas, J. Methods in Mammalian Reproduction (Academic, New York,
- McLaren, A. J. Reprod. Fert. 19, 341-346 (1969).
- Whitten, W. K. Adv. Biosci. 6, 129-141 (1971
- Hogan, B. & Tilly, R. Nature 265, 626-629 (1977). McLaren, A. J. exp. Biol. 33, 394-416 (1956).
- Weber, E. Grundriß der Biologischen Statistik 7th edn, Ch. 37, 268-270 (VEB Gustav
- 11. Falconer, D. S. Introduction to quantitative genetics (Oliver & Boyd, Edinburgh and London.
- 12. Grüneberg, H. The Pathology of Development (Blackwell, Oxford, 1963)

Population genetics of selfish DNA

Tomoko Ohta

National Institute of Genetics, Mishima, 411, Japan

Selfish DNA, which contains no genetic information but which is perpetuated in eukaryote genomes, has attracted much attention recently¹⁻⁴. Two classes of selfish DNA have been distinguished: tandemly repetitive sequences such as satellite DNA and dispersed repeated families. Unequal crossing-over is considered to be mainly responsible for spreading of the former, and an integration mechanism (as in bacterial transposons) for evolution of the latter. Here I derive equations and simulate distributions (using randomly generated numbers as data) to show how the numbers of tandem and dispersed repeat units of selfish DNA in a population change with time. I find that whereas the mean number of clustered and dispersed units remains constant, the distribution becomes increasingly dispersed with time.

Both unequal crossing-over and integration occur randomly, such that the overall mean change of unit number is zero. I assume that the number of units per genome in an evolving population changes by inter-genome exchange as well as by random sampling drift at reproduction. How does the mean number of units per genome of the population drift with time? In the previous study⁵, the relationship between the mean and variance of the number of units in the population was obtained when the above processes balance each other. I shall start from such an equilibrium situation. The basic premise of this study is that self-replication has a more profound effect on the evolution of selfish DNA than individual base substitution, on which evolution of ordinary stable gene loci depends.

The same model as in ref. 5 is used; however, the tandem and the dispersed repeated sequences are here treated separately. Each repeating unit of the dispersed class is assumed to have a constant probability α_1 of either forming an additional copy of itself or being deleted in one generation. Exchange of units between the genomes also takes place in each generation at rate β . At meiosis, the units are assumed to be randomly distributed to the two daughter cells by the normal methods of chromosomal segregation. Both unequal sister-chromatid exchange (rate per generation α_2) and unequal interchromosomal crossing-over change the amount of clustered selfish DNA. It is assumed that the mean number of duplicated or deleted units in one generation is proportional to n, the number of repeating units in a genome (constant of proportionality, a). Inter-chromosomal crossing-over between the homologous repeated DNA is complicated because the two genomes usually have different numbers of repeating units. Recombination can be either 'equal' or 'skewed' depending on where the smaller segment of repeated DNA comes in relation to the larger segment. Equal recombination occurs when the small segment falls completely within the larger segment, and skewed recombination when it does not. The equal recombination rate per generation is denoted by β_I and the skewed one, by β_1 . In addition to the above processes, random sampling of gametes occurs at reproduction (number of breeding individuals, N). The parameters of various processes for the two classes are summarized in Table 1.

Let x and y be the average number of units per genome in the populations of tandem and the dispersed repeated sequences respectively. They are random variables and, in the following, their distribution is investigated as function of time. In numerical experiments I generate data starting off with random numbers, and study the distribution obtained using the equations derived below.

The 'pseudosampling (PSV)' method of Kimura⁶ is used, which greatly facilitates the simulation. If x is the average number of units in the present population, then its number in the next generation, x', is given by

$$x' = x + \xi_{psv'} \tag{1}$$

where $\xi_{\rm psv}$ is a uniform random number with mean 0 and the variance expected after the occurrence of the various processes given in Table 1. The variance $(V_{\Delta x})$ of change of x $(\xi_{\rm psv})$ may be calculated using equations (5) and (12)–(14) of ref. 5, and by noting that x is the average of 2N variables. Note further that each genome undergoes, independently of the other, replication and exchange processes, before it is sampled for the next generation. Also, equation (13) of ref. 5 is equivalent to stating that the square of the average number of units increases by the absolute value of the right term of this equation by sampling of gametes. It becomes

$$V_{\Delta x} = \frac{x^2}{2N} \left(\alpha_2 a^2 + \frac{\beta_1'}{6} \right) \left\{ 1 + \frac{6(\alpha_2 a^2 + 1) - 2\beta_1 - \beta_1'}{2\beta_1 + \beta_1' + 3N^{-1} - 6\alpha_2 a^2} \right\}$$
 (2)

by assuming that $2\beta_1 + \beta_1' + 3N^{-1} > 6\alpha_2 a^2$. The simulation is continued, starting from a given value of x at time 0, for 50N generations, and it is repeated 1,000 times. Whenever x becomes <1/N, x is put to 0, and the population has lost the

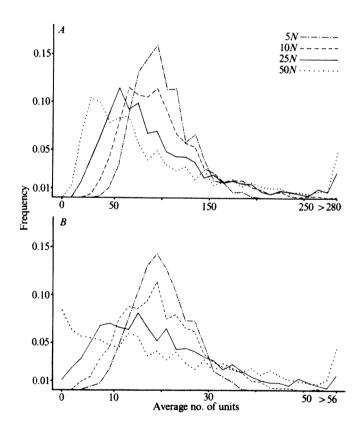


Fig. 1 A, results of simulations of the distribution of the average number of tandemly repeated units per genome in the population. Parameters are: initial number of units $(x_0) = 10^2$; unequal crossover rate $(\alpha_2) = 10^{-2}$; fraction of units duplicated or deleted (a) = 0.1; equal recombination rate $(\beta_1) = 10^{-2}$; skewed recombination rate $(\beta_1') = 10^{-3}$; and $N = 10^4$. Distribution was obtained for the intervals of 10 units, with terminal classes of 0 and >280 units. B, results of simulations for the dispersed repeating sequences. Parameters are: initial number of units $(y_0) = 20$; rate of duplication or deletion of each unit $(\alpha_1) = 10^{-2}$; rate of inter-genome exchange $(\beta) = 1$; and $N = 10^2$. The product, $N\alpha_1$, is chosen to be reasonably realistic. Distributions are for the intervals of 2 units with terminal classes of 0 units and >56 units. The distributions for t = 5N, 10N, 25N and 50N generations are given. They are the result of 1,000 independent trials.

Table 1 Processes responsible for the evolution of selfish DNA and the parameters used

	•	
Processes	Tandemly repeated sequences (x)	Dispersed repeated sequences (y)
Replication process	Unequal crossing-over by sister-chromatid exchange α_2 = rate per cluster per generation a = mean fraction of duplicated or deleted units at an unequal crossing-over in one cluster of repeated sequence	Integration mechanism α_1 = rate in one generation at which each unit is either duplicated or deleted independently
Inter-genome exchange process	Inter-chromosomal recombination at meiosis β_1 = rate of recombination in one generation which takes place in this sequence when the pairing of two chromosomes is equal β_1 = the above rate when the pairing of two chromosomes is skewed	Random distribution to the daughter cells at meiosis β = rate per generation at which the dispersed units are distributed
Random genetic drift	N = effective population size	N = effective population size

repeating sequence. In practice, ξ_{psv} may be obtained by choosing a random number rnd (uniformly distributed between 0 and 1) such that

$$\xi_{\text{psv}} = \sqrt{3V_{\Delta x}} (2rnd - 1) \tag{3}$$

(See ref. 6.)

The distribution of y (the mean number of units of the dispersed family) is similarly studied by using $y' = y + \xi_{psv}$. ξ_{psv} now follows a uniform distribution with mean 0 and variance that equals the variance of the change of y after the occurrence of the three processes of Table 1. The variance takes the following form from equations (3), (8), (13) and (14) of ref. 5:

$$V_{\Delta y} = \frac{y}{2N} \left(\alpha_1 + \frac{N \alpha_1 \beta}{1 + 2N \alpha_1} \right) \left(1 + \frac{2 - \beta}{\beta + N^{-1}} \right)$$
 (4)

From equations (2) and (4), it is possible to approximate the variance of x or y at any time, that is, the variance of the number of units among the isolated populations. They may be expressed as

$$V_{x,t} = V_{\Delta x_0} t \tag{5}$$

and

$$V_{\mathbf{y},t} = V_{\Delta \mathbf{y},0}t \tag{6}$$

where t denotes the tth generation after splitting of the populations, and $V_{\Delta x_0}$ and $V_{\Delta y_0}$ are obtained by replacing x of equation (2) with x_0 , and y of equation (4) with y_0 respectively, where x_0 and y_0 are the numbers of units of clustered and dispersed classes at t = 0. As β (in equation (4)) is likely to be unity in sexually reproducing species (because the rate at which the dispersed family is divided into the daughter cells at meiosis is unity), the product $N\alpha_1$ is the most crucial factor in determining the distribution of dispersed families.

Figure 1A,B shows the results of simulation. The values of the parameters used are not known exactly in real cases and are tentative suggestions. Note that several dispersed families are known in which the number of units per genome is of the order

Table 2 Comparison of the observed and expected variances of the average number of units per genome among the isolated populations

Time in	Tandemly	repeated	Dispersed	repeated
generations $(\times N)$	Observed variance	Expected variance	Observed variance	Éxpected variance
5	768	798	32.9	34.2
10	1,717	1,595	69.4	68.3
15	2,480	2,393	97.9	102.5
20	3,816	3,190	137.5	136.7
25	4,672	3,988	166.3	170.8
30	6,003	4,785	200.9	205.0
35	7,112	5,583	237.9	239.1
40	8,568	6,381	274.8	273.3
45	8,852	7,178	300.3	307.5
50	9,484	7,976	320.8	341.6

of 10 (refs 7-10) and that the parameters α_1 and N are chosen such that the product, $N\alpha_1$, is a realistic value (= 1). For the clustered families, reasonable values are also chosen11-14, except for N, which is too small but makes the simulation easier because of more rapid differentiation. The figure shows, starting from the initial number of units, how the distribution of x or y changes with time. For example, after 10N generations, the value of x ranges from 30 to 290 with the peak at about $x_0 = 100$, and y, from 3 to 53, with the peak at $y_0 = 20$. As t increases, the distribution becomes more and more dispersed in both classes. Nevertheless, the overall mean of 1,000 replicated lines stays constant; at the 50N th generation, $\bar{x} = 98.1$ and $\bar{y} = 19.9$. Such an outcome would be characteristic of random processes of this kind. Note that, at this generation, ~8% of 1,000 lines have lost the dispersed repeating sequence, and about the same proportion contained less than 20 copies of the clustered class. Such a proportion would increase with time even if the overall mean remained unchanged.

The variances of x and y have also been calculated and compared with the theoretical predictions from equations (5) and (6), as shown in Table 2. The agreement between the theoretical and simulated results is generally satisfactory; however, for the clustered families, equation (5) underestimates the true value when t gets large. This is because the number of units duplicating or being deleted is assumed to be proportional to the number of units, and therefore the variance of the change of x is proportional to x^2 , the expectation of which becomes larger than x_0^2 as t gets large.

It has been shown that whereas some species have the same α satellite DNA15, other closely related species have lost the sequence 16,17. It has thus been argued that this sequence may be important in speciation¹⁶. However, as can be seen from the present results, spreading and loss of repeated sequences occur randomly and it is extremely difficult to draw any conclusion from such an observation. Data on species comparisons of dispersed repeated sequences are also rapidly accumulating (see ref. 7 for review). Most examples seem to fit in with the framework of the present theory

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1. Doolittle, W. F. & Sapienza, C. Nature 284, 601-603 (1980). Orgel, L. E. & Crick, F. H. C. Nature 284, 604-607 (1980). Cavalier-Smith, T., Dover, G., Smith, T. F. & Reid, R. A. Nature 285, 617-620 (1980). Orgel, L. E. et al. Nature 288, 645-648 (1980) Ohta, T. & Kimura, M. Proc. natn. Acad. Sci. U.S.A. 78, 1129-1132 (1981). Kimura, M. Proc. natn. Acad. Sci. U.S.A. 77, 522–526 (1980) Calos, M. P. & Miller, J. H. Cell 20, 579–595 (1980). Catos, M. F. & Millet, J. H. Ceil 26, 379-393 (1909).
 Young, M. W. Proc. natn. Acad. Sci. U.S.A. 76, 6274-6278 (1979).
 Cameron, J. R., Loh, E. Y. & Davis, R. W. Cell 16, 739-751 (1979).
 Finnegan, D. J., Rubin, G. M., Young, M. W. & Hogness, D. S. Cold Spring Harb. Symp. quant. Biol. 42, 1053-1063 (1978).

- quant. Biol. 42, 1035-1035 (1976).
 Smith, G. P. Science 191, 528-535 (1976).
 Szostak, J. W. & Wu, R. Nature 284, 426-430 (1980).
 Petes, T. D. Cell 19, 765-774 (1980).
 Reis, R. J. S. & Goldstein, S. Cell 21, 739-749 (1980).

- Fry. K. & Salser, W. Cell 12, 1069-1084 (1977). Hennig, W. & Walker, P. M. B. Nature 225, 915-919 (1970).
- 17. Brown, S. D. M. & Dover, G. A. Nature 285, 47-49 (1980).

Inhibition of actin polymerization in blood platelets by cytochalasins

Joan E. B. Fox & David R. Phillips

Department of Biochemistry, St Jude Children's Research Hospital, Memphis, Tennessee 38101, USA

It has been suggested that the polymerization of actin to form actin filaments is critical for many functions in non-muscle cells including phagocytosis, cell division, secretion and cell motility1,2. In support of this suggestion, many of these functions are inhibited by cytochalasins3 which also inhibit the actin nucleiinduced polymerization of purified actin⁴⁻⁷. Recent studies suggest that the cytochalasins (cytochalasins B, D and E) inhibit polymerization by binding to the growing end of actin filaments and blocking the further addition of monomeric actin molecules^{4,7-9}. If the cytochalasins inhibit cellular contractile functions by a similar mechanism, they could be useful tools in determining whether actin polymerization is required for specific processes within cells. Blood platelets provide an excellent system for testing this as exposure of platelets to thrombin results in a rapid polymerization of actin^{10,11}. We have now studied the effects of the cytochalasins on thrombininduced actin polymerization and report here the first clear evidence that cytochalasins inhibit actin polymerization in intact

The filamentous actin content of platelet samples was determined in platelet lysates by the method of Blikstad *et al.*¹² who showed that nonfilamentous actin inhibits DNase I activity while filamentous actin has little effect unless depolymerized by guanidine hydrochloride. Platelets were lysed by adding an equal volume of Triton extraction buffer (2% Triton X-100 (v/v), 10 mM EDTA, 100 mM Tris-HCl, pH 7.4). This buffer, unlike that used elsewhere ^{10,12}, did not depolymerize actin filaments, so that identical inhibitory values were obtained whether the assay was performed 30 s or 2 h after lysis (J.E.B.F., M. E. Dockter & D.R.P., in preparation).

In Triton lysates from unstimulated platelets, the DNase I inhibitory activity in the absence of guanidine hydrochloride was ~50% of that in its presence. When platelets were activated with thrombin before the addition of Triton X-100, the amount of guanidine hydrochloride-dependent DNase I inhibitory activity increased to ~70% of the total. This stimulus-induced transformation was rapid and was almost complete within 15 s (Fig. 1). We conclude that $\sim 50\%$ of the total actin was in a filamentous form in unstimulated platelets and that this increased to $\sim 70\%$ on thrombin activation. In support of this conclusion, we found that only the guanidine hydrochloridedependent inhibitory activity was sedimented by centrifugation of lysates at 100,000 g for 3 h. Furthermore, sedimented material contained structures morphologically similar to actin filaments¹³. Finally, quantitation of actin in sediments and supernatants by densitometry of Coomassie blue-stained gels after SDS-polyacrylamide gel electrophoresis gave actin contents that were within 5% of those obtained by the DNase I inhibition assay. Thus, the amount of inhibition of DNase I activity in Triton lysates correlates directly with the amount of nonfilamentous actin (an assumption used throughout this study), and the amount of filamentous actin within platelets rapidly increases after exposure to thrombin.

Figure 1 shows that 10^{-6} M cytochalasin E added 100 min before thrombin inhibited the thrombin-induced increase in actin filament content. Cytochalasins D and B had similar effects. None of these cytochalasins had any effect when added

simultaneously with the Triton extraction buffer. As shown in Table 1, the inhibitory effect was rapid, as the same extent of inhibition was observed whether cytochalasin D was present for 100 min or for only 1 min before the addition of thrombin. Cytochalasin D did not affect the filament content in unstimulated platelets (Table 1). These data show that the decreased filament content in thrombin-treated platelets is due to an inhibition of thrombin-induced actin polymerization and not to

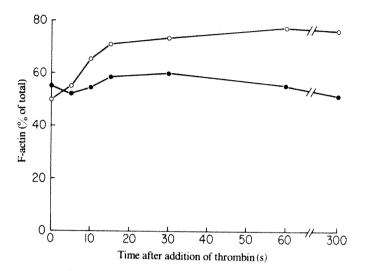


Fig. 1 Inhibition of thrombin-induced actin polymerization in human platelets by cytochalasin E. Human platelets from freshly drawn blood were washed as previously described²⁴ and suspended to a concentration of 2×10^8 per ml in 138 mM sodium chloride, 2.9 mM potassium chloride, 12 mM sodium bicarbonate, 0.36 mM sodium phosphate, 5.5 mM glucose and 1 mM EDTA, pH 7.4. The platelet suspension was incubated with 0.2% (v/v) DMSO (dimethyl sulphoxide) (O) or 10^{-6} M cytochalasin E in 0.2% DMSO () at 37 °C. After 100 min, thrombin was added (final concentration 0.1 NIH U ml⁻¹) and at intervals varying from 0 to 300 s, platelets were lysed by addition of an equal volume of Triton extraction buffer and a 10-µ1 aliquot removed for determination of the amount of unpolymerized actin by the DNase inhibition assay. The total actin content in lysates was measured after depolymerization of filamentous actin by addition of an equal volume of buffer containing 1.5 M guanidine-HCl, 1.0 M sodium acetate, 1.0 mM sodium ATP, 20 mM Tris-HCl and 6 mM calcium chloride, pH 8.6 (to yield a final pH of 7.4, determined experimentally). Kinetic analyses were initiated within 30-60 s after platelet lysis. The absorbance was continually monitored by a dual-beam spectrophotometer directly linked to a Hewlett Packard 9845S computer. The linear portion of the sigmoidal curve was determined by regression analysis and runs were discarded if the correlation coefficient was less that 0.999. Duplicate determinations of the DNase I inhibitory activity from an individual platelet sample usually agreed within 5%. The actin filament content (F-actin) is expressed as a percentage of the total actin.

a decreased filament content of unstimulated platelets. Similar increases in actin polymerization were observed when platelets were stimulated for 30 s with 0.4 μ M ionophore A23187 or for 60 s with 20 μ M ADP. Cytochalasin D also inhibited these stimuli-induced increases.

Although the cytochalasins may affect many metabolic processes within cells, two sites of action that could potentially affect actin polymerization have been described: one site is on the actin filaments⁴⁻⁹; the other is on the membrane-bound glucose transport system^{14,15}. However these sites have differing affinities for the cytochalasins. The site on actin filaments has been measured using both purified actin^{4,8,9} and actin-containing membrane complexes^{5,16}. In both cases, cytochalasins D and E bound with higher affinity that cytochalasin B with the binding

affinity corresponding to the inhibition of actin nuclei-induced polymerization. In contrast, the site of the membrane-bound glucose transport system has a higher affinity for cytochalasin B than for cytochalasin D or E which, unlike cytochalasin B, do not inhibit glucose transport into cells¹⁴⁻¹⁸.

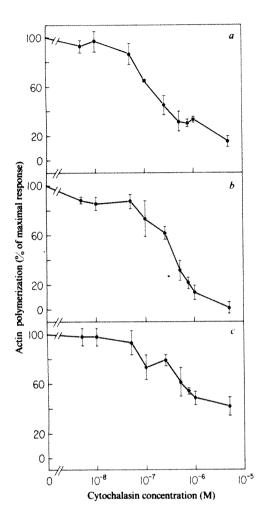


Fig. 2 Comparison of the effects of cytochalasins D(a), E(b) and B(c) on thrombin-induced actin polymerization in human platelets. Platelet suspension $(2\times10^8~{\rm per~ml}^{-1})$ was incubated at 37 °C with 0.5% ethanol (v/v) or with varying concentrations of the different cytochalasins in 0.5% ethanol. After 88 min, buffer or thrombin (final concentration 0.1 NIH U ml⁻¹) was added and 30 s later the platelets were lysed by addition of Triton extraction buffer. The actin filament content of lysates was determined as described in Fig. 1 legend and the thrombin-induced increase in actin filaments expressed as a percentage of the increase obtained when thrombin was added to platelets that had not been exposed to cytochalasin. Values shown are the mean \pm s.e.m: from four donors.

Figure 2 compares the effects of the three cytochalasins on thrombin-induced actin polymerization. At 5×10^{-6} M, cytochalasins D and E decreased the thrombin-induced actin polymerization by $\sim 85\%$ and 100%, respectively, while cytochalasin B inhibited polymerization by only $\sim 60\%$. Within the limits of experimental error, the concentrations of cytochalasins D and E required to produce half-maximal inhibition of polymerization were indistinguishable ($\sim 2\times 10^{-7}$ M) and were lower than the concentration of cytochalasin B required for a similar inhibition ($\sim 10^{-6}$ M). These concentrations are similar to those that bind to actin filaments and inhibit actin polymerization in purified systems 4.7.9. The relative order of potency

Table 1 Effect of time of preincubation of platelets with cytochalasin D on the actin filament content of unstimulated and thrombin-activated platelets

		Filan	nentous	actin	(% of t	otal)	
Addition during	Stimulatory	Time of preincubation					
preincubation	agent	1	5	20	70	100	
Ethanol	None	52.1	51.6	48.1	48.2	50.5	
	Thrombin	69.5	65.2	60.6	66.2	67.3	
Cytochalasin D in ethanol	None	49.7	49.1	47.2	48.1	50.1	
	Thrombin	54.4	56.1	51.6	55.4	56.3	

Platelet suspensions were incubated with 0.5% ethanol (v/v) or with 2.5×10^{-5} M cytochalasin D in 0.5% ethanol at 37 °C. At intervals from 1 to 100 min, buffer or thrombin was added and after 30 s, platelets were lysed with Triton extraction buffer and the actin filament content measured as described in Fig. 1 legend. Values given are the actin filament content as a percentage of the total platelet actin.

agrees with the notion that the cytochalasins inhibit actin polymerization by binding to preformed actin filaments and is inconsistent with a mechanism involving inhibition of glucose transport. In support of this view, the inhibitory effect of cytochalasin was rapid (Table 1), a result not expected if the drug inhibited glucose transport sites alone. In addition, concentrations of cytochalasin that completely inhibited thrombin-induced actin polymerization had no effect on the thrombin-induced phosphorylation of two platelet polypeptides¹⁹ or on the thrombin-induced association of myosin with platelet cytoskeletons¹³.

It has been proposed that actin filaments 'treadmill' where one end is a net polymerizing end and the other a net depolymerizing end^{20,25}. Experiments showing that the cytochalasins decrease the lengths of preformed actin filaments, unless the net depolymerizing ends are blocked, have been interpreted as evidence for such a mechanism8. If the cytochalasins inhibit thrombin-induced actin polymerization by binding to actin filaments, they must have access to the filaments before stimulation. Although the permeability of platelet plasma membranes to the cytochalasins has not been verified experimentally, we predict from their structure that they would readily cross plasma membranes. However, our results show that the cytochalasins had no measurable effect on the filament content when incubated for up to 100 min with unstimulated platelets (Table 1). This suggests that if 'treadmilling' occurs in unstimulated platelets, it occurs too slowly to be detected in the times used here, possibly because the depolymerizing ends are blocked.

Recently, Morris and Tannenbaum²¹ reported that cytochalasin D did not decrease the filament content of HEp-2 cells. These authors conclude that as cytochalasin D inhibits motile functions without decreasing filament content, the inhibitory effect is not due to a limitation of the availability of filaments. However, it is becoming increasingly clear that actin polymerization can occur on cellular stimulation 10,11,22,23. Such responses need not result in a net measurable increase in filament content as occurs within platelets, but may involve small increases in filament content within specialized areas of cells. Our observations that the cytochalasins inhibit actin polymerization in platelets suggest that cytochalasins may inhibit cellular processes³ by preventing the increased formation of actin filaments required for these processes. Experiments are now being done to determine whether the concentrations of cytochalasins that inhibit actin polymerization in platelets cause corresponding inhibition of platelet responses (shape change, aggregation, release of granule contents or clot retraction), and it is anticipated that similar studies will help to determine the role of stimuli-induced actin polymerization in other nonmuscle cells.

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- Clarke, M. & Spudich, J. A. A. Rev. Biochem. 46, 797–822 (1977).Korn, E. D. Proc. natn. Acad. Sci. U.S.A. 75, 588–599 (1978).Wessells, N. K. et al. Science 171, 135–143 (1971).

- Brown, S. S. & Spudich, J. A. J. Cell Biol. 83, 657-662 (1979). Lin, D. C. & Lin, S. Proc. natn. Acad. Sci. U.S.A. 76, 2345-2349 (1979).
- Brenner, S. L. & Korn, E. D. J. biol. Chem. 255, 1670-1676 (1980). MacLean-Fletcher, S. & Pollard, T. D. Cell 20, 329-341 (1980).
- Brenner, S. L. & Korn, E. D. J. biol. Chem. 254, 9982-9985 (1979) Flanagan, M. D. & Lin, S. J. biol. Chem. 255, 835-838 (1980).
- Carlsson, L., Markey, F., Blikstad, I., Persson, T. & Lindberg, U. Proc. natn. Acad. Sci. U.S.A. 76, 6376-6380 (1979).
- U.S.A. 76, 6376-6380 (1979).

 1. Phillips, D. R., Jennings, L. K. & Edwards, H. H. J. Cell Biol. 86, 77-86 (1980).

 12. Blikstad, I., Markey, F., Carlsson, L., Persson, T. & Lindberg, U. Cell 15, 935-943 (1978).

 13. Jennings, L. K., Fox, J. E. B., Edwards, H. H. & Phillips, D. R. J. biol. Chem. (in the press).

 14. Kletzien, R. F., Perdue, J. F. & Springer, A. J. biol. Chem. 247, 2964-2966 (1972).

 15. Plagemann, P. G. W. & Estensen, R. D. J. Cell Biol. 55, 179-185 (1972).

 16. Lin, S. & Snyder, C. E. J. biol. Chem. 252, 5464-5471 (1977).

- Lin, S., & Shyder, C. E. J. ann. Chem. 252, 3404–341 (1971).
 Lin, S., Santi, D. V. & Spudich, J. A. J. biol. Chem. 249, 2268–2274 (1974).
 Rampal, A. L., Pinkofsky, H. B. & Jung, C. Y. Biochemistry 19, 679–683 (1980).
 Lyons, R. M., Stanford, N. & Majerus, P. W. J. clin. Invest. 56, 924–936 (1975).
 Wegner, A. J. molec. Biol. 108, 139–150 (1976).

- Morris, A. & Tannenbaum, J. *Nature* **287**, 537-639 (1980). Swanston-Flatt, S. K., Carlsson, L. & Gylfe, E. *FEBS Lett.* **117**, 299-302 (1980).

- Hywell, S. L. & Tyhurst, M. Biochem. J. 192, 381–383 (1980).
 Phillips, D. R. & Agin, P. P. J. clin. Invest 60, 535–545 (1977).
 Pollard, T. D. & Mooseker, M. S. J. Cell Biol. 88, 654–659 (1981).

E. coli contains eight soluble proteolytic activities, one being ATP dependent

K. H. Sreedhara Swamy* & Alfred L. Goldberg

Department of Physiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115, USA

Escherichia coli selectively degrades polypeptides with highly abnormal conformations¹⁻⁴. Normal cell proteins are degraded more slowly, but this process increases when the bacteria lack required nutrients^{1,5-7}. The pathways by which aberrant or normal proteins are hydrolysed are not known^{1,3,4}, but these processes require metabolic energy^{3-5,8,9}. Recently, cell-free proteolytic systems have been obtained from E. coli^{4,10} and mammalian cells^{3,11-15} that are stimulated by ATP. To clarify the pathway of intracellular protein degradation and to determine whether ATP might directly influence specific proteolytic enzymes, we have now undertaken to define the various proteases in E. coli. Our studies demonstrate the existence of eight distinct soluble proteolytic activities in E. coli extracts. Seven seem to be new enzymes. Six are serine proteases that hydrolyse ¹⁴C-globin and ³H-casein, while two others are metalloenzymes that degrade 125 I-insulin but not these larger polypeptides. One of the globin-degrading enzymes is stimulated markedly by ATP and thus may be the rate-limiting enzyme for protein breakdown in vivo.

Although several reports have concerned the isolation of proteases from E. coli, this field is in a confusing state. The so-called 'protease I' and 'protease II', which cleave chromogenic substrates for chymotrypsin and trypsin respectively^{16,17}, show little or no hydrolytic activity against proteins 18,19

Furthermore, mutants lacking them have a normal capacity to degrade cell proteins¹⁹⁻²². Another 'protease' has been purified²³ using a chromogenic substrate of subtilisin, but its ability to hydrolyse proteins has not been established. In addition, an aminopeptidase (pepN) has been purified, which has been claimed to have some endoprotease activity²⁴. An activity that can degrade casein, named 'proteaseA', has been partially purified²⁵ but it is probably not a single enzyme (see below). Cheng and Zipser²⁶ have purified an enzyme, protease III, that hydrolyses the amino-terminal fragments of β -galactosidase ('auto- α '), but it is not essential for protein degradation in vivo²⁷. Finally, these bacteria contain various peptidases that are essential for growth on peptides and hydrolyse small peptides generated during intracellular protein breakdown^{28,29}.

We therefore undertook a systematic search for protease activities in E. coli. For substrates, we used 14C-methylapohaemoglobin, ³H-methyl-α-casein and ¹²⁵I-insulin. Because insulin is a small polypeptide, it may be degraded by enzymes involved only in the later steps in the complete degradation of cell proteins^{29,30}. Extracts of E. coli K-12 hydrolysed all three substrates, but only degradation of globin was significantly stimulated by ATP. The degree of stimulation by ATP (60%) in these extracts, which were prepared with the French press from frozen cells, is smaller than the two- to threefold effects reported previously with fresh cells lysed by gentler methods 10,36

To isolate the proteolytic activities responsible, we fractionated these extracts on DEAE-cellulose and eluted the adsorbed proteins with a NaCl gradient (Fig. 1). Five different peaks with proteolytic activity were found (designated I-V, according to their order of elution). These same peaks, and no additional ones, were found in several other E. coli K-12 strains that had been freshly grown and collected at either late log or stationary phase. Of special interest was the finding that one of the peaks that degraded globin and casein (IV) was stimulated dramatically by ATP, and this is presumably the activity responsible for the stimulation of globin and casein hydrolysis by ATP in the crude extracts¹⁰

Peak I shows strong hydrolytic activity against globin and casein, but very slight activity against insulin. Peak I was further characterized by chromatography on CM-cellulose (Fig. 2) and by gel filtration on Sepharose 6B (data not shown). Both techniques resolved two distinct activities, both of which hydrolyse globin and casein. Peak IA shows on gel filtration an unusually large molecular weight (\sim 500,000), whereas IB has a molecular weight of ~82,000. Both IA and IB are sensitive to diisopropyl fluorophosphate (DFP), and thus are serine proteases.

Peak II hydrolyses insulin, globin and casein. Isoelectric focusing (Fig. 3) and CM-cellulose chromatography at pH 5.2 (data not shown) can both resolve this fraction into three proteolytic peaks, one active against insulin and the other two against globin and casein. Both globin-degrading activities, IIB and IIC, seem to be serine proteases, but they differ in their relative sensitivities to DFP as well as in their heat stabilities³¹. The insulin-degrading enzyme (IIA) resembles in many respects protease III purified by Cheng and Zipser²⁶. Like protease III, peak IIA rapidly hydrolyses amino-terminal fragments of β galactosidase (auto- α) and is sensitive to metal-chelating agents (EDTA and o-phenanthroline). Furthermore, a mutant lacking protease III (ptr), isolated by Cheng et al.27, specifically lacked the insulin-degrading activity in peak II. These findings (K.H.S.S., C. H. Cheng and A.L.G., unpublished results) indicate that the insulin-degrading activity is identical to protease II.

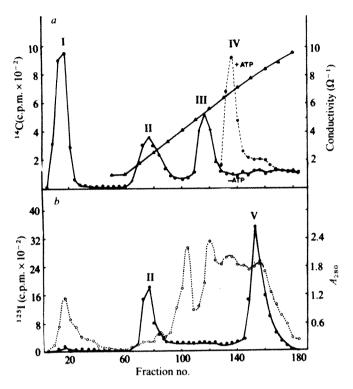
Peak III hydrolyses globin (Fig. 1) and casein but not insulin. Because it is sensitive to DFP, the enzyme also seems to be a serine protease.

Peak IV was the one ATP-stimulated activity. The stimulation of globin or casein degradation by ATP ranged from 4- to 10-fold in the various fractions and required Mg²⁺ ions. The low amount of globin hydrolysis evident in the absence of ATP decreased further on use of a narrower salt gradient or DEAE-Sepharose, which resulted in an enzyme that seemed to be

^{*}Present address: Department of Pharmacology, University of Vermont, College of Medicine, Burlington, Vermont 05405, USA

completely dependent on ATP. This enzyme showed no activity against insulin, which explains the finding that insulin hydrolysis was not stimulated by ATP in crude extracts. The ATP-stimulated activity shows the DFP-sensitivity characteristic of a serine protease.

Peak V contained insulin-degrading activity that was sensitive to o-phenanthroline but not to DFP. This peak did not digest



DEAE-cellulose chromatography of proteolytic enzymes from E. coli K-12. In a, fractions were assayed for proteolytic activity against 14C-globin in the absence (O or presence (●---●) of 3 mM ATP. In b, proteolytic activity against 125I-insulin (O--O) was assayed. Also shown in a is the conductivity (\blacktriangle — \blacktriangle) and in b, the A_{280} ($\bigcirc \cdots \bigcirc$). In all peaks except peak IV, the hydrolysis of 14 C-globin and 125 I-insulin was not stimulated by ATP (for clarity, these data were not shown). The globindegrading peaks also hydrolysed ³H-casein (data not shown). Cell-free extracts were prepared from 50 g of frozen E. coli K-12 cells (Grain Processing Co.). After thawing at 4 °C, the cells were suspended in 100 ml of 50 mM Tris-HCl, pH 7.8, 10 mM MgCl₂ and 200 mM KCl, and disrupted in a French press at 14,000 p.s.i. The homogenate was centrifuged at 30,000g for 30 min, and the supernatant centrifuged again at 130,000g for 3 h. The supernatant was dialysed overnight against 10 mM Tris-HCl, pH 7.8, containing 5 mM MgCl₂. The dialysed extract was adsorbed to a DEAE-cellulose column (2.5×27 cm) equilibrated with 10 mM Tris-HCl, pH 7.8. containing 5 mM MgCl2. The column was washed with the same buffer until the A_{280} of the eluate was <0.05. The adsorbed proteins were then eluted with 1,600 ml of a linear NaCl gradient (0-0.25 M NaCl). The flow rate was 100 ml h⁻¹ and 12-ml fractions were collected. Proteolytic activity was estimated by following the degradation of ¹⁴C-globin (2-4×10⁶ c.p.m. per mg), ³H-casein (1×10⁷ c.p.m. per mg) or ¹²⁵I-insulin (125-140 mCi per mg, Cambridge Nuclear and Radiopharmaceutical Corporation) to products soluble in 10% trichloroacetic acid. ¹⁴C-globin was prepared by methylating crystalline beef haemoglobin (Sigma) with ¹⁴C-formaldehyde (40– 60 mCi mmol⁻¹, NEN) and bovine α -casein with ³H-formaldehyde (40–60 mCi mmol⁻¹) as described previously⁴⁸. To increase its proteolytic susceptibility, haem was extracted from the haemoglobin using methyletone⁴⁹ All the assaus ware corried out in 50 methyletone. . All the assays were carried out in 50 mM Tris-HCl, pH 7.8, ethylketone 10 mM MgCl₂, in the presence or absence of 3 mM ATP. When assayed with globin or casein as a substrate, the incubation mixture contained $100-200~\mu l$ of enzyme preparation and 4–5 μg of ¹⁴C-globin or 25 μg of ³H-casein in a final volume of 0.5 ml. Assays using insulin as a substrate contained 12.5 μ g insulin (Lilly), a trace amount of ¹²⁵I-insulin (12–15,000 c.p.m.) and the enzyme preparation in a final volume of 0.5 ml. The incubations were performed for 2 h at 30 °C when globin or casein was the substrate, and for 1 h at 37 °C when insulin was the substrate. After incubation, 40 μ l of bovine serum albumin (30 mg ml⁻¹) was added as a carrier and 60 µl of 100% trichloroacetic acid to precipitate the proteins. The assay tubes were kept on ice for 30 min, and after centrifugation, 0.4 ml of acid-soluble products from ¹⁴C-globin or ³H-casein hydrolysis were counted in 4 ml of Liquiscint (National Diagnostics). The products of ¹²⁵I-insulin hydrolysis are counted in an autogamma spectrometer. The protein was determined by the method of Lowry et al. 50 using crystalline bovine serum albumin as standard.

casein or globin. No additional proteolytic activity could be eluted with higher concentrations of NaCl.

These studies and related ones demonstrate the existence of eight soluble proteolytic activities in E. coli, all of which have now been purified to near homogeneity. The complete purification and characterization of these enzymes will be reported elsewhere (refs 31-33 and K.H.S.S., C. H. Chung and A.L.G., manuscripts in preparation). Of these, the six globindegrading enzymes seem to be serine proteases. By contrast, the enzymes that degrade insulin (IIA and V) and similarly sized polypeptides are metalloproteases (Table 1). All these activities represent new enzymes31 except for the insulin-degrading enzyme (IIA), which seems to be identical to protease III26. All these activities are also found in mutant strains lacking proteases I and II and aminopeptidase N. These enzymes are also distinct from the recA gene product, which catalyses the ATP-dependent proteolytic cleavage of the \(\lambda\) repressor during prophage induction^{34,35}, but does not hydrolyse casein or globin to acidsoluble material (data not shown). Regnier and Thang25 partially purified a casein-hydrolysing activity, named protease A, which was eluted from DEAE-cellulose at the same ionic strength as peak II. This peak contains three enzymes that differ in their isoelectric points, sensitivities to inhibitors and substrate specificities (Fig. 3). Thus, protease A is a mixture of proteases, rather than a single enzyme.

The marked stimulation (4-40-fold) of peak IV protease by ATP can account for the energy requirement for protein breakdown in vivo $^{1.8,9}$. In crude extracts, a much smaller stimulation of proteolysis by ATP (60-200%) was observed 10 , probably because of the five additional globin-degrading enzymes active in the absence of ATP. Previous studies 8,30 indicate that the initial endoproteolytic steps in protein breakdown require ATP, and it is therefore likely that these steps are catalysed by the ATP-stimulated protease in peak IV. Recently, this enzyme has been shown to be the product of the lon gene (also called CapR and deg) 32,33,36,37 , and lon mutants have a reduced capacity to degrade abnormal proteins 36,37 .

When cells are disrupted by gentler methods than those used here, a large amount of ATP-stimulated proteolytic activity is associated with the cell membrane³⁰. It is not clear whether the ATP-stimulated activities in the soluble and membrane fractions are different enzymes or whether the soluble activity is

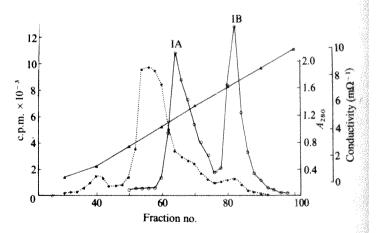


Fig. 2 CM-cellulose chromatography of proteases in peak I. Hydrolysis of ${}^3\mathrm{H}$ -casein (O——O), A_{280} (\bullet · · · • •) and conductivity (\bullet —— \bullet) are shown. The pooled fractions from peak I of the DEAE-cellulose column Fig. 1) were concentrated and dialysed against 10 mM sodium acetate buffer, $p\mathrm{H}\,5.6$. After removing the precipitated material by centrifugation, the proteins were loaded onto a CM-cellulose column (1.5 × 12 cm), equilibrated with 10 mM sodium acetate buffer, $p\mathrm{H}\,5.6$. The adsorbed proteins were eluted with 400 ml of 0–0.3 M linear NaCl gradient. The flow rate was 35 ml h⁻¹ and 4-ml fractions were collected.

Table 1 Proteolytic activities from Escherichia coli

Stimulation	Tu bibita un	Suggested
Oy ALL	minionors	nomenclature
ein-degrading en	zymes	
	DFP	Do
	DFP	Re
	DFP	Mi
	DFP	Fa
-	DFP	So
+	DFP	La
ng enzymes		
	EDTA, o-phenanthroline	Pi (periplasmic)
*****	o-Phenanthroline	Ci (cytoplasmic)
	by ATP ein-degrading en	by ATP Inhibitors ein-degrading enzymes

released from a membrane-associated form during cell breakage. Some of the other soluble enzymes described here may also be derived from the inner or outer membranes during cell

A major task for future research will be to clarify which of these enzymes are involved in the degradation of abnormal proteins and in the breakdown of normal cell constituents in starving cells. These proteases may also have critical roles in other physiological processes, such as the processing of secretory or membrane proteins³⁸⁻⁴¹, utilization of exogenous peptides²⁸, phage morphogenesis^{28,42}, breakdown of peptides²⁸, phage morphogenesis^{28,42}, breakdown of colicins⁴³⁻⁴⁵ and inactivation of regulatory proteins^{34,42,46}. One important clue to the physiological roles of these enzymes is their subcellular distributions. Elsewhere we shall demonstrate that one of the insulin-hydrolysing activities is found in the periplasm (IIA) and one in the cytoplasm (V). One globindegrading activity (IIB) is localized in the periplasm, one (IB) seems to be distributed equally between periplasm and cytoplasm, and the others (IA, IIC, III, IV) are cytoplasmic. Presumably, these latter enzymes are involved in the catabolism of intracellular proteins.

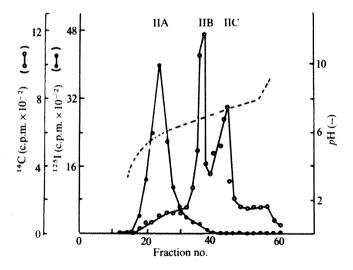


Fig. 3 Isoelectric focusing of peak II. The peak II fractions from DEAEcellulose column (see Fig. 1) were combined and concentrated by precipitating the proteins by adding solid ammonium sulphate to 95% saturation. After centrifugation at 31,000g for 30 min, the pellet was dissolved, dialysed against 1% glycine overnight and applied to the isoelectric focusing column. Isoelectric focusing was performed using a LKB 110-ml column in a sucrose gradient with 1% solution of Ampholine in the pH range 5-8. Electrofocusing was conducted for 22 h at 4 °C with a constant voltage of 1,600 V. Fractions (2 ml) were collected from the bottom. The pH of the fractions was measured at 4 °C (----) and 0.2-ml aliquots were assayed for activity against 125 I-insulin (•—•) and 14 C-globin (O——O). The peaks of IIA, IIB and IIC have isoelectric points (pI) of 5.75, 6.75 and 7.36, respectively.

The discovery of seven new enzymes necessitates the choice of a useful nomenclature. Unfortunately, the lack of precise information about their physiological functions or chemical specificities precludes informative designations. Therefore, we have decided to introduce a novel nomenclature for these enzymes. As the two metalloenzymes that degrade insulin have distinct compartmentalizations in the cell⁴⁷, we suggest the name Pi for the periplasmic insulin-degrading enzymes (IIA) and Ci for the cytoplasmic insulin-degrading enzyme (V) (Table 1). Such a scheme, based on subcellular distribution, is not useful for the enzymes that degrade globin and casein. We would like to introduce an easily remembered nomenclature for these six enzymes: proteases Do, Re, Mi, Fa, So and La in their order of elution from DEAE-cellulose and CM-cellulose or isoelectric focusing (Table 1). This nomenclature also permits an easily remembered designation for their genetic loci. When more is known about the properties or roles of these enzymes, a more descriptive nomenclature will be possible.

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- Goldberg, A. L. & St. John, A. C. A. Rev. Biochem. 45, 747-803 (1976).
 Pine, M. J. A. Rev. Microbiol. 26, 103-126 (1972).
 Goldberg, A. L., Kowit, J. D., Etlinger, J. D. & Klemes, Y. in Protein Turnover and Lysosomal Function (eds Segal, H. & Doyle, D.) 171-196 (Academic, New York, 1978).
 Goldberg, A. L., Voellmy, R. & Sreedhara Swamy, K. H. in Biological Functions of Proteinases (eds Holzer, H. & Tschesche, H.) 35-48 (Springer, Berlin, 1979).

- Mandelstam, J. Bact. Rev. 24, 289-308 (1960). St. John, A. C., Conklin, K., Rosenthal, E. & Goldberg, A. L. J. biol. Chem. 253, 3945-3951
- Voellmy, R. & Goldberg, A. L. J. biol. Chem. 255, 1008-1015 (1980)
- Voeilin, R. & Goldberg, A. L. J. biol. Chem. 255, 1008-1013 (1980). Kowit, J. D. & Goldberg, A. L. J. biol. Chem. 252, 8350-8357 (1977). Olden, K. & Goldberg, A. L. Biochim. biophys. Acta 542, 385-398 (1978). Murakami, K., Voellmy, R. & Goldberg, A. L. J. biol. Chem. 254, 8194-8200 (1979).
- 11. Etlinger, J. D. & Goldberg, A. L. Proc. natn. Acad. Sci. U.S.A. 74, 54-58 (1977)
- 12. Hershko, A., Ciechanover, A. & Rose, I. A. Proc. natn. Acad. Sci. U.S.A. 76, 3107-3110 (1979).
- Boches, F. S. & Goldberg, A. L. Fedn Proc. 39, 1682 (1980).
 DeMartino, G. N. & Goldberg, A. L. J. biol. Chem. 254, 3712-3715 (1979).
 Goldberg, A. L., Strnad, N. P. & Sreedhara Swamy, K. H. CIBA Fdn Symp. 75, 227-251
- Pacaud, M. & Uriel, J. Eur. J. Biochem. 23, 435-442 (1971).
 Pacaud, M. & Richaud, C. J. biol. Chem. 250, 7771-7779 (1975)

- Pacaud, M. & Richaud, C. J. biol. Chem. 250, 771-779 (1975).
 Pacaud, M., Sibilli, L. & LeBrass, G. Eur. J. Biochem. 69, 141-151 (1976).
 Kowit, J. D., Choy, W. N., Champe, S. P. & Goldberg, A. L. J. Bact. 128, 776-784 (1976).
 Miller, C. G., Heiman, C. & Yen, C. J. Bact. 127, 490-497 (1976).
 Miller, C. G. & Zipser, D. J. Bact. 130, 347-353 (1977).
- Heiman, C. & Miller, C. G. J. Bact. 135, 588-594 (1978)
- 23. Strongin, A. Y., Gorodetsky, D. L. & Stepanov, V. M. J. gen. Microbiol. 110, 443-451
- Lazdunski, C., Busuttil, J. & Lazdunski, A. Eur. J. Biochem. 60, 363-369 (1975).
- Regnier, P. & Thang, M. N. Eur. J. Biochem. 54, 445-451 (1975) Cheng, Y. E. & Zipser, D. J. biol. Chem. 254, 4698-4706 (1979)
- Cheng, Y. E., Zipser, D., Cheng, C. & Rolseth, S. J. J. Bact. 140, 125-130 (1979).
 Miller, C. G. A. Rev. Microbiol. 29, 485-504 (1975).
- Yen, C., Green, L. & Miller, C. Y. J. molec. Biol. 143, 21-48 (1980). Voellmy, R. & Goldberg, A. L. Nature 290, 419-421 (1981).
- Goldberg, A. L., Sreedhara Swamy, K. H., Chung, C. H. & Larimore, F. S. in Proteolytic Enzymes Pt C (ed. Lorand, L.) (Academic, New York, in the press).
- 32. Charette, M. F., Henderson, G. W. & Markovitz, A. Proc. natn. Acad. Sci. U.S.A. 78, No. 8
- Chung, C. H. & Goldberg, A. L. Proc. natn. Acad. Sci. U.S.A. 78, No. 8 (1981).
 Roberts, J. W., Roberts, C. W. & Craig, N. L. Proc. natn. Acad. Sci. U.S.A. 75, 4714–4718 (1978)
- Craig, N. L. & Roberts, J. W. Nature 283, 26-30 (1979).
- Bukhari, A. I. & Zipser, D. Nature new Biol. 243, 238-241 (1973). Gottesman, S. & Zipser, D. J. Bact. 133, 844-851 (1978).
- Wickner, W. A. Rev. Biochem. 48, 23-45 (1979)
- Wickner, W. A. Rev. Biochem. 48, 25-45 (1979).
 Davis, B. D. & Tai, P. C. Nature 283, 433-438 (1980).
 Chang, C. N., Inouye, H., Model, P. & Beckwith, J. J. Bact. 142, 726-728 (1980).
 Zwizinski, C. & Wickner, W. J. biol. Chem. 255, 7973-7977 (1980).
 Mount, D. W. A. Rev. Genet. 14, 279-319 (1980).

- Cavard, D. & Lazdunski, C. Eur. J. Biochem. 96, 525-533 (1979) Watson, D. H. & Sherratt, D. J. Nature 278, 362-364 (1979).
- Matson, D. I. & Shellar, D. J. Pacel, 145, 668-671 (1981).
 Bowles, L. K. & Konisksy, J. J. Bact, 145, 668-671 (1981).
 Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. Proc. natn. Acad. Sci. U.S.A. 77, 3225-3229 (1980).
- 47. Swamy, K. H. S. & Goldberg, A. L. J. Bact. (submitted)
- 48. Rice, R. H. & Means, G. E. J. biol. Chem. 246, 831-832 (1971). 49. Teale, F. W. J. Biochim. biophys. Acta 35, 543 (1959).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. J. biol. Chem. 193, 265-275 (1951).

Requirement of nifV gene for production of wild-type nitrogenase enzyme in Klebsiella pneumoniae

P. A. McLean & R. A. Dixon

ARC Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ, UK

Klebsiella pneumoniae nitrogenase is a complex enzyme consisting of two component proteins, the tetrameric MoFe protein (Kp1) and the dimeric Fe protein (Kp2)1. Together these catalyse the ATP-dependent reduction of N2 and of several triple-bonded substrates such as C2H2, CN, N3 and CH₃NC (refs 2, 3). In the absence of substrate, protons are reduced to H2 and all substrates compete with protons for reduction. Carbon monoxide inhibits all reductions except H2 evolution4. Seventeen genes are required for the synthesis of nitrogenase and expression of its activity in K. pneumoniae 5-8, some of which are believed to be involved in processing of the nitrogenase proteins during synthesis 9,10. Here we describe studies of the nitrogenase of bacteria mutated at one of these genes-nifV. These mutants produce nitrogenase which does not reduce N2 in vivo, although it does in vitro in conditions of high electron flux. We show that the defect is in the Kp1 component, and suggest that the nifV gene product modifies the Kp1 component so that N2 reduction will occur at the lower electron fluxes present in vivo, and that this might have been involved in the possible evolutionary development of the nitrogenase from a cvanide-detoxifying enzyme.

nifV mutants were originally isolated by their inability to grow anaerobically (anaerobic conditions are required because oxygen represses nitrogenase production) on nitrogen-free medium, but all nifV mutants were shown to give a leaky phenotype when assayed for C_2H_2 reduction^{5,6}, a test normally accepted as indicative of N_2 -reducing ability. Indeed, some nifV::Tn7 mutants gave 50–100% of wild-type C_2H_2 -reducing levels even though such mutations should completely inactivate the nifV gene product in most cases⁶.

To test the true nitrogen-fixing ability of nifV strains, derepressed cultures were incubated under an atmosphere of 15N2 and after 11 h cell pellets were collected for 15N analysis (Table 1). The two nifV::Tn7 mutants gave no significant incorporation of ¹⁵N into cell material whereas these mutants significantly reduced C₂H₂, indicating that derepression had occurred (Table 1). Failure to incorporate ¹⁵N₂ was not due to defective assimilation of fixed nitrogen^{11,12}, because both mutants utilized histidine, arginine, tryptophan and nitrate as sole nitrogen sources aerobically and nitrate and arginine anaerobically. If N₂ cannot act as a substrate for the enzyme the presence of N2 might not affect C₂H₂ reduction. Figure 1 shows the effect of N₂ on in vivo C₂H₂ reduction by whole cells of a nifV mutant (UNF1613) and a wild-type control (UNF3001). C₂H₂ reduction by wildtype cells was noticeably inhibited by 0.1 atm and 1 atm N₂ whereas C₂H₂ reduction by the mutant was not. UNF3001 and UNF1613 had a K_m for C_2H_2 of 4.2 and 4.6 atm, respectively. We conclude that nifV insertion mutants are unable to reduce

This inability of nifV mutants to reduce N_2 could be due to an excess of Kp1 over Kp2, resulting in a situation favouring C_2H_2 reduction $^{13.14}$. Crude extracts of nifV and wild-type strains were thus tested for the relative levels of the two nitrogenase proteins by rocket immunoelectrophoresis using purified proteins as standards. These showed that UNF3001, UNF1610 and UNF1613 crude extracts had an $in\ vivo\ Kp2$: Kp1 molar ratio of 6.6, 5.2 and 4.7, respectively. These results are not significantly different and confirm the conclusion 15 that there is an effective excess of Kp2 $in\ vivo$. However, this assumes that all cross-reacting material is catalytically active. Unfortunately, direct activity measurements on the individual nitrogenase proteins in crude extracts is complicated by their interdependence for

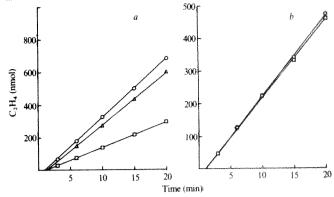


Fig. 1 Time course for in vivo C_2H_2 reduction by wild-type (UNF3001) (a) and nifV mutant (UNF1613) (b) cells at 0.0074 atm C_2H_2 . Cultures were grown in NFDM+arginine (1 g l⁻¹) for 20 h at 30 °C with constant N_2 bubbling. They were collected by centrifugation under argon and resuspended in cold argon-bubbled NFDM+chloramphenicol (0.1 g l⁻¹) and kept on ice under argon. Samples (5 ml) were taken by syringe into 30-ml flasks flushed with either argon or N_2 and incubated for 10 min at 30 °C before addition of 0.25 ml C_2H_2 to start the reaction. Headspace samples (0.5 ml) were taken at intervals and analysed by gas chromatography. Assays under 0.1 atm N_2 were set up by adding 3.0 ml N_2 to the 29-ml headspace. \bigcirc , 1 atm A_1 ; \triangle , 0.1 atm N_2 in A_1 (wild type only); \square , 1 atm N_2 .

substrate reduction. An indirect estimate of the extent to which Kp1 is saturated with Kp2 in crude extracts can be measured by observing the increase in C_2H_2 -reducing activity on addition of excess purified Kp2. Figure 2a shows the effect of adding a >10-fold molar excess of purified Kp2 protein to crude extracts of a wild-type and nifV strain. Kp2 addition stimulated C_2H_2 -reducing activity to approximately the same extent in both crude extracts. Thus, an unfavourable Kp2 Kp1 ratio is unlikely to account for the inability of nifV cells to fix N_2 in vivo.

Figure 2b shows hydrogen evolution by crude extracts under various atmospheres and the effect of added Kp2. Control assays omitting either ATP or dithionite showed that <10% of H₂ evolved in assays with added Kp2 was attributable to other hydrogenase activity. Nitrogen failed to compete with protons in assays with mutant extracts even with the addition of a sevenfold molar excess of Kp2 (maximum 6% inhibition of H2 evolution) whereas significant competition (63% inhibition) was seen in assays of the wild-type extract even without added Kp2. Thus, the nifV crude extract is apparently defective in reducing N2 as a substrate, although it reduced CN and CH3NC (data not shown). CO alone or CO plus C2H2 did not significantly affect H2 evolution in the wild-type extract (Fig. 2b). (The combination of CO plus C₂H₂ inhibits reversible hydrogenase without affecting H₂ evolution by nitrogenase¹⁶.) However, hydrogen evolution by nifV crude extracts was inhibited 50% in the presence of 3%

Table 1 Incorporation of ¹⁵N₂ by cultures of K. pneumoniae nifV mutants

Strain	Genotype	Specific activity of C ₂ H ₂ reduction (% of wild-type)	Atom % 15N enrichment
UNF2036	$nif\Delta$	<1	< 0.4
UNF3001	nif ⁺ (wild-type)	100	21
UNF1610	nifV2249::Tn7	61	0.7
UNF1613	nifV2253::Tn7	81	0.5

Strains were grown under a stream of N_2 for 8 h at 30 °C in nitrogenfree Davis and Mingcoli medium $(NFDM)^6$ arginine $(1\ g\ l^{-1})$ to permit derepression. Cells were collected by centrifugation, resuspended in fresh Argon-bubbled NFDM+ arginine $(1\ g\ l^{-1})$ and shaken for 11 h at 30 °C under 63% N_2 (70% enriched for $^{15}N_2$) in argon. Cell pellets were collected by centrifugation and digested for ^{15}N analysis by mass spectroscopy. C_2H_2 reduction was measured before incubation under $^{15}N_2$ as in Fig. 1 except that 2.0 ml of culture was used.

Table 2 Effect of varying the molar ratio of purified Kp2 and nifV Kp1 on substrate reduction

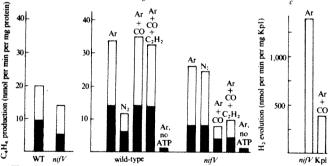
	Kp2:Kp1			of substrate reducemin per mg Kp1)	tion	% Inhibition	Electron flow under N ₂
Source of Kp1	molar ratio	H ₂ under argon	H ₂ under N ₂	NH ⁺ production	C ₂ H ₂ reduction	of H ₂ evolution by N ₂	Electron flow under Ar
UNF1613 nifV::Tn7	1:1	182	155	17.8	152	-3 - 2	
UNF1613 nifV::Tn7	2::1	399	340	73.5	152	15	100
UNF1613 nifV::Tn7	4::1	763	503	157	320	15	113
UNF1613 nifV::Tn7	7::1	1.022	717		620	34	97
UNF1613 nifV::Tn7	10::1	1,022		259	843	30	108
UNF1613 nifV::Tn7	25::1	,	842	318	1,044	32	106
MSal wild type		1,532	938	370	1,262	39	97
	1::1	249	94.4	113	247	62	102
M5al wild type	10::1	1,527	471	725	1,127	69	102

Assays were done with 0.22 µM MoFe protein either under 1 atm Ar, 0.13 atm C₂H₂ in Ar or 1 atm N₂ at 30 °C, and were linear with time. H₂, C₂H₄ and NH⁺₄ were determined as described previously21. Specific activities in nmol C2H4 reduced per min per mg protein in optimal conditions were nifV Kp1, 1,262; wild-type Kp1, 1,240; wild-type Kp2, 1,150. Component ratios were calculated assuming a maximum specific activity for C2H2 reduction of 1,800 nmol per min per mg for both Kp1 and Kp2.

CO or CO plus C₂H₂. Thus ATP- and reductant-dependent H₂ evolution by nifV nitrogenase is inhibited by carbon monoxide, in contrast to wild-type enzyme.

The failure of added Kp2 to stimulate N₂ reduction might be due to inhibition by defective Kp2 in the mutant crude extract, a situation analogous to certain heterologous combinations of nitrogenase components from different organisms 17-20. Following purification of Kp1 and Kp2 from UNF1613 by conventional procedures 19,21,22, Kp2 from the nifV mutant showed wild-type properties with respect to N₂ reduction and CO insensitivity of H₂ evolution when complemented by wild-type Kp1 protein. The nifV Kp1 showed CO inhibition of H₂ evolution when complemented with wild-type Kp2 (Fig. 2c). These mutants therefore have a defective MoFe protein.

Table 2 shows the effect of the Kp2: nifV Kp1 molar ratio on substrate reduction. The rate of substrate reduction increased with increasing Kp2: Kp1 ratio. The rate of NH₄ production by the mutant Kp1 increased with ratio but was less than half the wild-type rate at a 10:1 ratio. Even at a 25:1 ratio only 39% of the electron flow was utilized for NH₄ production, compared with 62% for a 1:1 ratio with wild-type Kp1. However, the rate of electron flow through both mutant and wild-type enzyme was the same under either argon or nitrogen (Table 2 and ref. 23). Clearly, N₂ reduction by nifV Kp1 only competes effectively with hydrogen evolution at non-physiological molar ratios of the two proteins, whereas C₂H₂ reduction seems to be normal. The



a, C₂H₂ reduction by UNF3001 (wild type, WT) and UNF1613 (nifV) crude extracts with (unshaded) and without (shaded) added Kp2; b, H2 evolution by crude extracts under argon; N₂; Ar+3% CO; Ar+CO+14% C₂H₂. 20-min assays were done as in ref. 21. Cells for crude extract preparation were grown for 17-18 h in 20 l Ar-bubbled NFDM + NH₄Cl (0.24 g l^{-1}) Sodium aspartate was added (50 mg l^{-1}) and the pH adjusted to 7.2. After 5 h derepression, cells were collected by centrifugation anaerobically, resuspended in 30-45 ml 50 mM HEPES pH 8.7, 0.65 mM dithiothreitol and 2 mM $Na_2S_2O_4$, crushed at 12,000 Pa in a French pressure cell and cleared by centrifugation at 38,000g for 45 min. The supernatant was frozen in liquid N₂ and stored at -196 °C. Assays²¹ contained 1.03 mg (UNF3001) and 1.54 mg contained 1.03 mg (UNF3001) and 1.54 mg (UNF1613) protein and were linear with added protein. Kp2 (specific activity 900 nmol C₂H₂ per min per mg) was added at 0.151 mg per assay where appropriate. c, H_2 evolution by purified nif V Kp1 and a 10-fold molar excess of wild-type Kp2 under argon with and without 5.8 matm carbon monoxide.

discrepancy between the lack of N2 reduction in vivo and in crude extracts compared with the measurable rates with purified enzyme may reflect the presence of other proteins in the former which affect the rate of nitrogen reduction.

Recent in vitro studies with purified nitrogenase components have shown that the distribution of electrons between competing substrates depends on the rate of electron flux through the MoFe protein and that this is influenced by the ATP concentration and the Fe: MoFe protein ratio 13,14,24. The electron flux increases with increasing Fe protein: MoFe protein ratio 24,25 and whereas low electron fluxes favour proton reduction, progressively higher fluxes favour C₂H₂ and N₂ reduction in that 25. As nifV Kp1 does not respond normally to Kp2 addition, what is the function of the nifV protein? We conclude that it processes the MoFe protein, and that this modification renders H₂ evolution insensitive to CO inhibition. Furthermore, the nifV product seems to modify the catalytic ability of Kp1 so that N₂ reduction will occur at a lowered electron flux. It has been suggested that nitrogenase was originally a cyanide detoxifying enzyme²⁶. We suggest that the nifV modification might be a later evolutionary development which converted the original enzyme into an efficient nitrogenase. Finally, like other workers^{14,17}, we stress the need for caution when using the C₂H₂-reduction test as a measure of nitrogen fixation. The study of the nifV MoFe protein may provide useful insights into the structure of the MoFe protein of nitrogenase and its mechanism of substrate reduction.

We thank Professor J. R. Postgate and Dr R. Richards for helpful suggestions, Dr B. E. Smith for useful discussions and for donating purified wild-type nitrogenase components, E. Kavanagh for performing the ¹⁵N digestion and analysis, and Mr K. Baker for growth of cells for protein purification. P.A.McL. is the recipient of a research assistantship from the Agricultural Research Council.

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- Mortenson, L. E. & Thorneley, R. N. F. A. Rev. Biochem, 48, 387-418 (1979).
- Zumft, W. G. & Mortenson, L. E. Biochim. biophys. Acta 416, 1-52 (1975).
 Hardy, R. W. F. in A Treatise on Dinitrogen Fixation, Sections I, II (eds Hardy, R. W. F., Bottomley, F. & Burns, R. C.) 515-568 (Wiley-Interscience, New York, 1979).
 Hwang, J. C., Chen, C. H. & Burris, R. H. Biochim. biophys. Acta 292, 256-270 (1973).

- MacNeil, T. et al. J. Bact. 136, 253-266 (1978). Merrick, M. et al. J. gen. Microbiol. 117, 509-520 (1980). Elmerich, C. et al. Molec. gen. Genet. 165, 181-189 (1978).
- Klipp, W. & Pühler, A. in Biological Metabolism of Inorganic and Sulphur Compounds (eds Bothe, H. & Trebst, A.) (Springer, Berlin, in the press).
 St. John, R. T., Shah, V. K. & Brill, W. J. J. Bact. 119, 266-269 (1975).
 Roberts, G. P. et al. J. Bact. 136, 267-279 (1978).
- Brenchley, J. E., Prival, M. J. & Magasanik, B. J. biol. Chem. 248, 6122-6128 (1973). Tyler, B. A. Rev. Biochem. 47, 1127-1162 (1978).

- Net, B. A. Reb. Backett. 41, 1127-1102 (1978).

 Thorneley, R. N. F. & Eady, R. R. Biochem. J. 167, 457-461 (1977).

 Davis, L. C., Shah, V. K. & Brill, W. J. Biochim. biophys. Acta 403, 67-78 (1975).

 Davis, L. C. & Wang, Y.-L. J. Bact. 141, 1230-1238 (1980).

 Smith, L. A., Hill, S. & Yates, M. G. Nature 262, 209-210 (1976).

 Smith, B. E. et al. Biochem. J. 157, 439-447 (1976).

 Elmerich, D. W. & Burris, R. H. Proc. nam. Acad. Sci. U.S.A. 73, 4369-4373 (1976).

 Smith, B. E. et al. in Paper Development in Williams Fixed in Add National Williams.
- Smith, B. E. et al. in Recent Developments in Nitrogen Fixation (eds Newton, W., Postgate, J. R. & Rodriguez-Barrueco, R.) 191-203 (Academic, New York, 1977).
- Elmerich, D. W., Ljones, T. & Burris, R. H. Biochim. biophys. Acta 527, 359-369 (1978).
 Eady, R. R. et al. Biochem. J. 128, 655-675 (1972).
 Shah, V. K. & Brill, W. J. Proc. natn. Acad. Sci. U.S.A. 74, 3249-3253 (1977).
- Liones, T. Biochim, biophys. Acta 321, 103-113 (1973).

 Shah, V. K., Davis, L. C. & Brill, W. J. Biochim. biophys. Acta 384, 353-359 (1975).
- Hageman, R. V. & Burris, R. H. Biochim. biophys. Acta 591, 63-75 (1980). Silver, W. S. & Postgate, J. R. J. theor. Biol. 40, 1-10 (1973).

BOOK REVIEWS

Synaptic patterns of the mind

H.B. Barlow

THIS book is based on a Work Session of the Neurosciences Research Program that was held in May 1978, but although it is thus based on papers given at a conference three years ago it is very much alive and worth reading by those interested in theoretical approaches to the nervous system. The editors have evidently made a determined effort to present the material as a coherent whole in a readable form, and to a large extent they have succeeded. They divide the material up into three levels: the nature of the computations that are needed for the sensory and motor tasks we perform, the algorithms by which the computations are achieved, and the biological mechanisms which perform them. These are dealt with in reverse order. neural mechanisms first and the nature of the computation last; the recognition of the three levels provides a convincing framework for describing selected aspects of the enormously complicated processes that underlie our perceptions and control our movements. The book not only has a unified viewpoint but also an original feature here visible, I think, for the first time. This is the attempt to provide a theoretical background, together with a shorthand graphical representation, for the non-linear interactions on which the crucial logical operations of the nervous system depend. However let us first consider the review chapters, which are still useful even though not fully up to date.

The first section has four chapters on how the nervous system performs its computational algorithms. The first two are useful general reviews by T. Lamb and D. Baylor on the biophysics of transduction in photoreceptors and the integration and transmission of this information through the retina. These look upon signal generation and transmission as linear processes, but the next two chapters by T. Poggio and V. Torre are the ones in which a biophysical mechanism for the non-linear interactions that must underlie pattern selective mechanisms are proposed, and I shall return to them for they form the book's kernel of originality.

The next section has seven chapters at the level of "algorithms and interactions" in neural systems. It contains up-dating reviews on various aspects of the visual system by Victor, Poggio, Reichardt, Wilson, Julesz, Hubel and Cowan. As the editors seem to admit, it is not quite right to apply the term "algorithm" to what is here described. An algorithm surely has a clearly defined purpose, but we are almost completely ignorant of the purposes of

Theoretical Approaches in Neurobiology. Edited by W.E. Reichardt and T. Poggio. Pp.252. ISBN 0-262-18100-2. (MIT Press: 1981.) \$27, £14.

non-linear interactions in the cat's retina, cooperative processes in texture perception or the columnar organization of the visual cortex. All the same one needs a level of description intermediate between biophysics and artifical intelligence or cognitive modelling. Anyone who has tried to understand someone else's computer program, or even one of his own, will recognize the dazed feeling that accompanies the question "What in the world does this page of nonsense accomplish?", and that is the feeling one has when reading much of this middle section of the book. Of course something is accomplished, but the interpretive keys are in many cases unknown, or at any rate not divulged.

The level of the final section is that of describing the nature of computations. Two of them deal with visual control of flight in flies (Reichardt and Poggio), and of limb pointing in monkeys. The other two are concerned with human perception. Ullman deals at a theoretical level with the remarkable human capacity to interpret the relative motions of a few isolated spots of light in terms of the coherent motion of a single rigid object. Marr's chapter gives a brief summary of his approach to vision, and it would form a very useful introduction to his work for someone who has not read his lengthier papers. It is clear that this book as a whole owes a great deal to Marr's recognition of the different levels of description that are required in order to understand visual perception.

Tomaso Poggio, as well as editing the book, is author or co-author of at least 25 per cent of the material in it, and in these sections an important new approach is set out. As Boole realized when he wrote the "Laws of Thought" more than 100 years ago, higher mental processes require logical operations that are essentially nonlinear and more like multiplications than addition or subtraction. He was of course thinking of mental processes at a verbal level, but the lesson must also apply for the pre-verbal processes that underlie pattern selectivity and motor control, and it is the mechanism and organization of these processes at the level of the synaptic interactions between cells that Poggio and Torre attack in the biophysical section of this book. The intracellular potential of a particular cell has customarily been regarded as summating the positive and

negative influences of all the neurones that make excitatory and inhibitory synaptic connections with it. Poggio and Torre point out that synapses situated close to each other on one of a cell's dendrites will mutually influence each other, and the effects produced at a distant point will not simply represent the sum of the two. For instance, if one synapse is inhibitory and acts by increasing conductance to an ion whose concentration ratio is close to that corresponding to the cell's resting potential, then its activation will contribute very little potential change at a distant point in the cell. Nevertheless it may have a very powerful effect in stabilizing the potential at that point in the cell when a neighbouring excitatory synapse becomes active. This shunting effect can be very powerful, but will only be local. They propose that this may be the mechanism whereby directional selectivity is achieved in retinal ganglion cells, and it certainly fits two of the main requirements deduced from physiological analysis, namely that the interactions responsible are between local sub-units of the receptive field of a retinal ganglion cell, and that they are inhibitory in nature. The logical operation is thus equivalent to "and not", or one input vetoing another, rather than to the conjunctive "and".

The development of this idea is given in some detail, made more digestible by placing much of the mathematics in an appendix, as is also done in the rest of the book. Unfortunately, though the ideas are important for a large number of people, it is not very easy to follow. The authors have sought clarity in exactitude, whereas a certain amount of over-simplification might have been more effective. They also invent an interesting graphical notation which has symbolic representations for conductance changes as synaptic inputs, for the currents thereby produced and the potential changes resulting from the filterlike action of decremental electrotonic conduction, and most important, for the multiplicative effect of a conductance change on a voltage. If this notation is developed, and is adopted, one may be able to "read" a neural circuit in the same way that one can transistor circuits. It may even be possible to relate such a graphical form of neural circuit to the synaptic anatomy visible by electron microscopy, for many important quantities determining nonlinear interaction are dependent upon anatomical variables such as proximity and dendritic diameter.

Neurophysiology is mainly a simple

science, and I am not sure that Poggio has expressed his ideas in a simple enough form for us to understand. But this part of the book is certainly a novel attack on a most important problem, and one leaves the book as a whole in a rather more optimistic mood than is usually the case after a dose of brain theory. Instead of feeling that the subject matter is incoherent and totally unapproachable, one feels that, within the framework set out, the brain may, ultimately, become comprehensible.

H.B. Barlow is Royal Society Research
Professor in the Physiological Laboratory of the
University of Cambridge.

Not quite Allard

C.N. Law

The Theory of Plant Breeding. By O. Mayo. Pp.293. ISBN 0-19-854536-3. (Clarendon/Oxford University Press: 1981.) £30, \$89.

PLANT breeding has long required an upto-date and authoritative textbook. It is now over 20 years since what many regarded as a good book on the subject, Principles of Plant Breeding by R.W. Allard (Wiley, 1960), first appeared. Since that time numerous attempts have been made to write a worthy replacement but with limited success. This book on the theory of plant breeding, by O. Mayo, is likely to receive a similar assessment. This is a pity, since the book has merit and touches on much that needs to be said about plant breeding and related sciences.

The book has 17 chapters, of which seven of the longest describe methods of genetic analysis, the properties of genetic systems in inbreeding and outbreeding crops, the response of such systems to environmental variables and different selection pressures. These are then followed by several short chapters on induced mutation, disease resistance, somatic cell genetics and, as if as an afterthought, further cytogenetical manipulations. The final chapters discuss the conservation of germplasm and aspects of plant breeding strategy.

A number of errors occur, mainly of omission. Thus, the author is rather cursory in his treatment of the covariance-variance analysis of diallel crosses, and is apparently not aware that the creators of this analysis have been at pains to point out its weaknesses in detecting certain types of gene action. Likewise, the treatment of the genetical causes of heterosis leaves much to be desired, particularly since the author appears to labour under the misconception that heterosis is a phenomenon still awaiting a genetical explanation.

Far too frequently important methods and issues are mentioned en passant, when

Entomology — and how the West was won

Philip S. Corbet

From Arsenic to DDT: A History of Entomology in Western Canada. By Paul W. Riegert. Pp.357. ISBN 0-8020-5499-4. (University of Toronto: 1981.) \$30. To be published in the UK on September 24, £18.

THE history of Canadian entomology has received close and expert scrutiny before. Compared with two previous accounts by R. Glen (Can. Ent. 88, 290-371; 1956) and G.J. Spencer (Can. Ent. 96, 33-59; 1963) - Professor Riegert's attractively presented book is narrower in scope but much more detailed. It traces the development of entomology (mainly applied entomology) in the four western provinces of Canada, from the arrival of the first European colonists until just after the Second World War, when synthetic organic chemical insecticides were starting to transform the practice of pest control. (The history of forest entomology is omitted because Riegert discovered that such a document had recently been prepared.) As it stands, this book provides a wealth of detail on subjects as varied as the daunting mosquito attacks recorded by traders in the early seventeenth century, instructions for making and applying "Criddle Mixture" (a horse-manure-based poisoned bait for grasshoppers) and the progressive evolution and enforcement of interprovince quarantine regulations.

The five main parts of the book are devoted to encounters between European colonists and insects; the first professional entomologists; the insects of British Columbia — mainly mosquitoes and orchard pests; the insects of the prairies — including the wheat-stem sawfly, grass-hoppers, cutworms and wireworms; and specialized topics such as medical and veterinary pests, stored-products insects, and the teaching of entomology in universities.

A brief, admirably succinct, epilogue reviews the main personalities and events which attended the growth of applied entomology in western Canada from the 1880s to the 1950s — "from arsenic to DDT" — and ends by listing some of the lessons to be learnt from this richly documented set of case studies.

The author's style tends to be somewhat florid for my taste, and his inclusion of diverse anecdotal and factual material at times impairs the fluency of the text, although this seems a modest price to pay for the exhaustive background information he provides. A map would have been a useful adjunct to the excellent contemporary photographs.

This book will certainly appeal to those wishing to learn, or to be reminded of, the circumstances and individuals that have helped Canadian entomology to become so vigorous and effective; and, for the sake of the discipline's continuing health, one may wish that such readers will include those who decide the resources to be allocated to entomology by future Canadian governments! As a contribution to the existing literature on the history of entomology, Riegert's book has undoubted value in several other respects. Using a canvas of manageable size, he delineates the changing pattern of agricultural and veterinary entomology in a developed, though recently settled, country which is prone to severe pest problems. The extremely detailed description of these problems - including contemporary recipes for their mitigation and the frank but sensitive description of the men and institutions concerned with pest management, may attract the interest of social historians as well as applied entomologists. One may hope that those destined to practise and administer applied entomology in Canada and elsewhere properly appreciate the realities of largescale pest suppression when synthetic organic chemical pesticides are not available. People who read this book will surely gain such an appreciation; and in doing so they will come to share the author's high regard for the immensely able and resourceful pioneer entomologists who successfully tackled some of the most intense insect attacks ever to be endured by human beings.

Philip S. Corbet is Professor of Zoology at the University of Dundee, and was formerly an entomologist in Canada.

more detailed and penetrating accounts are demanded. This is a disappointing feature which probably arises from the stated objectives of the book, which are to give an account of plant breeding theory but not to provide extensive descriptions of proofs or methods. However, one questions whether this is a wise decision when such an important aspect as breeding for disease resistance receives only three pages of text. The impression emerges that the author has much to contribute in writing about the

theory of plant breeding; but either he has been overly constrained by limitations on the size of the book or he has been perhaps too unambitious in his objectives. He should be encouraged to build upon the firmer foundations of this book as well as to include views about the practice of plant breeding in any future edition.

C.N. Law is Head of the Cytogenetics Department at the Plant Breeding Institute, Cambridge.

Illiterate lamp men and other problems in geodesy

William M. Kaula

Geodesy, 4th Edn. By G. Bomford. Pp.855. ISBN 0-19-851946-X. (Oxford University Press: 1980.) £49. \$139.

In 1952 I was reassigned from a combat engineer battalion in the Fulda Gap to the Ohio State University in order to study geodesy. Upon arriving in Columbus that June, I met Professor W. A. Heiskanen, who said: "I'm going to Finland tomorrow for the summer. Here, study this book". "This book" was the first edition of Bomford's Geodesy, just published. So that summer I spent many hours between classes under the trees (now gone) on the east bank of the Olentangy, gleaning geodesy from Geodesy.

Geodesy is a compendium, rather than a textbook or a treatise. It is the principal geodetic reference book in English. The main parts of this new edition are: horizontal control (196 pp.), vertical control (57 pp.), physical geodesy (118 pp.), artificial satellites (139 pp.), and appendixes and bibliography (186 pp.).

Virtues of the book and its author include comprehensiveness within geodesy, a strong geometrical intuition, keen insights about instrumentation and observations, a sensitivity to measuring errors - and good writing. Bomford has much better command of language than most scientists and engineers. His discussions of such topics as the effects of atmospheric refraction on theodolite or spirit-level measurements are a pleasure to read, being syntheses of practical experience, an analytical attitude and fluency of expression. His Indian past is manifest in passages such as "When supplies and transport are easy and with lamp men who can find their own way about, the programme presents no special problems, but in uninhabited country, or with illiterate lamp men, it calls for very careful thought . . . ". Bomford's careful selection of tangential topics, practical (e.g. star catalogues) or scientific (e.g. geophysical implications of gravity anomalies) is also evident.

However, he is less at ease with the abstract and the mathematical. His treatments of potential fields and orbital dynamics are somewhat perfunctory, and these topics are made unnecessarily obscure by quoting complicated formulae, rather than discussing underlying principles. A saving grace is the frequent referencing; anyone trying to use Geodesy as a text (as I did) must resort to looking up the references to understand whence came

The first of a new series, Current Reviews in Biomedicine, has been published by Elsevier/North-Holland. The paperback Towards Understanding Receptors, edited by J.W. Lamble, is a well-organized collection of 33 reviews largely taken from Trends in Pharmacological Sciences, and costs £7, \$25.

many of the prickly-looking equations. Oddly incongruous with the quoted formulae in the main text are most of the appendixes, which include rather elementary explanations of matrix algebra, theory of errors, vectors and so on. Some of this matter is outmoded: for example, eight pages on the solution of matrix equations make no mention of square-root techniques, while four pages on harmonic analysis reference only books written before 1930, and do not mention fast Fourier transform methods. Such archaisms may unduly deter those who have had modern engineering educations from using the book.

The revisions between the 1971 and 1980 editions are major. The type appears to have been completely reset and the size of the book has risen by 17 per cent. This increase is almost entirely explicable by the greater space accorded to artificial satellite methods, which has not only more than tripled but has also been rearranged with respect to the other material. However, going through the two editions in parallel reveals many other detailed changes, such as increases in the space devoted to crustal movements, time standards, MSL-geoid difference and gyro-theodolites which are balanced by decreases in that allocated to map projections, mechanical chronographs, astrolabes etc. In the earlier parts, particularly, this compressive zeal was paragraph by paragraph. A loss of flavour sometimes results. Thus, what was in the 1952 edition:

Such an apparatus was used in India in 1880 [150], p.35, and another was used with the prismatic astrolabe in 1927-9 [151], but neither was entirely satisfactory. Possibly the principle was sound, but the mechanism imperfect. Recently R. Woolley (of the Commonwealth Observatory, Canberra) has made an apparatus on similar lines, which is reported to be satisfactory in the observatory, although possibly not easily portable. Full reports have not yet been published.

has now shrunk to: "Such an apparatus has been constructed from time to time, but no great use of the method has been reported". Unfortunately, this drive to prune and mend apparently flagged by the time the appendixes were reached.

Bomford's experience and perception in geodetic survey is particularly applicable to current problems of using past geodetic measurements to infer crustal movements. The relevant cautionary remarks appear in both the sections on control and those on crustal movements: for example "The differences cannot lightly be ascribed to crustal movements", and "Levelling has sometimes been less good than it looks". Several likely systematic errors are discussed, but the bite of specific examples is lacking, perhaps because the Survey of India was always stricter in its practices

than was the US Coast and Geodetic Survey.

I must report that I found no errors, other than a few misprints. The defects are much more ones of selection and exclusion. In addition to those mentioned above, an oddity is the omission of the determination of longitudinal variations in the gravity field from satellite orbit perturbations, which is both conceptually simpler and more fruitful of geodetic results than the resonant effects which are included.

Nonetheless, if you have a geodetic problem, look it up in Bomford. If he doesn't give you the answer, he'll tell you where else to look.

William M. Kaula is a Professor of Geophysics at the University of California, Los Angeles.

Material attraction

P. J. Grundy

Ferromagnetic Materials: A Handbook on the Properties of Magnetically Ordered Substances. Vols 1 and 2. Edited by E.P. Wohlfarth. Vol.1 pp.634, ISBN 0-444-85311-1. Vol.2 pp.600, ISBN 0-444-85312-X. (North-Holland: 1980.) Each volume Dfl.210, \$102.50.

Many textbooks and technical handbooks lay claim to the title of the definitive text. However, in order to prove its superiority over all comers, a book, like all championship contenders, must have all the necessary qualities. If all the promised material — a further two volumes are planned — is provided and published within a reasonable time, this work should, indeed, prove to be a champion.

The handbook is designed to cover as wide a range of material properties as possible. Its restrictive title of Ferromagnetic Materials is quickly extended by the subtitle to take account of the other varieties of magnetic ordering, and the subjects covered by the first two volumes are varied indeed. The contributing authors have been well chosen, are "experts" in their specialist fields and most are household names in magnetism.

Understandably, there is no obvious overall plan to the layout of the chapters, except that the first is by the editor and reflects his deep interest and involvement with work on the trio of ferromagnetic transition metals. The second chapter of the first volume, by Mydosh and Nieuwenhuys on dilute transition metal alloys, deals authoritatively with the complex and fascinating range of effects arising from the concentration changes and differing magnetic interactions of the

magnetic impurity in these spin glass systems. This first volume also contains contributions on rare earth metals and alloys and compounds by Legvold, Buschow and Clark. Each of these authorities has worked on these fundamentally important, numerous (Buschow lists details and references to at least 1,000 binary and ternary compounds) and potentially useful materials since the 1960s. The journey through the periodic table is completed by Trzebiatowski's contribution on actinide elements and compounds. Even for many people active in magnetism, these materials are somewhat enigmatic. However, their properties are clearly dealt with and no text could claim to be comprehensive without their inclusion. As might be expected, the chapter on amorphous magnetic materials prepared by rapid quenching from the melt is an especially authoritative and lucid account; Luborsky and co-workers have been pace-makers in the study of the magnetic properties and preparation methods of these alloys, especially those suitable for the application and production of commercially useful material.

The second volume is dominated by contributions from the research and development laboratories of large industrial corporations. This is an important and welcome feature for several reasons. One is that it brings home to the reader, especially in the chapters on bubble domain materials by Eschenfelder, where charts in which interrelated material properties and parameters can be crosschecked are given, the special approach to research which is concerned with designing and using materials in a real application. Another is that articles, such as those by Chin and Wernick on soft magnetic materials and Bate on magnetic recording materials, are extremely comprehensive and are accompanied by exhaustive reference lists — presumably the product of excellent information retrieval systems. On the other hand, the smaller corpus of knowledge on other materials, and perhaps a more selective approach, is evident in some of the other contributions. This also has its advantages in that it can be said that part of the author's responsibility is to guide the reader and select the important contributions to his subject.

The four articles by Gilleo, Slick, Nicolas and Eschenfelder on the magnetic ferrites and garnets cover the ground admirably. The final chapter by Charles and Popplewell is, not surprisingly, the only contribution on materials which are effectively liquids. "Ferrofluids" present a challenge in thought as to what uses can be made of magnetizable colloidal suspensions. Several applications have already been addressed but one severe problem seems to be the rapid departure from liquid behaviour accompanying only modest increases in concentrations and magnetization.

There is no doubt that these volumes

should be available in all research and development laboratories concerned with magnetic materials and material properties in general. Their value as sourcebooks to both graduate students and seasoned researchers is obvious, and their appearance will help to promote the important messages that magnetic materials display a bewildering variety of properties and that the applications of magnetism

permeate the whole of modern society by providing electric power, communications and information storage. I am sure the international magnetism community awaits with anticipation the volumes yet to appear.

P. J. Grundy is Senior Lecturer in the Department of Pure and Applied Physics at the University of Salford.

Mathematical stimulus for biologists

J.D. Murray

The Geometry of Biological Time. By Arthur T. Winfree. Pp.530. ISBN 0-387-09373-7/3-540-09373. (Springer-Verlag: 1980.) DM 59.50, \$32.80.

GENUINE interdisciplinary research can often produce spectacular and exciting results. Winfree, a biologist, knows a lot of mathematics and this book reflects his appreciation of interesting problems in both fields. Amongst other things he gives some excellent case histories of what interdisciplinary endeavour can achieve. There is no doubt that the development of models and model mechanisms as an aid in elucidating biological phenomena with a view to determining basic principles is a flourishing, useful and fascinating area of research. (The interdisciplinary mathematics-biology fields are now recovering from the ravages caused by catastrophe theory.) In many countries the generous support from research funding organizations reflects this growth area.

This book is a major contribution to theoretical biology and will become, I am sure, an essential reference book for those, theoreticians as well as experimentalists, interested in modelling and understanding periodic behaviour in biology. From a pedagogical point of view it does not require much detailed knowledge of mathematics as such, except for selected chapters, but it does require a mathematical appreciation and understanding which most bioscientists do not have. However, those with firmity of purpose. who persevere with most of the more instructive first nine chapters, will reap rich rewards. Those with less time or weaker will can usefully go to those parts of the second half of the book which deal with more specific phenomena. Many of the ideas and concepts described are illustrated with relevant, and often ingenious experiments.

Winfree first discusses periodic behaviour in general, phases, phase singularities and phase resetting. The latter is shown to be an important concept of relevance to experimental observation. The effect of interactions between oscillators is then examined. In particular, where they are spatially distributed they can result in a variety of unexpected wave phenomena. One of Winfree's aims is to try to give a picture of observed events and natural phenomena which are not dependent on specific models. Generally he uses a geometric conceptual approach.

The second half of the book deals mainly with specific phenomena and experiments which exemplify some of the ideas developed earlier, but can be read, more or less, independently from the first half. Here Winfree discusses several major topics of current biological research and among other things gives his personal views on them. For example, such topics as the Belousov-Zhabotinskii reaction (emphasizing the wave phenomenon aspect), slime mould aggregation, circadian rhythms in insect eclosion, the female cycle and the cell mitotic cycle are surveyed and discussed. Researchers in each of these areas, and others covered, will no doubt feel that the views expressed do not give the whole picture of current thinking (and how could they in a single book?) or present a biased picture, and so on. This indeed so, but whatever their views, it will be difficult to ignore many of Winfree's ideas with impunity.

This book exhibits a remarkably wide spectrum of biomedical problems of current research interest. With Winfree's talent in presenting succinctly the essential interesting features of a phenomenon, it is accessible to most mathematicians, physical scientists, and with somewhat more effort, bioscientists who believe in the use of models in biology (and few can now admit to a contrary view). Parts of it can be usefully given to both undergraduates and graduate students. I strongly recommend it for those seriously or casually interested in modelling and understanding biological oscillators and their role in nature: the book is full of unanswered questions and research ideas. As an introduction for the uninitiated, and a stimulant for the cognoscenti, it is excellent, lucidly and anecdotally written, and has a well-vetted bibliography.

J.D. Murray is Reader in Mathematics at the University of Oxford with research interests in theories of biological pattern formation.

ANNOUNCEMENTS

Awards

Prof. Choh Hao Li (Director of the UC-San Francisco Hormone Research Laboratory) has received the Fred Conrad Koch Award research on the hormones of the pituitary gland.

The Geological Society of London has made the following awards: The Wollaston Medal to Prof. R.M. Garrels; The Murchison Medal to Prof. G.M. Brown; The Lyell Medal to Dr W.S. McKerrow; The Prestwich Medal to Dr H.G. Reading; The Bigsby Medal to Dr A.G. Smith; The Wollaston Fund to Prof. John Murray; The Murchison Fund to Dr A.H.G. Mitchell; Moieties of the Lyell Fund to Dr M.G. Bassett and Dr B.J. Bluck; President's Awards to Dr A. Saunders and Dr A. Scott; The R.H. Worth Prize to Mr E.H. Shackleton.

Through the Irish Foundation for Human Development, the Curt P. Richter Prize in Psychoneuroendocrinology has been established for meritorious research in the area of Psychoneuroendocrinology. The sum of \$1,000 will be awarded annually for the best essay or manuscript submitted by a scientist or physician under 40 years of age by I January 1982. Manuscripts (in quadruplicate) to: Dr Fleur L. Strand, Secretary, International Society of Psychoneuroendocrinology, Biology Department, New York University, 952 Brown, 100 Washington Square, New York, New York 10003, USA.

Churchill Travelling Fellowships are open to all UK citizens of any age or occupation; no educational or professional qualifications are needed. The awards enable candidates to gain a better understanding of the lives and work of people in countries overseas, and to bring back useful knowledge for the benefit of the community. Grants are offered in different categories each year (for example "Medical Research into the Relief of Pain" will interest scientists, doctors and research workers associated with the development of new methods to alleviate suffering; and "Animal Welfare Legislation" will interest those concerned with the preparation and application of animal welfare legislation relating to husbandry, laboratory, game laws and wildlife conservation. The grant will cover return air fare, plus travel and living expenses abroad for a period of up to three months. To apply send your name and address only on a postcard between August and Mid-October to the Winston Churchill Memorial Trust, 15 Queen's Gate Terrace, London SW7, UK.

Meetings

29 September — 1 October, **Analytical Conference**, London (Tony Goodall, Pye Unicam Ltd, York St, Cambridge, UK).

13 October, The Short and Long Term Biological Consequences of Exposure to Radiation, London (Prof. J.H. Martin, Dept of Medical Biophysics, University of Dundee, UK).

29 October, Frontiers of Analytical Techniques and their Applications, Richmond (B. Mack, Philip Morris U.S.A., 100 Park Ave, New York, New York 10017, USA).

4-6 November, Managing and Motivating Scientists and Engineers, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

17-20 November, Separation of Solids from Liquids, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

7-10 December, Industrial Corrosion, Amsterdam (The Center for Professional Advancement, Postbus 19865, 1000 GW Amsterdam, The Netherlands).

5-7 January, Factors and Mechanisms Influencing Bone Growth, Los Angeles (A.D. Dixon, Dental Research Institute, Center for Health Sciences, University of California Los Angeles, Los Angeles, California 90024, USA).

11-15 January, From Gene to Protein: Translation into Biochemistry, Miami (Miami Winter Symposium, PO Box 016129, Miami, Florida 33101, USA).

26 January, Quantities and Units for Use in Radiation Protection, Teddington (Prof. J.H. Martin, Dept of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

1-3 February, Pan-African Crustal Evolution in Arabia and Northeast Africa, Jeddah (Dr A.A.M. Al Shanti, IGCP-64, PO Box 1744, Jeddah, Saudi Arabia).

4-5 February, Water and Waste Engineering in Asia, Madras (J. Pickford, WEDC, University of Technology, Loughborough, LIK

7-17 February, **Heavy Crude and Tar Sands**, Caracas (UNITAR, 801 United Nations Plaza, Rm 316, New York, New York 10017, USA).

8-12 February, Viral Diseases in South-East Asia and the Western Pacific, Canberra (Assoc. Prof. J.S. Mackenzie, Dept of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009, Australia).

14-26 February, Molecular Biology Approach to the Neurosciences, Rehovot (H. Soreq, Dept of Neurobiology, Weizmann Institute of Science, Rehovot, Israel 76100). 22-26 February, Marine Mineral Resources, London (Dr D.S. Cronan, Dept of Geology, Imperial College, London SW7, UK).

28 February-6 March, B and T Cell Tumours: Biological and Clinical Aspects, Squaw Valley (UCLA Symposia, Molecular Biology Institute, University of California, Los Angeles, California 90024, USA).

2-5 March, Oceanology International '82, Brighton (Spearhead Exhibitions Ltd, Rowe House, 55/59 Fife Rd, Kingston upon Thames, Surrey, UK).

8-12 March, Analytical Chemistry and Applied Spectroscopy, New Jersey Mrs M.V. Senneway, 437 Donald Rd, Pitsburgh, Pennsylvania 15235, USA).

12-17 March, Applications and Technology of Ionizing Radiations, Riyadh (R.C. Barrall, Cancer Therapy Institute, King Fiasal Hospital, PO Box 3354, Riyadh, Saudi Arabia).

22-25 march, Solid State Physics, Manchester (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

23-25 March, Electro-optics/Laser International '82 UK, Brighton Dr F.L. Morritz, Industrial & Scientific Conf. Management Inc. 222 W. Adams St, Chicago, Illinois 60606, USA).

25-26 March, Food and the Consumer—the Next 50 Years, London (G.D. Brown, c/o RHM Research Ltd, Lincoln Rd, High Wycombe, Bucks, UK).

29-31 March, Vacuum '82, Chester (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

29 March — 2 April, 3rd International Congress of Thermology, Bath (Marlet Conference Services, 24 Preston St, Brighton, Sussex, UK).

5-7 April, Control Systems Concepts and Approaches in Clinical Medicine, Sussex (M.J. Yates, Institute of Measurement and Control, 20 Peel St, London W8, UK).

13-16 April, Crystal Growth Processes in Sedimentary Environments, Madrid (Dr. R. Clemete, Instituto de Geologia Consejo Superior de Investigaciones Cientificas c/Jose Gutierrez Abascal, 2 Madrid 6, Spain).

19-21 April, International Recycling Congress, Berlin (Heckman GMBH, Kappellenstrasse 47, D? 6200 Wiesbaden, FRG).

19-22 April, **Hazardous Spills Conference**, Milwaukee (CMA, 2501 M St, NW, Washington DC 20037, USA).

25-29 April, Cell Function and Differentiation, Athens (Secretariat, Special FEBS Meeting, Nuclear Research Center Democritos, Dept of Biology, Aghia Paraskevi, Attakis, Greece).

27-30 April, Analytica '82, Munich (SIMA, 8 Leicester St, Leicester Square, London WC2, UK).

FACULTY POSITION

Tenure track Assistant Professorship

for Eukaryotic Molecular Cell Bio-

logist with interests in some facet of

immunology. Preference will be

given to an individual who has potential for and commitment to

developing an independent research

program and who has an interest in

and an aptitude for teaching

graduate, medical and dental students. Position available during

Salary commensurate with

Applicants should submit a letter

experience but not less than \$26,000

outlining current and future research

interests, curriculum vitae and biblio-

for a twelve-month appointment.

1981-82 academic year.

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CHICAGO Didier & Broderick (312) 498 4520 ● BOSTON CEL Assoc (617) 848 9306

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UNIVERSITY OF IOWA DEPARTMENT OF CHEMISTRY

POSTDOCTORAL POSITION

Isolation, physiochemical studies, and biological assay of trace metal complexes. Species are naturally occurring and deficiencies are related to maturity-onset diabetes and ischemic heart disease. Must have experience in biochemical Isolation. Position available immediately

Send curriculum vitae and names of two references to: Harold Goff, Department of Chemistry, University of Iowa, Iowa City, Iowa 52242. equal opportunity employer/ affirmative action employer (NW815)

TRACE METAL **METABOLISM TECHNICIAN**

Applications are invited for the post of technician to work on a two year project to study trace metal absorption by premature babies. Experience with atomic absorption spectrophotometry desirable but not essential. Starting date 1st October 1981. Salary up to £5,759 inclusive of London Weighting.

Copies of qualifications, curriculum vitae and the names, addresses and telephone numbers of two referees to be submitted, within three weeks of the publication of this advertisement to Personnel Officer (Technical Staff GP1) University College London, Gower Street, London WC1E 6BT.

(9310)A

MANCHESTER AREA **HEALTH AUTHORITY** (Teaching) — Central District

DEPARTMENT OF MEDICAL GENETICS SENIOR SCIENTIFIC **OFFICER**

To join a team in well-equipped HLA and Cellular Immunogenetics Laboratories providing services for renal transplantation and disease.

Applicants should hold PhD or an Honours BSc in some aspect of HLA, cellular immunology or cell biology and have an interest in genetics.

This is a permanent post providing wide postdoctoral experience and many research opportunities.

Salary scale: £7,674 to £9,921 per annum depending on age and qualifications.

Informal enquiries to Dr G M Taylor on 061-224 9633 ext 472.

Curriculum Vitae and names of two referees to Dr G M Taylor, Department of Medical Genetics, Saint Mary's Hospital, Hathersage Road, Manchester M13 0JH as soon as possible.

nature

seeks a

WASHINGTON CORRESPONDENT

to succeed David Dickson, who will be leaving his post in Washington on 31 March 1982. The post is central to Nature's reporting of developments affecting science in North America and elsewhere.

Applicants should be English-speaking, preferably with some first-hand knowledge of the United States and should also have

a university degree (preferably in science)

at least two years' experience of written journalism (preferably with a weekly or daily periodical, but not necessarily scientific)

a broad interest in current affairs.

The successful applicant will be employed by Nature America Inc., initially for a fixed term (to be negotiated).

To allow for a spell in the London office of Nature and a period of overlap in Washington, applicants should be able to be free no later than 1 March 1982.

Salary, travel allowances and other conditions of employment by negotiation.

Applications, including a full curriculum vitae, should be plainly marked "Job application" and addressed to the Editor, Nature, Macmillan Journals, 4 Little Essex St, London WC2R 3LF or Nature, 15 East 26 St, New York, NY 10010, to arrive before 30 September 1981. (9324)A

graphy and should arrange for three letters of reference to be sent to: Dr Roy Curtiss III, Department of Microbiology, Box 11 SDB, The University of Alabama in Birmingham, Birmingham, Alabama 35294. An Equal Opportunity/ Alabama Affirmative Action Employer.

(NW806)A

UNIVERSITY OF BRISTOL DEPARTMENT OF

VETERINARY MEDICINE POST-DOCTORAL MICROBIOLOGIST

Applications are invited for a postdoctoral research worker to join a team investigating mucosal pro-tection to respiratory herpesvirus infection in cattle and cats. Previous experience in virology is essential; experience in immunological techniques is desirable.

The project, funded by the Wellcome Trust is for a three year period in the first instance with a possible extension for a further two

Salary in the range £6,475 to £7,290 per annum.

Applications accompanied by a curriculum vitae and the names and addresses of two referees should be sent to Professor F J Bourne, Department of Veterinary Medicine, Langford House, Langford, Bristol BS18 7DU by August 29th, from whom further particulars may (9327)A

MASSEY UNIVERSITY Palmerston North, New Zealand LECTURER IN MICROBIOLOGY

(Medical Bacteriology)

Applications are invited for the above-mentioned position in the Department of Microbiology and Genetics within the Science Faculty. The Department is responsible for courses leading to the BSc, BSc (Hons), MSc, and PhD degrees in microbiology.

Applicants who have experience in, and research interests relating to diagnostic bacteriology will be given favourable consideration. The level of appointment will be commen-surate with experience and qualifications within the range: NZ\$19,140 -NZ\$23,520.

Further details of this position and of the University, together with the general conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF, or from the Registrar of the University.

Applications close on 16 October 1981 (9341)A

Hoechst



is planning to extend considerably its gene technology group in Frankfurt (Main), Germany involved in basic research with special regard to health care.

We are inviting applicants for

Molecular Biology

with several years experience in recombinant DNA research and nucleic acid biochemistry.

Salary will be competitive and in accordance with experience. Applicants should have basic knowledge in German language. Applications, including curriculum vitae (with a description of previous experience) should be sent to:

Hoechst Aktiengesellschaft, Personalabteilung T, Referat Naturwissenshaftler, Postfach 80 03 20. 6230 Frankfurt (Main) 80

(W403)A

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts AL5 2JQ PLANT PROTOPLASTS

Applications are invited from graduate biologists to join a team working in the Biochemistry Department on the genetic manipulation of crop plants. The successful applicant will be expected to develop techniques for the regeneration of crop plants from isolated protoplasts. Applicants must have a proven record of success in plant regeneration and protoplast technology and preference will be given to those with a PhD and some post-doctoral experience.

Qualifications: 1st or Upper 2nd class honours degree.

The successful applicant will be appointed in one of the following grades according to qualifications and experience: Higher Scientific Officer (£6,075 — £7,999); Senior Scientific Officer (£7,644 — £9,619); Principal Scientific Officer (£9,690 — £12,540).

At least 2 years relevant experience is required for HSO grading, and 4 years for SSO. Grading at PSO level will only be considered for exceptional candidates. Pay award pending. Non-contributory superannuation.

Apply in writing to the Secretary naming two referees and quoting Ref. 459 by 19th September 1981. Further details on request.

(X9326)A

UNIVERSITY OF THE WITWATERSRAND

Johannesburg

DEPARTMENT OF CHEMISTRY

LECTURER

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin, for appointment as Lecturer in the Department of Chemistry. Duties are to be assumed as soon as possible.

Special consideration will be given to applicants with a strong interest in education, who would be able to participate in teacher training and to prosecute research in chemical education, and who would involve themselves with educational developments in the Department.

The salary attached to the post will be in the range of R10,995 — R19,230 per annum. The initial salary will be determined according to qualifications and experience of the successful applicant.

Prospective applicants should send a curriculum vitae giving full details of qualifications, experience, present salary and interests, to the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WCIV 7HE, or to the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa 2001, by 30 September 1981. An information sheet relating to this post is available from the above addresses. (9305)A

UNIVERSITY OF NAIROBI Kenya

Applications are invited for

2 SENIOR LECTURESHIPS IN THE

DEPARTMENT OF BOTANY

in any of the following ecological disciplines: Terrestrial Ecology, Marine Ecology, Aquatic Ecology Applicants must have a PhD with considerable teaching and research experience.

The appointees will be expected to participate in the teaching of both undergraduate and postgraduate courses and to develop research programmes in their areas of specialisation. Salary scale: K£3,300 — K£4,950 pa. (K£1 = £1.19 sterling). It is unlikely that the British Government will provide salary supplementation and associated benefits. FSSU. Non-contributory medical scheme; subsidised housing or housing allowance; family passages.

Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent to the Registrar (Recruitment and Training), University of Nairobi, PO Box 30197, Nairobi, Kenya, to arrive no later than 15 September 1981. Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London W1P ODT. Further details are available from either address.

(9308)A

UNIVERSITY OF ZIMBABWE

Applications are invited for these posts:

SENIOR LECTURESHIP/ LECTURESHIPS BIOCHEMISTRY CIVIL ENGINEERING (Structural engineering) FOOD TECHNOLOGY

MATHEMATICS
(Pure or Applied or Engineering
Mathematics)
SCIENCE EDUCATION
CENTRE

Salary Scales: Lecturer Grade II \$7,008 \times 504 - \$9,528 \times \$528 -\$12,168. Lecturer Grade I \$12,720 \times \$528 - \$14,832. Senior Lecturer \$14,040 \times \$528 -\$15,624 \times \$540 - \$18,324. (£1stg = Z\$1.35 approx).

Conditions of Service: Both permanent pensionable terms and short-term contracts are offered for academic posts.

Further Particulars on the above posts, on conditions of service and on method of application should be obtained prior to submitting an application from Director, Appeintments and Personnel, University of Zimbabwe, PO Box MP167, Mount Pleasant, Salisbury, Zimbabwe, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF.

Applications should be submitted by 31 August 1981. (9293)A



Senior Cardiovascular **Pharmacologist** (Haemodynamics)

Haessle is a rapidly expanding research and marketing organisation within Astra Pharmaceuticals, Sweden.

Our activities are internationally oriented and concentrate on the development and marketing of drugs for cardiovascular and gastrointestinal disease. The research organisation currently has a gastrointestinal disease. The research organisation currently disease. The research organisation currently staff of about 300. We now urgently need to fill a new(?) position of Senior Pharmacologist (Section Head) within the department of Cardiovascular Pharmacology (Head: B.AABLAD). The person in this position will be responsible for the section investigating the integrated haemodynamic effects of drugs in experimental animals.

Applicants should hold an advanced degree in science, medicine or veterinary medicine and have appropriate experience in cardiovascular pharmacological or physiological experimental research. Documented ability to conduct independent experimental work on integrated cardiovascular control mechanism will be required. Prior knowledge of Swedish is not necessary.

Cardiovascular research at Haessle is conducted by teams working in close cooperation with internal and external specialists and is concentrated on pharmacodynamic studies in the fields of hypertension, angina, cardiac failure and cardiac arrhythmias. We provide excellent opportunities to develop and maintain international scientific contacts, as studies on basic cardiovascular mechanisms and drug action are conducted in parallel with drug development. Furthermore, we work in very close contact with University based research groups, particularly at the University of Gothenburg.

Haessle laboratories have excellent facilities for experimental work, including computer based control and data acquisition systems for integrated haemodynamic studies. Our laboratories are situated in Molndal, just outside the city of Gothenburg on the west coast of Sweden. The surroundings of the city are noted for their scenic attractions and the area provides ample opportunities for those interested in cultural events or outdoor pursuits at all times of the

For additional information on the position, the company or just life in Sweden, please contact:

or Dr. Nick Boves Astra Pharmaceuticals Ltd. St. Peter's House 2 Bricket Road St. Albans, Herts. phone 0727 33241

Dr. Tony Pottage Haessle Research Laboratories Moindai Sweden

'phone 010-46-31/87 01 20

Applications, including a full curriculum vitae with list of publications and the names of two referees, should be sent before 30th September, 1981 to: AB Haessle, Personnel Department, S-43183 Moindal, Sweden. (9288)A

ASTRA/Haessle.

TECHNICAL EDITOR WRITER - PROOFREADER **BRUSSELS, BELGIUM**

Here is an opportunity for a young scientist to join a small dynamic consulting company where rapid advancement is possible for a real producer. We need a medically oriented science graduate who has excellent communication skills to edit, rewrite and check our reports for English, logic mathematics and accuracy. The ideal age range is 24 to 34 and the location is Brussels, Belgium. The salary will be competitive and will be based upon age, relevant qualifications and experience. We will pay moving expenses. Please send C.V., two references and your telephone number.

ROBERT S. FIRST, INC. 19A Avenue Marnix, 1050 Brussels, Belgium. ≧

The University of Wollongong DEPARTMENT OF BIOLOGY

Lecturer in Ecology

(Limited term appointment of 2 years)

The appointee will be responsible for a third year course in Ecology and will be expected to contribute to other courses in the biology syllabus. Applicants should possess a higher degree and preference may be given to a candidate with postgraduate experience in Ecology. It expected that the appointee should take up the appointment on 1st February, 1982. A tenurable lectureship in this field may be advertised towards the end of 1983. Further information may be obtained from Professor A.D. Brown, Chairman, Department of

Commencing salary, according to qualifications and experience will be in the range \$A19,821 \$A26,037 per annum. Fares to Wollongong for the appointee and dependents will be paid and a relocation allowance is payable. The University will consider contributing to the cost of repatriation on conclusion of the appointment. Applications should contain full details of qualifications, employment history, research interests, publications list, and the names and addresses of three referees and should be forwarded to the University Secretary, the University of Wollongong, Box 1144, PO Wollongong, NSW 2500, Australia. Please mark envelope 'Confidential — Appointment'. Applications close 1st September (9323)A

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY SENIOR RESEARCH **ASSISTANT**

Applications are invited from suitably qualified postdoctoral research staff with particular interests and expertise in plant tissue culture and protoplast techniques, plant virology or molecular biology to work on an SRC-funded project entitled "Virus-cell interactions and the temporal control of gene expression upon infection of tobacco protoplasts with TMV".

The post is tenable for a period of up to 16 months from 1st September, 1981 at an initial salary within the range £6,070 — £6,880 per annum.

Applications, together with the names of three referees, should be received not later than 28th August, 1981, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote (9295)A ref. RV/862/N.

UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for the post of

SENIOR LECTURER/LECTURER IN PHYSIOLOGY

in the Department of Human Biology in the Faculty of Medicine. It is expected that the appointee will share in the teaching of Physiology, undertake research and be actively involved in the National Staff Development Programme. The Department of Human Biology is responsible for teaching basic medical sciences to undergraduate and postgraduate medical and dental students. Courses offered include gross anatomy, histology, embryology, neurosciences, medical physiology, biochemistry and pharmacology, A degree in physiology and or medicine and a higher qualification with teaching experience is required. Teaching and/or research experience in a developing country would be an advantage. Salaries: Senior Lecturer K17,895 pa. Lecturer Grade II K16,045 pa. Lecturer Grade I K14,195 pa. In addition, a clinical allowance of up to K2,000 pa may be payable to registrable medically qualified staff who accept some clinical responsibilities. (£1 sterling = K1.28). Medical graduates have to supply evidence of their qualifications and status for registration by the Papua New Guinea Board.

Details will be sent on application. Three-year contract; gratuity; support for approved research; rentfree accommodation; family passages; baggage allowance; leave fares after 18 months service; education subsidies; salary continuation scheme to cover extended illness or disability.

Applicants who wish to arrange secondment from their home institutions will be welcomed. Detailed applications (2 copies), including a curriculum vitae, a recent including a curriculum vitae, a recent small photograph and naming 3 referees, should be sent to the Assistant Secretary (Staffing), University of Papue New Guinea, Box 4820, University PO, Papua New Guinea to arrive no later than 16 September 1981. Applicants resident in UK should also send I copy to the m UK should also send I copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London W1P 0DT. Further details are available from either address.

Please mention

nature

when replying to these advertisements

UNIVERSITY COLLEGE **CARDIFF**

DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for an SRC-Sponsored Postdoctoral appointment for work on Bacterial Alcohol Dehydrogenase Enzymes. The appointment will be for 3 years and the appointee will join Professor K S Dodgson's research group in Studies on the Biodegradation of Surfactants. Salary within R & A Staff Range IA £6,070 — £6,880 pa. Duties to commence 1st October or as soon as possible.

Applications, (two copies), together with the names and addresses of two referees should be to the Vice-Principal (Administration) and Registrar, University College, PO Box 78, Cardiff CF1 1XL, from whom further particulars can be obtained. Closing date 10th September 1981. Ref: 2262. (9291)A

UNIVERSITY OF WESTERN AUSTRALIA Perth

BOTANY

Applications are invited for appointment as

RESEARCH OFFICER/RESEARCH FELLOW (TEMPORARY) IN

SEAGRASS ECOLOGY

in the Department of Botany. This appointment, for twelve months in the first instance, with the prospect of continuation for a further twelve months, will be to study the ecology of seagrasses in Shark Bay, Western Australia and will be required to give attention to plant production and leaf turnover rates, at specific sites, and from these assess the significance of the seagrasses to carbon, nitrogen and phosphorus cycling in the system. The appointee will join a small interdisciplinary team working especially on carbon flux, and concerned with water movement, grazing, community structure, and contributions to sedimentation. Applicants must have a relevant degree and practical experience. A higher degree is necessary for appointment at Research Fellow level. Further information may be obtained from the Head of the Department of Botany.

Current salary ranges: Research Officer — \$A14,351 — \$A18,134 per annum; Research Fellow — \$A17,083 — \$A19,821 per annum. Some financial assistance may be available for fares and other appointment expenses.

Applications in duplicate stating full personal particulars, qualifica-tions, experience and earliest possible starting date should reach the Acting Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009 by 19th September 1981. Candidates should request two referees to write immediately to the Acting Staffing Officer. (9317)A

THE UNIVERSITY **OF LEEDS DEPARTMENT OF PLANT SCIENCES**

Applications are invited for a post of **RESEARCH FELLOW**

in Cell Recognition to join an established research group in the above Department concerned with gamete recognition systems in algae. The work will involve isolation and characterization of fertilization receptors. The appointment will be made for a fixed period of up to one year and six months. Applicants should have, or shortly expect to receive, a PhD, preferably in the area of cell biology or bio chemistry (plants or animals).

Salary within the range £6,070 to £7,290 on the IA scale for Research and Analogous Staff (£6,070 to £10,575)

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 53/18/D. Closing date for applications 3rd September 1981. (9331)A

LINCOLN COLLEGE Canterbury, New Zealand

LECTURER/SENIOR LECTURER IN ANIMAL **SCIENCE**

(Growth and Development)

The Council of Lincoln College, University College of Agriculture, Canterbury, New Zealand, invites applications for appointment to the position of Lecturer/Senior Lecturer in Animal Science (Growth and Development) in the Animal Science Department within the Animal Sciences Group.

Applicants should hold a degree in Agricultural Science or its equivalent and postgraduate experience in the field of growth and development in animals. The appointee will be expected to contribute to the teaching programme of animal science to students at degree and postgraduate levels, and to contribute to the research programmes of the department in the fields of animal nutrition and disease and sheep

The successful candidate will be appointed as Lecturer or Senior Lecturer at a salary commensurate with his/her qualifications and experience. Current academic salary scales are: Lecturer NZ\$19,140 — \$23,520; Senior Lecturer NZ\$24,110 \$27,589 Bar — NZ\$30,035.

Travel and removal expenses will be reimbursed to specific limits. New Zealand Government Superannuation will be available.

Conditions of Appointment giving further details of this position may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H0PF, or from the Registrar of the College.

Applications close on 16 October 1981. (9269)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

RESEARCH SCHOOL OF EARTH SCIENCES

Fellow in Mineral Physics

An appointment is sought in the Petrophysics Group in the field of mineral physics, specifically in elastic and anelastic properties of rocks and minerals. The successful applicant will be expected to be responsible for research in this area and in particular to develop high pressure, high temperature experimental studies relevant to the velocities and attenuation of seismic waves in rocks. Previous experience is essential. The laboratory is well-equipped for work at ultrasonic frequencies and equipment is being built for seismic frequencies

CLOSING DATE: 30 September 1981.

RESEARCH SCHOOL OF PHYSICAL SCIENCES

Professorial Fellow in Nuclear Physics

The Department of Nuclear Physics carries out work in nuclear structure physics and has an academic staff of 18 headed by Professor J.O. Newton, FAA. At present the principal fields of study are heavy ion included Coulomb excitation and reactions, including fusion, fission and transfer, and high angular momentum phenomena through heavy ion, xn reactions. The major accelerator is the 14UD pellertron tandem (14 MV terminal). An Enge splitpole spectrometer, equipped with focal plane detector, is available. The successful candidate will be expected to provide leadership in the first of these fields. Appointment will take effect from early 1982. CLOSING DATE: 30 September 1981.

Senior Fellow/Fellow in **Astrophysics**

The Mount Stromlo and Siding Spring Observatories (Director: Professor D.S. Mathewson) has its main lines of research on the evolutionary problems in global clusters, the interstellar medium, galactic structure, the Magellanic Clouds, external galaxies and radio sources. Applicants should be in the field of observational extragalactic astronomy. Appointment will take effect from early 1982. CLOSING DATE: 31 August 1981, or as soon as possible thereafter.

Postdoctoral Fellows

A number of Postdoctoral Fellowships are available in the Research School of Physical Sciences. In addition to the above, the School has Departments of Applied Mathematics, Engineering Physics, Mathematics, Solid State Physics, Theoretical Physics and the Plasma Research Laboratory and the Atomic and Molecular Physics Laboratories, the latter comprises three units — the Diffusion Research Unit, the Electron and Ion Diffusion Unit and the Ultraviolet Physics Unit. Applicants should hold, or expect to have completed by mid-1982, a PhD degree or equivalent qualification and normally be under the age of 30 at the time of application. Appointments will take effect from mid-1982 and will be for two years only. CLOSING DATE: 30 September 1981.

Salary on appointment will be in accordance with qualifications and experience within the ranges: Professorial Fellow \$A38562 p.a; Senior Fellow \$A30994-\$36125 p.a; Fellow \$A23104-\$30952 p.a; Postdoctoral Fellow — a fixed point within the range \$A19821-\$25871 p.a. Present exchange rates are: \$A1 = £Stg.0.60; \$US1.14.

Appointment, unless otherwise stated, will be: Professorial Fellow immediately to retiring age; Senior Fellow/Fellow initially for 5 years with the possibility after reviewing of reappointment to retiring age.

Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation benefits are available for applicants eligible to contribute. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Registrar, PO Box 4, Canberra ACT 2600, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF before submitting applications.

(9318)A

Drug Discovery GRADUATE BIOCHEMIST

Hoechst, one of the largest pharmaceutical companies in the world, seeks a Biochemist to work in its modern research laboratories at Milton Keynes.

The position will be suitable for a graduate in biochemistry with an interest in the biological evaluation of compounds and, ideally, possessing 2-3 years industrial experience associated with the use of in vitro biochemical procedures

The person, male or female, whom we select will have the opportunity to play an important role in a multi-disciplinary team of biochemists. pharmacologists and synthetic chemists seeking to identify potential drugs with new modes of action.

Salaries and conditions are of the high order to be expected from a world leader and include a contributory pension scheme with free life assurance. free membership of PPP, subsidised staff restaurant, flexitime working and a generous allowance for relocation where appropriate.

Accommodation for rent or for purchase is available in the new town of Milton Keynes.

Please write or telephone for an application form to: Adrian Forrest. Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

Hoechst



(9334)A

Southampton THE UNIVERSITY

DEPARTMENT OF CHEMISTRY **POST DOCTORAL RESEARCH FELLOW**

Applications are invited for two Post Doctoral Research Fellow posts commencing 1 October, 1981 on two year contracts. The first (ref PP1) will involve work on the synthesis of anti microbial dolabellane natural products, the post will also involve some supervision of research students. The second post (ref PP2) will involve work on the synthesis of quassinoids as potential anti cancer agents.

Starting salaries in the range £6,070 to £6,880 per annum plus USS benefits

Applications (2 copies) with curriculum vitae and the names of 2 referees should be sent as soon as possible to Dr P Parsons, Department of Chemistry, The University, Highfield, South-ampton SO9 5NH. Please quote ref:N (9337)A

MICHIGAN STATE UNIVERSITY **POSTDOCTORAL RESEARCH POSITIONS** ENVIRONMENTAL CARCINOGENESIS-MUTAGENESIS

Two positions available immediately (as well as two later) for persons with strong background in molecular biology, biochemistry, biophysics or microbiology to investigate in diploid human cells in culture the molecular mechanisms of radiation and chemical carcinogen-induced mutagenesis and oncogenic transformation. The multidisciplinary approaches used include comparative studies in cells which differ in DNA repair capabilities; determination of the nature of the DNA lesions responsible for the biological effects; gene isolation, cloning and sequencing; and site specific mutagenesis.

Send application, including curriculum vitae, reprints, and letters of recommendation to: Drs. J. Justin McCormick or Veronica M. Maher, Co-Directors Carcinogenesis Laboratory-Fee Hall, Michigan State University, East Lansing, MI 48824. MSU is an Affirmative Action/Equal Opport-(NW812)A unity Institution.

THE UNIVERSITY OF LEEDS COMPUTER SIMULATION OF **COLLOIDAL DISPERSIONS**

Applications are invited for a post of POSTDOCTORAL RESEARCH **FELLOW**

in the Procter Department of Food Science for work on an SERC funded project on the simulation of particle dynamics in concentrated colloidal dispersions. A PhD. together with experience in computer simulation and/or statistical mechanics is required. Previous experience in colloid chemistry is desirable but not essential. The post is available from 1 October 1981 for a fixed period of up to two

Salary in the range £6,070 — £7,700 on IA scale for Research and Analogous Staff (£6,070 -£10,575), according to age, qualifications and experience.

Informal enquiries may be made to Dr E Dickinson (0532 31751 ext

Application forms and further parriculars may be obtained from the Registrar, The University, Leeds LS2 0JT, quoting reference number 72/8/D. Closing date for applications 4 September 1981

(9319)A

THE AUSTRALIAN

NATIONAL UNIVERSITY THE JOHN CURTIN SCHOOL OF MEDICAL RESEARCH DEPARTMENT OF CLINICAL SCIENCE SENIOR RESEARCH FELLOWS OR RESEARCH FELLOWS

(3 posts) Three new positions are offered in the developing research areas of intestinal inflammation and the molecular basis of tissue injury in human beings. Proven ability in original research is essential and experience in one of the following areas will be an advantage: (1) regulation of secretion and activity of neutral proteases from inflammatory cells; (2) cell-mediated aspects of mucosal immunity; (3) integration of hormonal control of intestinal motility.

Appointment: two years initially, with possible extension to maximum of five years. Salary in accordance with qualifications and experience within the range: Senior Research Fellow \$A27,684 — \$A33,021 pa; Research Fellow \$A19,821 — \$A25,871 pa. Salary loading of up to \$A5,000 may be paid for appropriate medical qualifications. Current exchange rate: \$A1 = £Stg.0.60.

Reasonable appointment expenses paid, superannuation scheme and assistance with finding accommodation.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Further information from Registrar of the University, PO Box 4. Canberra ACT 2600, Australia, or from the Association of Common-wealth Universities (Appts), 36 Gordon Square, London WC1H

Applications to the Registrar by 30 September 1981. (9340)A

UNIVERSITY OF BRISTOL

DEPARTMENT OF ANATOMY Applications are invited from a **GRADUATE**

in biomedical sciences for the post of Research Assistant, Department of Anatomy. The successful applicant would join a small research team investigating the role of the nervous system in birth. No previous experience is necessary but a basic knowledge of nervous physiology would be beneficial. The post is supported by the Medical Research Council for three years. Starting salary £5,285 per annum.

Applications, including cv and names of two referees, should be sent to Dr A J S Summerlee, School of Veterinary Science, Park Row, Bristol BS1 5LS by 21st August, 1981. For further information please telephone Bristol (0272) 24161 ext 616, mornings only.

THE ROYAL SOCIETY GUEST RESEARCH FELLOWS

The Council of the Royal Society invites applications for the support of Guest Research Fellows. The object of this scheme, established in 1980, is to assist outstanding leaders in research in the United Kingdom to invite guests of proven ability from overseas to come and work with them for periods of at least four months and not exceeding two years. The aim is to meet the research needs of an individual through international collaboration and the emphasis in selection will be on the abilities and field of research of applicants.

Applicants (who must be working in laboratories in United Kingdom) should provide:

(a) An outline in not more than 1000 words of their present research and their proposals for developing it, together with a brief curriculum vitae (including date of birth and nationality) and list of their last six major publications.

(b) A statement explaining why their research would be significantly assisted by the presence of a Guest Research Fellow or Fellows. Initially it is not essential for applicants to name the person it is proposed to invite, but they should state clearly why international collaboration is needed. This may or may not involve identifying the laboratory from which the Guest Research Fellow will come

(c) The proposed dates of the visit with an estimate of costs and an indication of likely support from other sources. Royal Society support may include a basic maintenance allowance related to university lecturer scales, one return air fare and an additional one if the visit is for a full academic year or longer, and other expenses such as local travel and special research expenses.

(d) The names of two referees external to each applicant's group.

Applications should be made by letter addressed to the Executive Secretary (CRF/UMAM), The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and be received not later than 30 September 1981.

(9301)A

FRESHWATER BIOLOGICAL ASSOCIATION BIOMETRICIAN/ STATISTICIAN

required for Windermere Laboratory to initiate research in biometry as applied to pupulation dynamics of benthos, plankton and fish, sampling freshwater organisms and chemical/physical variables and experimental studies in collaboration with biologists and chemists. Minimum of 2.1 honours degree in statistics/biometry/mathematics or science degree plus statistical training or experience. Conditions analogous to HSO/SSO in Civil Service, starting salary between £6,075 and £7,644 pa.

Full details: Secretary, FBA, The Ferry House, Ambleside, Cumbria LA22 0LP (096 62 2468). Closes 7 Sept. (9314)A

UNIVERSITY OF PAPUA NEW GUINEA

Applications are invited for the post of

SENIOR LECTURER/ LECTURER IN PHYSICS (Electronics and General Physics)

tenable from January 1982. The appointee will be expected to teach undergraduate courses in electronics and related topics, and to share in teaching introductory Physics. Applicants should preferably have teaching experience in these areas. The Department has a well equipped electronics workshop and has research activity in upper atmosphere physics, agricultural meteorology, thunderstorms and geomagnetism An interest and experience of one of these, while not essential, will be considered and advantage.

Salaries: Senior Lecturer K17,895 pa. Lecturer Grade II K16,045 pa. Lecturer I K14,195 pa. (£1 sterling = K1.28). Three-year contract; gratuity; support for approved research; rent-free accommodation; family passages; baggage allowance; leave fares after 18 months service; education subsidies; salary continuation scheme to cover extended illness or disability.

Applicants who wish to arrange secondment from their home institutions will be welcomed. Detailed applications (2 copies), including a curriculum vitae, a recent small photograph and naming 3 referees, should be sent to the Assistant Secretary (Staffing), University of Papua New Guinea, Box 4820, University PO, Papua New Guinea to arrive no later than 15th September 1981. Applicants resident in UK should also send 1 copy to the Committee for International Cooperation in Higher Education, The British Council, Higher Education Division, 90/91 Tottenham Court Road, London W1P 0DT. Further details are available from either address.

(9309)A

MANCHESTER AREA HEALTH AUTHORITY (TEACHING) South District CHRISTIE HOSPITAL AND

CHRISTIE HOSPITAL AND HOLT RADIUM INSTITUTE JUNIOR B TECHNICIAN

to work on the measurement of steroid hormone receptors in breast cancer. The successful applicant will join a team studying this disease and will be responsible for the routine assay of clinical samples. Previous experience in steroid receptor analysis or radioimmunoassay would be an advantage but is not essential.

Salary dependent upon qualifications and experience. Day release for further study would be granted.

For an application form please apply to the Sector Administrator, Christie Hospital and Holt Radium Institute, Wilmslow Road, Withington, Manchester M20 9BX. Tel: 061-445 8123 ext 206. Closing date: 4th September, 1981.

(9313)A

Synthetic Organic Chemist

Carbohydrate Research

This is an outstanding career opportunity for an innovative and highly self-motivated research specialist at the newly established Lord Zuckerman Research Centre, set-up by Cadbury Schweppes on the campus of the University of Reading, one of Europe's leading centres of food science and technology.

The Centre, a major investment vital to the company's future growth, is responsible for fundamental research into projects relevant to Cadbury Schweppes' business as a whole and for new projects which fall outside the scope of the operating Divisions

The successful man or woman will undertake a major project and will be given every opportunity to develop and progress, both personally and professionally.

Applicants should have a good first degree in chemistry, ideally followed by a PhD, plus at least 5 years' proven experience in synthetic carbohydrate chemistry.

The salary and benefits are extremely competitive and will reflect the importance we attach to this position.



Please write with brief personal and career details to Mrs P M Carvosso, Personnei Department, Cadbury Schweppes Limited, Leconfield House, Carzon Street, London WIV 7FP. (9335)A

Cadbury Schweppes

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY TECHNICIANS

required to work in the research group under the direction of Professor G Burnstock

Grade 3: required to work on a three-year grant-supported project involving several techniques. Experience in biochemistry, histology, electrophysiology or electronmicroscopy would be advantageous. Starting salary £4,933 pa plus London Weighting £1,016. Quote reference FB.5.

Grade 6: with experience in biochemical assays to work in established techniques for the estimation of neurotransmitters and related enzymes and to take part in the development of new assays. Will be required to assist in the routine maintenance of a neurochemistry laboratory. This post is grant-supported and is for a period of one year in the first instance with the possibility of renewal. Starting salary £6,532 pa plus London Weighting £1,016. Quote reference FB.6.

Applications, together with curriculum vitae and the names of two referees to Personnel Officer (Technical Staff), University College London, Gower Street, London WC1E 6BT. (9292)A

THE UNIVERSITY OF SHEFFIELD CASE STUDENTSHIP IN THE DEPARTMENT OF PHYSICS

Applications are invited from graduates with a Class I or III honours degree in physics, materials science or electronic engineering, for a SERC CASE award in collaboration with AERE, Harwell, to study ion implantation in amorphous silicon and related The successful materials. candidate will join an established group in the field of amorphous semiconductors especially interested in the study of photo and electro-luminescence, using laser and related opto-electronic techniques. The objective is to clarify the relationships of impurity and defect states in amorphous materials. The group collaborates actively with a number of workers in the device field, interested, for example, in solar cells and large area displays. The CASE project will involve a wide range of solid state techniques with emphasis on the physics of these materials and should lead to a PhD. Starting date: 1 October 1981, Stipend: SERC postgraduate rate plus about £600 p.a. Apply to Dr T.M. Searle/I.G. Austin with cv, Department of Physics, the University, Sheffield S102TN, Quote ref R623/G, (9332)F



Shell has an immediate opportunity for a director of clinical research as the Cetus-Shell Interferon Program moves into clinical testing. This director will have overall responsibility for developing strategy, and for conducting and managing clinical studies on interferons. The clinical director will also provide advice and recommendations to the Shell and Cetus managements on all aspects of the program, including the development of *in vitro* tests for product efficacy.

Requirements: M.D. with experience in Phase I, Phase II drug development. Background in oncology/virology is advantageous.

Letter of application and curriculum vitae should be sent in confidence to: R&D Director—Interferon, Shell Oil Company, 901 Grayson, Berkeley, California 94710. (NW805)A

Equal Opportunity Employer M/F

UNIVERSITY OF OKLAHOMA

DEPARTMENT OF CHEMISTRY

CRYSTALLOGRAPHER

Applications are invited for this permanent, non-tenure track staff position to operate and maintain x-ray diffractometer (nonity cad-4) with primary responsibility for performing single-crystal x-ray structure determinations for chemistry research groups.

Salary commensurate with experience (18,000 dollars - 20,000 dollars per year). Successful applicant should have PhD or equivalent in chemical crystallography, experience in structure determination using single crystal x-ray diffraction, ability to operate and maintain an diffractometer, automatic knowledge in diffractometry and diffraction equipment, experience with mini-computer software applicable to automatic diffractometers and in writing analytical software for larger computers, ability to communicate with students and faculty about crystallographic problems, and experience in independent research, research publication in x-ray diffraction and in writing for research publications.

Positions available immediately. Send résumé and three letters of recommendation to chairman, Crystallographic Search Committee, Department of Chemistry, University of Oklahoma, 620 Parrington Oval, Norman, Oklahoma 73019 by October 1, 1981.

An affirmative action/equal opportunity employer. (NW814)A

UNIVERSITY OF MUNICH DEPARTMENT OF ZOOLOGY

C2 PROFESSOR IN DEVELOPMENTAL BIOLOGY

Applicants should have PhD, post-doctoral research and teaching experience (Habilitation or equivalent) and be fluent in German. Research interests should involve genetic or biochemical aspects of cell differentiation and pattern formation in lower eucaryotes.

Send curriculum vitae, list of publications, a short description of research interests and the names of 3 references before September 30, 1981, to the Dean, Faculty of Biology, University of Munich, Zoology Department, Luisenstraße 14, D-8000 München 2.

(W399)A

UNIVERSITY COLLEGE CARDIFF DEPARTMENT OF

ANATOMY BURSARY

Applications are invited from UK graduates for a Bursary in the above Department tenable in the first instance for one year but renewable up to a total of three years. While researching within the field of the Anatomical Sciences the incumbent would have the opportunity to work for a higher degree. Index-linked stipend of £2,460 includes a fee for limited teaching duties.

Applications, together with the names and addresses of three referees, should be forwarded to Professor J D Lever, Department of Anatomy, University College, PO Box 78, Cardiff CF1 1XL by 28th August 1981. Ref: 2260. (9339)A

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL TUBERCULOSIS RESEARCH INSTITUTE

(Pretoria, South Africa)

Applications are invited from suitably qualified persons for the following two posts:

POST 1: MICROBIOLOGIST/ IMMUNOLOGIST

A vacancy exists for a Chief Research Officer to participate in research with a multi-disciplinary team consisting of bacteriologists, epidemiologists, a statistician and consulting clinicians and radiologists.

Requirements: A PhD or DSc, experience in medical microbilogy or immunology will be a recommendation

POST 2: BIOLOGIST/DATA ANALYST

A Research Officer is required to participate in and later manage projects in connection with health surveys, disease risks and the analysis of data obtained from medical and paramedical services.

Requirements: Msc, BSc (Hons) graduate or BSc with experience in one of the biological fields such as microbiology, physiology, zoology, etc with a knowledge of data analysis. Computer programming ability would be useful (but is not essential).

Further particulars and application forms are obtainable from the South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE. Tel: 242 1766.

General: The MRC is nationally responsible for medical research in South Africa in terms of an Act of Parliament. Its head office is near Cape Town with research institutes and laboratories situated throughout the country.

The MRC offers a five-day week, an academic environment, excellent opportunities for research and further study, group life insurance scheme, pension and medical schemes, a service bonus, generous vacation and sick leave and a housing subsidy for those who qualify.

(9303)A

UNIVERSITY OF EAST ANGLIA Norwich POSTDOCTORAL SENIOR RESEARCH ASSOCIATE

required in the School of Biological Sciences to investigate genetics of mating recognition by song within and between sibling species of grasshopper and its relation to allozyme differences. An interest in quantitative genetics is desirable but not essential. The appointment is supported by a 3 year SERC grant and salary in the range £6,880 — £7,700 per annum.

Applications including a curriculum vitae and naming two referees should be sent to Dr G M Hewitt, Biological Sciences, UEA, Norwich NR4 7TJ by 28th August, 1981. (9342)A

UNIVERSITY OF FORT HARE Republic of South Africa PROFESSOR AND HEAD OF THE DEPARTMENT OF

MICROBIOLOGY
Applications are invited from suitably qualified candidates for the above post.

Minimum requirements: A Doctorate and preferably teaching experience. Candidates must be able to lecture through the medium of English.

Assumption of duty: As soon as possible. Closing date for applications: 4th September 1981.

General: The University of Fort Hare is situated near Alice in the Ciskei. Persons who are not South African Citizens can only be appointed on contract basis, which is usually for an initial period of three years renewable thereafter for further three year periods.

Salary: Professor — R17,100 × R750 — R18,600 × R900 — R22,200.

Additional Fringe Benefits: The following benefits are available subject to certain conditions: Group life assurance; medical aid; pension scheme; service bonus; territorial allowance; generous leave and sick leave benefits and generous assistance towards university training of employees' children in the Republic of South Africa.

Applications including a complete curriculum vitae and the names and addresses of at least three referees, should be addressed to: The Staff Officer, University of Fort Hare, Private Bag X1314, Alice 5700, Republic of South Africa. Telephone: 281 Alice, Telegraphic address: Unifort Alice. (9302)A

POST DOCTORAL POSITION

To study structure/function relationships in contractile proteins. Experience in protein modification and characterisation desirable.

Send curriculum vitae and two letters of reference to Dr M Burke, Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106. (NW813)A



UNIVERSITY OF THE WITWATERSRAND Johannesburg DEPARTMENT OF BOTANY SENIOR LECTURER/ LECTURER IN PLANT **PHYSIOLOGY**

Applications are invited from suitably qualified persons, regardless of sex, race, colour or national origin, for appointment to the above

Preference will be given to candidates with specialist interests in one or more of the following areas; physiology of growth substances and plant growth and development; ecophysiology; and plant water relations, as well as a good general botanical background in order to participate in the first year teaching programme.

Duties are to be assumed on 1st Feburary 1982, or as soon as possible thereafter.

The salary attached to the appointment will be in the ranges: Senior Lecturer: R14,370 — R20,850 pa. Lecturer: R10,995 — R19,230 pa.

The initial salary and level of appointment will be determined according to the qualifications and experience of the successful candidate. An annual service bonus in accordance with existing Government regulations is also payable.

Interested persons are invited to obtain the information sheet relating to this post from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WCIV 7HE, or from the Registrar (Staffing), University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa 2001, with whom applications should be lodged by 30 September 1981. (9306)A

UNIVERSITY OF LONDON Institute of Neurology **DEPARTMENT OF** NEUROCHEMISTRY POST-DOCTORAL WORKER (Grade 1A)

required in a team working on metabolism and role of 5-HT (and related topics) and supported until July 1984 by MRC programme grant. Experience in at least one of the following fields essential: neurochemistry, neuropharmacology, behaviour. The successful applicant will mainly study the effects of hormonal and nutritional status on transmitter metabolism and on behaviours due to transmitter release. Initial salary on scale £7,037
— £11,542 including London Allowance.

Applications with CV and names of two referees to Professor G Curzon, 33 John's Mews, London WC1, by 15 September 1981

(9325)A

THE LINNEAN SOCIETY OF LONDON

PLANT TAXONOMIST

Applications are invited for the post of Plant Taxonomist to undertake the compilation of a critical catalogue, for eventual publication, of the type specimens of Linnaean plant species. The work, which will result in a much needed source of reference for taxonomic botanists throughout the world, will provide an exceptional training opportunity in formal taxonomy and nomenclature.

The project is supported by a grant from the SRC and will be based in the Department of Botany, British Museum (Natural History), under the supervision of Professor W T Stearn and Dr N K B Robson. Employment will be on the basis of a three-year contract, with the possibility of further extension.

Starting salary will be £6,986 (under review) plus £1,016 London Weighting, and work will commence at the earliest possible date after 1st October 1981. The successful candidate will probably have a degree in Botany or in some related field, but graduate qualifications are not essential. Practical experience as a taxonomist dealing with day to day problems in nomenclature would be a special recommendation. Background experience in librarianship and bibliography might also be

Applications with full curriculum vitae and two references should be sent by 7th September 1981 to: The Executive Secretary, The Linnean Society of London, Burlington House, Piccadilly, London WIV OLQ. Further information on the project can be obtained from Dr Robson or Mr Cannon at the British Museum (Natural History). Tel: 01-589 6323. (9329)A 01-589 6323.

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS **POST GRADUATE** RESEARCH ASSISTANT

Applications are invited for this MRC-funded post tenable for 3 years from 1 October, 1981 to work on the biochemical and genetic analysis of in vitro genetic recombination in bacterial viruses under the supervision of Professor D A Ritchie. Candidates should have a good Honours degree in Biochemistry, Genetics or Microbiology and an interest in microbial genetics. There will be an opportunity to register for a higher degree.

Initial salary £5,285 per annum.

Applications, together with the names of three referees, should be received as soon as possible. The Registrar, The University, PO Box , Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/860. (9290)A

Graduate Chemist

£8,959 - £9,811

for the New River Head Laboratories of the Directorate of Scientific Services. You will be concerned with the analysis of trace organic compounds in water and you should have proven experience of organic analysis in general and of modern chromatography and related techniques in particular. Experience in gas chromatography/mass spectrometry would be an advantage.

Much of the work to be carried out forms part of the Authority's research programme and together with other duties, will be under the direction of the Manager, Metropolitan Water Services.

The post is open to men and women and application forms may be obtained from the Regional Manager (Manpower), New River Head, London EC1R 4TP telephone 01-837 3300 ext 2024. (9307

Thames Water

DARTMOUTH MEDICAL **SCHOOL**

Hanover, New Hampshire **IMMUNOLOGY PROGRAM** POSTDOCTORAL RESEARCH ASSOCIATE

Two postdoctoral positions are open as of January-July, 1982 for research on T-cell growth factor and the regulation of T- and B-cell function. Experience in cellular immunology, long-term culture of T-cells, biochemistry, and/or hormone-receptor interaction is desirable. The appointment will be for a minimum of two years. Salary is negotiable depending on experience.

Send curriculum vitae and two letters of reference to: Dr Kendall A Smith, The Basel Institute for Immunology, 487 Grenzacherstrasse, CH 4058 Basel, Switzerland.

An Equal Opportunity/Affirmative Action Émployer. (NW807)A

UNIVERSITY OF BRADFORD RESEARCH ASSISTANT in Chemical Engineering (fixed-term appointment -3 years)

Applications are invited from graduates in Science, Electrical Engineering or Chemical Engineering to work as part of a team on a project concerned with liquid separation in electrostatic fields. The appointment is supported by the SERC. Opportunity to register for a higher degree. Starting salary, £5,285 pa. Superannuable.

Further particulars and application forms are available from the Personnel Secretary, University of Bradford, West Yorkshire BD7 1DP. (Ref: RA/CHE/SERC/3/N). Informal enquiries to Dr P J Bailes, School of Chemical Engineering (9312)A

HILL FARMING RESEARCH OFGANISATION

Scientific Officer/Higher Scientific Officer

Applications are invited for a Scientific Officer/Higher Scientific Officer to join a research group working on the nutrition of hill and upland beef

The successful applicant will be expected to take up an innovative role within a small group working in the Beef Production Section. This research programme is largely concerned with the effects of nutrition on the components of cattle production. There will be opportunities for studies of both juvenile and adult animals, to engage in metabolic investigations and to become involved in work on systems research.

Qualifications: First or upper second class honours degree or equivalent in a relevant branch of science, preferably including Ruminant Nutrition or a related subject and some relevant post qualifying experience. At least two years relevant post qualifying experience required for

Salary: SCIENTIFIC OFFICER, according to qualifications within the scale £4,809 to £6,480.

HIGHER SCIENTIFIC OFFICER, commencing £6,075. The scale is from £6,075 to £7,999.

The salary scales are at present under review. Other Conditions: Non contributory Superannuation Scheme.

Scientific Officer – 20 days annual leave
Higher Scientific Officer – 22 days annual leave
Further particulars and application forms from the Secretary,
Hill Farming Research Organisation, Bush Estate, Penicuik,
Midlothian EH26 OPY to whom they should be returned not later than 11th September 1981.

M.R.C. CLINICAL RESEARCH CENTRE (NORTHWICK PARK HOSPITAL) **Watford Road** Harrow Middx HA13UJ

Senior Technician

On 1st October 1981, following a retirement, a vacancy will arise for the post of Senior Technician in the Division of Anaesthesia. Apart from the administrative responsibilities of Senior Technician; the successful applicant will be expected to participate in some aspects of the research work, which is broadly based within the field of anaesthesia and includes hyperbaric studies.

Applicants should have substantial experience in physics, electronics, neurophysiology or neurochemistry and be qualified to HNC/degree level or equivalent in an appropriate subject.

Salary/£6,656 to £8,622 inc L.W. dependant upon qualifications and

For application forms and further details, please write to Miss J. Cottam Personnel Section, quoting ref 101/2/3835.

Closing date 3 weeks from appearance of this advertisement.

(9297)A

IMMUNOLOGIST/ IMMUNOCHEMIST

Staff research position available for experienced researcher with PhD or MD interested in applying new technologies to the study of human disease

Experience with monoclonal antibody production and isolation of cellular antigens is desirable. Send CV and references to:

Dr. Robert Colvin, Immunopathology - Cox 5, Mass. General Hospital, Boston, MA 02114. (NW798)A

POST DOCTORAL RESEARCH ASSOCIATE **POSITION**

Available immediatly for candidate with a demonstrated interest and technological expertise developmental and molecular biology. Experience in hematopoietic stem cell growth in vitro desirable. Excellent research opportunity in the area of haemoglobin synthesis in man and baboon model systems. Salary negotiable and competitive.

Candidates would be employee of the University of Illinois. Career posibilities. Send curriculum vitae and names of three referees to: Doctor Joseph De Simone, Haemotology Research Laboratory, Veterans Administration, West Side Medical Centre, 820 South Danien Avenue, Chicago, Illinois 60612. An Affirmative Action/Equal Opport-(NW809)A unity Employer.

UNIVERSITY OF CAPE TOWN

Junior Lecturer in Geology

Applications are invited for the above post, vacant as from 1st January 1982. Appointment, according to qualifications and experience, will be made in the salary range R6 810 — R8 970 per annum.

The successful candidate will be required to give lectures and practicals carrying a total contact load of approximately 100 hours, either spread throughout the year or concentrated in one semester. The position is suitable for a graduate who wishes to engage in independent research in the department, or for a recent graduate wishing to register in the department towards a higher degree. Appointment is made on a year-to-year basis, and is normally renewed annually.

While candidates with special interests in any of the subdisciplines of geology will be considered, and selection will be on merit, preference will be given to candidates with experience and interest in sedimentology and/or the economic geology of sedimentary deposits.

Applicants should submit a curriculum vitae, stating present salary, research interests and publications, when available if appointed, and the names and addresses of three referees.

Further information should be obtained from the Head, Department of Geology, University of Cape Town, Rondebosch, 7700. Closing date 12th October 1981 but late applications may be accepted. Please quote reference number (AC.7).

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable from the Registrar. (W400)A

UNIVERSITY OF FORT HARE Republic of South Africa PROFESSOR/SENIOR LECTURER IN MICROBIOLOGY

Applications are invited from suitably qualified candidates for the above post.

Minimum requirements: Doctorate and preferably teaching experience. Candidates must be able to lecture through the medium of English.

Assumption of duty: As soon as possible. Closing date applications: 25th September 1981.

General: The University of Fort Hare is situated near Alice in the Ciskei. Persons who are not South African Citizens can only be appointed on contract basis, which is usually for an initial period of three years renewable thereafter for further three year periods.

Salary: Professor - R20,040 - $R20.850 \times R900 - R26.250$.

Additional Fringe Benefits: The following benefits are available subject to certain conditions: Group life assurance; medical aid; pension scheme; service bonus; territorial allowance; generous leave and sick leave benefits and generous assistance towards university training of employees' children in the Republic of South Africa.

Applications including a complete curriculum vitae and the names and addresses of at least three referees, should be addressed to: The Staff Officer, University of Fort Hare, Private Bag X1314, Alice 5700, Republic of South Africa. Telephone: 281 Alice, Telegraphic address: Unifort Alice. (9304)A

TECHNICAL AND NATURAL SCIENCES (Technisch-Naturwissenschaftliche Fakultät) of the Johannes Kepler University Linz, Austria the position of a full

IN THE FACULTY OF

PROFESSOR IN BIOPHYSICS

is vacant

The candidate for this post will be expected to devote his research activites to experimental membrane biophysics as his main effort. His teaching should be focussed on courses, which enable graduate students in physics, chemistry, computer sciences and mathematics to acquire knowledge in biophysics. Applicants should be proficient in German.

Applications including a curriculum and a list of publications should be sent before October 30th, 1981, to the dean (Dekan) of the Faculty, Altenbergerstr. 69, A-4040 Linz, Austria. Dean Prof Dr Bruno (W397)A Buchberger.

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) A CHIEF TECHNICIAN

is required to work in the Anatomy Department. The successful candidate will supervise technical services and the technicians in the department and will also be responsible for the Anatomy laboratories. The Department of Anatomy is currently situated in Hunter Street, but will be moving to new accommodation in the Medical School Building at Hampstead in the near future.

Salary on scale £8,159 - £9,385 inclusive (under review). 37 Hour week. 34 days leave including public & customary days. Interest free annual season ticket loan scheme.

Further particulars and application forms are available from the School Secretary, RFHSM, 8 Hunter Street, London WC1N 1BP or tele-phone 01-837 5385 ext 10. Closing date: 28 August 1981. Reference AD/CT. (9321)A

RESEARCH ASSOCIATE

Position available immediately for PhD to work on biochemical and physiological mechanisms in the crystalline lens in relation to cataract development. Experience in membrane or protein bio-chemistry would be most advantageous. Send Curriculum vitae and names of two references to Dr C A Paterson, Box B 205 University of Colorado Health Sciences Center, 4200 E 9th Ave., Denver, Colorado 80262. USA. The University of Colorado is an equal opport-(NW810)A unity employer.

ASSISTANTSHIPS

KING'S COLLEGE LONDON (University of London)

DEPARTMENT OF ANATOMY Applications are invited for a three year

RESEARCH **ASSISTANTSHIP**

from 1st October, 1981, funded by the Arthritis and Rheumatism Council, to investigate the mechanics of the arthritic foot and ankle joint and the influence of progressive pathological changes within the foot and ankle joint on gait. In addition to the appropriate biological background, the person appointed will be expected to have a reasonable knowledge of statistics and computing

Starting salary will be in the rage £5,285 — £6,070, depending on age and experience, plus London Weighting of £967.

Applications including a full curriculum vitae and names and addresses of 2 referees to Dr R W Soames, Department of Anatomy, King's College London, Strand, London WC2R 2LS by 28th August, 1981.

continued on page xvii

ASSISTANTSHIPS

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CERAMICS. GLASSES AND POLYMERS

RESEARCH **ASSISTANTSHIP**

Applications are invited from men and women for the above post to study the process of transformation of nitch-like materials and single aromatic hydrocarbons to carbon by pyrolysis. The process occurs via a liquid crystalline (mesophase) the growth and coalescence of which determines the microstructure of the carbon product. The approach will be to construct pseudo-phase diagrams for the transformation after which the properties of various compositions in the different phase fields will be studied. Emphasis will be placed on the rheological properties. This project, supervised by Dr B. Rand, is financed by SRC. Tenable for 3 years from 1 October 1981 at an initial salary up to £6,880 a year depending on age and experience. An honours degree in a physical science subject and research experience (preferably to PhD level) required. Experience in carbon technology, liquid crystals or polymers rheology would be relevant. Particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications including the names and addresses of two referees, should be sent by 24 August 1981. Quote ref: R 621/G.

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY THREE POSTDOCTORAL RESEARCH ASSISTANTSHIPS

Applications are invited for three postdoctoral research assistantships (supported by SRC) as follows:

- 1. To study stereochemical effects on nuclear spin coupling between directly bonded carbon atoms (for one year);
- 2. To develop and exploit a stopped flow laser polarimeter for heavy atom kinetic isotope effects (for one year);
- 3. To re-examine proximity ('steric') isotope effects (for two years).

Applicants should have experience in one or more of the following:

a) Synthesise with stable isotopes:b) F T nmr (posts 1 and 3);

c) Electronics (post 2).

The appointments are for one year ffrom 1st October 1981, or up to 3 to 6 months later by agreement) with post 3 being renewable for a second year. The starting salary will be in the range £6,070 to £6,880 pa (Research Support Grade 1A) plus USS,

according to age and experience.

Applications (one copy) with curriculum vitae and names and addresses of at least two referees should be sent as soon as possible to Dr M J T Robinson, Dyson Perrins Laboratory, South Parks Road, Oxford OX13QY. (9299)P

STUDENTSHIPS

UNIVERSITY OF LEEDS DEPARTMENT OF PLANT SCIENCES AN SERC (CASE) STUDENTSHIP

in conjunction with International Paint Marine Services is available for a good honours graduate in biology, microbiology or biochemistry. The investigation involves aspects of the biology and metabolism of selected diatom species, in relation to the problem

of tanker fouling.

Interested persons should contact Dr L V Evans, Department of Plant Sciences, The University, Leeds LS29JT by telephone (Leeds 31751 ext 6576), or submit a curriculum vitae to him with the names of two academic referees as soon as possible.

RESEARCH STUDENTSHIP EAST MALLING RESEARCH STATION

Applications are invited from candidates with a first or upper second-class honours degree in biochemistry on plant physiology for a studentship leading to a PhD on hormone regulation of pear fruit development.

Further details of this award, which is tenable for three years from October 1981, are available from the Deputy Secretary, East Malling Research Station, Maidstone, Kent ME196BJ. (9311)F

UNIVERSITY of Aston in Birmingham, Department of Biological Sciences — Fish Culture Unit. Research Studentship. Applications are invited from good Honours graduates in Biology, Biochemistry and Physiology, for a 3-year Research Studentship sponsored by the Tropical Products Institute. The project is for research on "The effects of the protein quality of feed ingredients of tropical origin on the growth and carcase characteristics of Tilapia". The general conditions for the Research Studentship are Analogous to those of the Science Research Council. Application forms are available from The Secretary, Department of Biological Sciences, University of Aston, Gosta Green, Birmingham B4 7ET and must be returned completed by Friday September 1981. (9343)F

ANNOUNCEMENTS

STANFORD UNIVERSITY **ANNOUNCEMENT**

Stanford University, in cooperation with the University of California, announces that patent licenses are now available on a non-exclusive basis for the genetic engineering inventions of Dr Herbert W Boyer of the University of California and Dr Stanley N Cohen of Stanford University (US Patent No 4,237,224 issued December 2, 1980 and pending US Patent Application No 959,288 filed November 9, 1978). gratefully acknowledge the financial support of the American Cancer Society, the National Institutes of Health and the National Science Foundation to the fundamental research programs from which the inventions resulted.

Farned royalties for end products are scaled, based on sales volume, from 1.0% to 0.5% of sales, for annual sales over \$10 million. To encourage companies to license early, a multiple of the minimum annual fees of \$10,000 can be credited against earned royalties. For example, for licenses signed before 15 December 1981, licensees may credit \$50,000 (against earned royalties otherwise payable in a future year) for each \$10,000 annual fee paid before commercial sales begin or 1987, whichever occurs first. Terms for licenses signed after 15 December 1981 will be less favorable to the licensee.

The license also requires that licensees specifically express intent to comply with the physical and biological containment standards of the NIH guidelines for recombinant DNA research.

Pursuant to 19USC337a, patent licenses are applicable to sales in the US of products derived from practice of methods of the patents outside of

The Universities intend to use royalty revenue for educational and research purposes which, in turn, may result in yet other scientific advances for public use and benefit.

For a copy of the license agreement, write to the Office of Technology Licensing, 105 Encina Hall, Stanford University, Hall, Stanford, California 94305.

(NW808)G:

should be sent as soon as possible to Professor J E Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. (9300)P UNIVERSITY

UNIVERSITY

OF OXFORD

DYSON PERRINS (ORGANIC

CHEMISTRY) LABORATORY

POSTDOCTORAL

RESEARCH

ASSISTANTSHIPS

Applications are invited from suitably qualified and experienced

organic chemists for a postdoctoral

research assistantship concerned with

biosynthetic studies of triple bonds.

The appointment, in Grade 1A, supported by SRC would be for two

starting salary up to £6,880 pa

(according to age and experience)

Applications, quoting Reference

R81/2, with curriculum vitae and the

names and addresses of two referees:

years from October 1981,

plus USS.

OF BRISTOL
H H WILLS PHYSICS LABORATORY
POST-DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a threeyear post-doctoral assistantship by the Science Research Council to investigate the isotopic dependence of neutron scattering lengths for a range of elements. This is a collaborative programme with the University of Munich and access to facilities at FRM Reaktorstation Garching will be made available to the successful candidate.

We are seeking a postgraduate with a good background in materials science. Experience in electron microscopy would be particularly useful. A knowledge of low energy nuclear physics or neutron scattering techniques is not required as training in these areas will be given.

The post will provide a good opportunity for a young physicist to broaden his or her experience. The starting salary will be at point 3 (£6,880) on the RA/1B scale, and the position is available from 1st October 1981.

Applications, which should Applications, which should include the names of two referees, should be sent to Professor J E Enderby, H H Wills Physics Laboratory, University of Bristol, Royal Fort, Tyndall Avenue, Bristol BS8 ITL. (9328)P

GRANTS and SCHOLARSHIPS

ARMAGH OBSERVATORY Armagh, Northern Ireland Applications are invited for a **SCHOLARSHIP**

at Armagh Observatory from candidates holding a good honours degree in physics, or astronomy, or a closely related field. Excellent opportunities for working towards a PhD thesis are being offered.

Applications stating qualifications, research interests, and names of two referees should be received not later than September 30, 1981 by the Secretary, Armagh Observatory, Armagh, N Ireland BT61 9DG, from whom further particulars may be obtained. (9298)H

IMPERIAL CANCER RESEARCH FUND Research Studentship (Bursary)

Applications are invited from recent honour graduates for a Bursary in the Transcription Laboratory to study the molecular biology of the DNA tumour virus polyoma.

The award, for full time studies leading to a higher degree (PhD) will be tenable for three years with a non-pensionable grant of £4,140 a year (subject to tax) and in some cases additional allowances. Candidates should have a first or upper second class honours degree in one of the biological sciences if they are UK graduates, but non-U.K. residents are not necessarily excluded. A strong background in molecular biology will be important and some training in recombinant DNA techniques would be particularly desirable.

For further information telephone Dr R. Kamen (01-242 0200 ext 277). Application forms are available from The Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields London WC2. Tel. as above ext 305. Completed application forms should reach the Fund no later than 1st September 1981.

(9294)H

FELLOWSHIPS

MULTIPLE SCLEROSIS RESEARCH TRAINING **FELLOWSHIP**

Tenure 3 years. To work in the Neurovirology Unit, Department of Neurology, St. Thomas' Hospital, London SEI 7EH. On "Mechanisms of pathogenesis of CNS virus diseases with special reference to demyelination". Current research is related to the virology, immunology, pathology, electron-microscopy and biocemical abnormalities associated with these infections. Applicants should apply by October 1, 1981 to Dr H E Webb giving a Curriculum Vitae with photograph and 2 referees. Salary will be by arrangement according to age and experience up to £8,000 a year. Anticipated starting date. (9333)E February 1, 1982.

UNIVERSITY OF WARWICK

POSTDOCTORAL FELLOWSHIP IN **MOLECULAR VIROLOGY**

Applications are invited from microbial geneticists, virologists and other suitably qualified candidates to work in the Department of Biological Sciences on an MRC funded project concerned with the molecular cloning of rotaviruses. Candidates with recombinant DNA experience are particularly welcome to apply. The appointment is for three years and is available immediately.

Salary in the range £6,070 to £10,575 pa. For arther details please write direct to Dr M A McCrae, Department of Biological Sciences.

Written applications should include a curriculum vitae and the names and addresses of two referees to the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref No: 51/A/81/0. Closing date for receipt of applications 28th August, 1981. (9338)E

POSTDOCTORAL FELLOWSHIP IN CARDIAC CELLULAR **ELECTROPHYSIOLOGY**

Two traineeships are available (2-3 years) to study (1) action potential conduction mechanisms and intercellular junctional coupling and (2) membrane ionic conductance mechanisms in isolated cell and tissue cultured model cardiac prepara-tions. Conventional microelectrode methods, voltage clamp and patch-electrode techniques will be employed as well as impedance/ noise analysis. Training will be provided in these techniques and in the preparation and manipulation of isolated cell systems. Support and excellent facilities are provided through an active interdepartmental program project.

Send curriculum vitae and names of three professional references to: Dr R L DeHaan, Department of Anatomy, Emory University School of Medicine, Atlanta, GA 30322. Emory University is an Equal Opportunity/Affirmative Action (NW811)E Employer.

UNIVERSITY OF CAPE TOWN

Research Fellowships

The University of Cape Town invites applications for Post-Doctoral Fellowships to be held at the University of Cape Town.

The Fellowships are tenable for 12 months and the stipend attached to the Fellowship is R16,000. Successful candidates from abroad will receive an additional travel grant up to a maximum of R1,500. There is no restrictions to any particular field of research.

Applications must include full details of the applicant's research programme in addition to a full curriculum vitae and the names of two referees to whom the University may refer. The policy of the University is not to discriminate in the appointment of staff on the grounds of sex race, colour or national origin. The dates at which the successful candidates will be expected to take up their duties are flexible and can be arranged to suit the special circumstances of the candidate.

Closing date for the receipt of applications is 30 September 1981.

Applications (quoting ref. number AC7) should be addressed to the Chief Administrative Officer, Research Administration, University of Cape Town, Private Bag, Rondebosch, 7700, South (W402)E Africa.

UNIVERSITY OF WESTERN AUSTRALIA Perth

UNIVERSITY RESEARCH FELLOWSHIPS (Post-Doctoral)

Up to two Research Fellowships will be offered, to be taken up in 1982. Appointment will be for one year in the first instance with the possibility of renewal for a second year: Fellowships may be renewed for a third year, but only in competition with new applications. The Fellowships will be tenable in the following academic Departments for work in the broad areas stated below:

Biochemistry - Hormonal Control of Cytochrome Synthesis in Mitochrondria of Developing Rat Ovary, Medicine — Studies of Ovary. Medicine -Patterns of Disease in Migrants to Australia, Microbiology - Genetic Analysis and Modification of the Fungus Phytophthora cinnamomi. Physical and Inorganic Chemistry Theoretical Calculations of Atomic Polarizabilities using the Method of Configurational Interaction.

The Fellowships are intended for PhD graduates, or those with equivalent qualifications, who by publication and in other ways have demonstrated significant research capability. Preference will normally be given to graduates of universities other than the University of Western Australia. Salary will be within the range \$A17083 — \$A19570 per annum.

Applications in duplicate setting out full personal particulars, qualifications and experience, and the proposal for research during the tenure of the Fellowship should reach the Acting Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009 by 2nd October 1981. Candidates should request three referees to write immediately to the Acting Staffing Officer from whom any further specific information may be obtained (9330)E

IMPERIAL CANCER RESEARCH FUND POSTDOCTORAL RESEARCH **FELLOWSHIP**

Applications are invited for a three year, postdoctoral appointment in the Viral Leukaemogenesis Labo-ratory at Lincoln's Inn Fields to work on control of expression of membrane proteins during differentiation of erythroblasts transformed by avian retrovirus, in particular avian erythrobalstosis

Salary range: £7,700 to £9,750, plus London Allowance of £967 a year, with entry point according to qualifications and experience.

Further information from Dr. M. Hayman (telephone 01-242 0200 extension 448). Applications including full curriculum vitae and names and addresses of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, by 31st Imperial Cancer August, 1981, quoting reference 121/81. (9244)E 121/81.

IMPERIAL CANCER RESEARCH FUND POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a three vear postdoctoral appointment in the joint laboratories of Tumour Virology and Viral Leukaemogenesis at Lincoln's Inn Fields to apply recombinant DNA techniques to the study of virus-induced oncogenesis. Molecular cloning of RNA tumour viruses is being undertaken to investigate, by a combination of restriction enzyme analysis, DNA sequencing and DNA transfection, the structure and function of mutant and wild type viral transforming genes and their interaction with host cell sequences in which they integrate. Salary range £7,700 — £9,750 plus London Allowance £967 a year, with entry point according to qualifications and experience. Further information from Dr. John Wyke (tel. 01-242 0200 ext 270). Applications with curriculum vitae and names and addresses of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 by 31st August, 1981 quoting reference

CONFERENCES and COURSES

1981 quoting reference (9243)E

THE HATFIELD POLYTECHNIC MSc in **ASTRONOMY AND**

ASTRONAUTICS (Part time, two evenings per week

for three years) PRELIMINARY COURSE starts 1 October 1981 MSc COURSE

starts 16 February 1982 For further details:

Telephone - Hatfield 68100 ext 3214 or

Write to

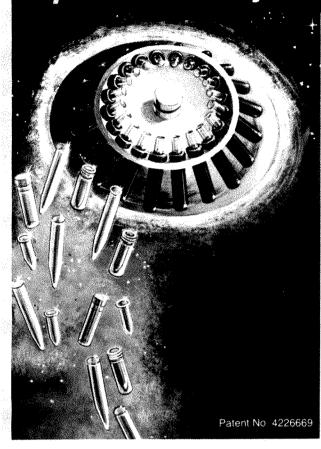
The Secretary (MSc) Hatfield Polytechnic Observatory, Bayfordbury House, Hertford, Herts SG13 8LD (9289)C

nature LONDON OFFICE Jean Neville
Jean Neville
4 Little Essex Street
4 London WC2R 3LF
Tel: 01 240 1101
Telex: 262024 NEW YORK OFFICE NEW YURK OFFICE Cathy Moore 15 East 26 Street 15 East 26 NY 10010 New York, NY 10010 Tel: (212) 689 5900

TORONTO OFFICE Peter Drake Associates Peter Drake Associates 32 Front Street West 32 Toronto. Ontario M511C5 Tel: (416) 364 1623

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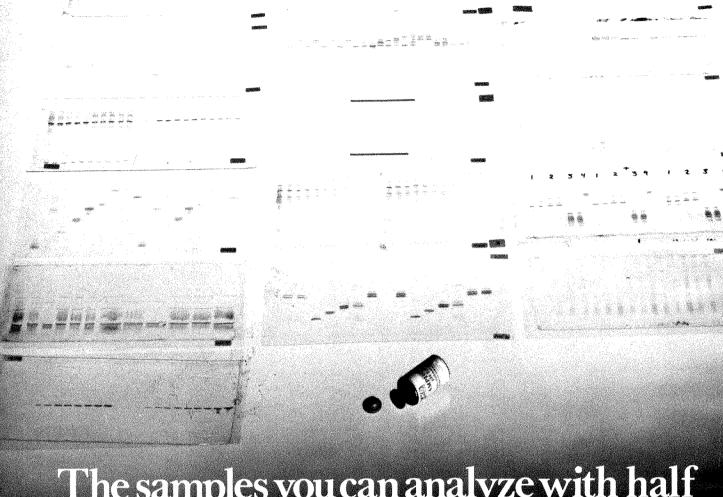
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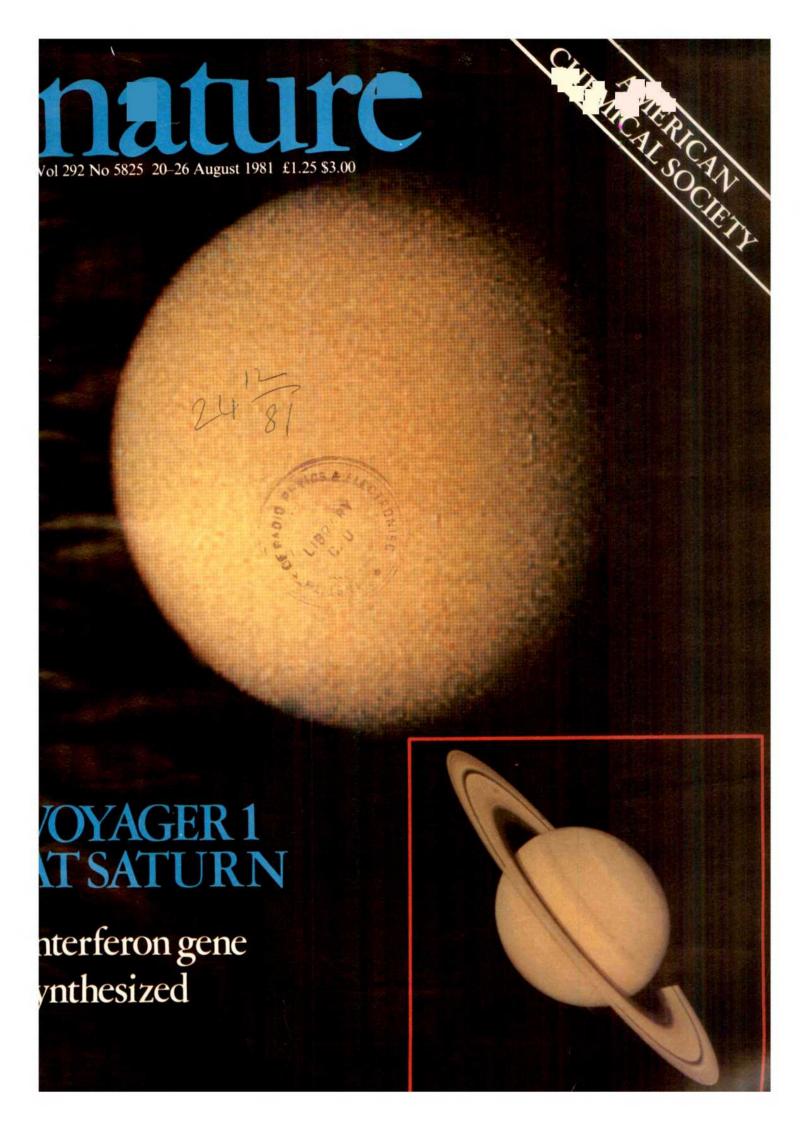


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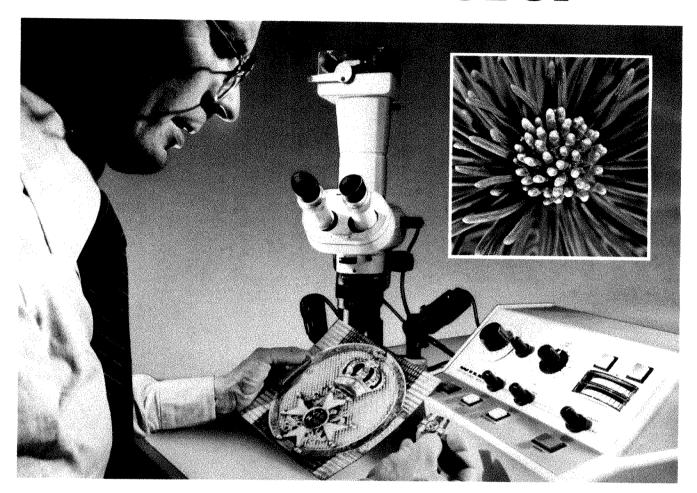
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¹Tu, C.-P.D. and Cohen, S.N., Gene, 10: 177, 1980

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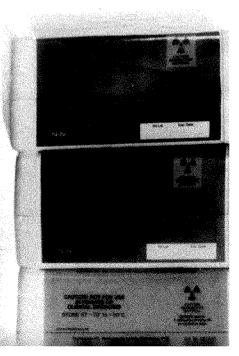
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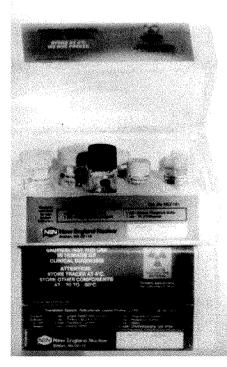
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faxam, A.M. and Gilbert, W., Methods in nzymology, 65 (1980)



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³Manley, J.L., Fire, A., Cano, A., Sharp, P.A., and Gefter, M.L., *PNAS* (U.S.A.), **77:** 3855 (1980)

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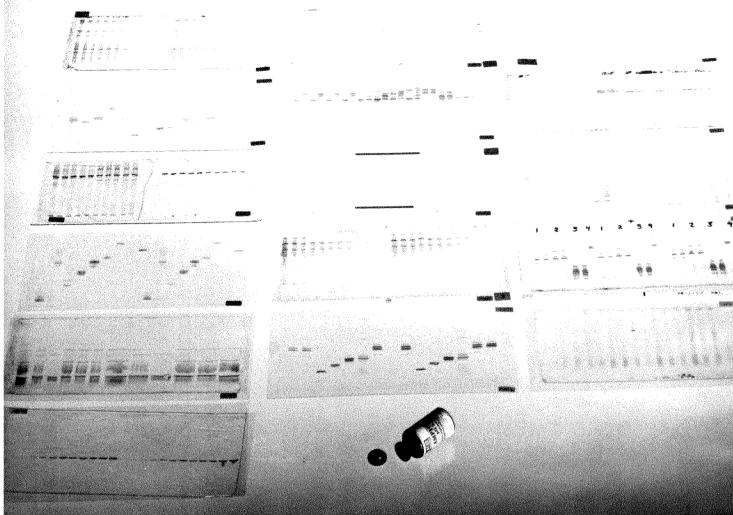
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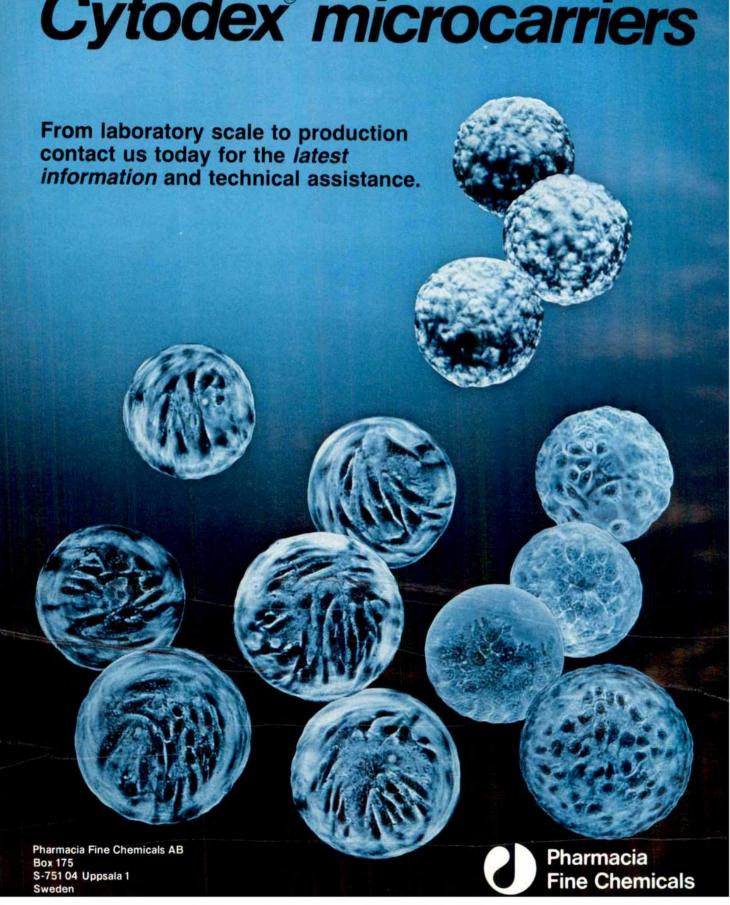
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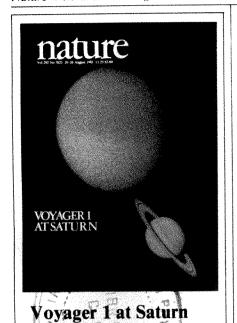
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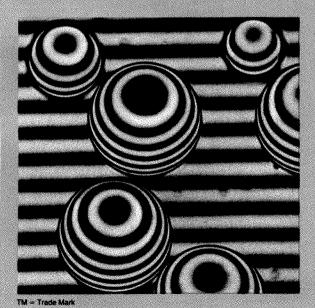
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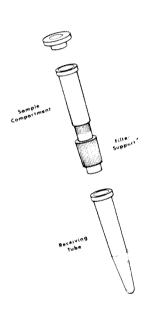
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EDITORIAL OFFICES

London 4 Little Essex Street, WC2R 3LF Telephone: (01) 836 6633 Telex: 262024 Telegrams: Phusis London WC2R 3LF

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Washington News Bureau

801 National Press Building, DC 20045 Telephone: (202) 737-2355 Telex: 64280 David Dickson (Washington News Editor)

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Features Advertising Manager: Marion Delaney
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New York 15 East 26 Street, New York, NY 10010 Telephone: (212) 689-5900

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nature

20 August 1981



Saving antibiotics from themselves

For how much longer will antibiotics remain at the disposal of physicians? This is the question that will be raised in the public mind by the gloomy statement put out earlier this month from Boston by a group of participants in the conference on the Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids held in Santo Domingo last January. The statement, in which Professor Stuart B. Levy of Tufts University has played a prominent part, points to the spread of resistance to antibiotics among bacteria in recent years, says that the "worldwide public health problem" is "due in large part to the indiscriminate use of antibiotics" and goes on to urge that "national and international committees" should be set up so as to issue "directives for prudent antibiotic use". Dr Levy's group is anxious that attention should widely be paid to its clarion call.

Nobody dissents. The problem of antibiotic resistance, although something of a surprise because thirty years ago nobody anticipated the versatility of bacterial plasmids, is, nevertheless, not new. Good sense argues in the same direction as Dr Levy's group: plainly everybody - not merely physicians but public health officials and farmers - should be more discriminating in the use which they make of a class of compounds, for the time being invaluable, whose value is being eroded as the years go by. Less vitally (in the human sense), the same process has been happening with insecticides, the other principal biochemical legacy of the 1940s. Organisms of all kinds, not only bacteria but insects as well, constantly surprise us by their biochemical versatility. (So, too, do higher organisms such as human beings, which have demonstrated a remarkable capacity for survival in circumstances in which self-adulteration with materials such as alcohol is common, although there is no reason to suppose that selection has played any significant part in this process.) How do we learn to live with such surprises?

The question is every bit as important as the statement earlier this month makes out. It is more than merely wasteful, but tragic as well, that the benefits of technological innovations (antibiotics, insecticides) should irreversibly be misused. Superficially, these sombre prospects are more galling than the waste of the potential benefits of mechanical technology — air transport (hamstrung by protective regulation), telecommunications (often hamstrung by protective legislation) and computer technology (hamstrung by collective inertia). For there, the argument goes, the promise remains, waiting merely on some kind of reform of people's resolve to make sensible use of the tools that science and technology have provided for them. But is it not also tragic that in present circumstances the productive wealth of the industrialized nations should be constrained, with all the human consequences that are entailed — and measured by the growing proportions of the unemployed — and that the gap between the prosperity of the rich and the poor should, through mutual impoverishment, be sharpened? Society, in other words, is as much at risk from the under-use as from the over-use of the technology at its disposal.

How, against this background, should enlightened policies on antibiotics be designed? Dr Stuart Levy's group, recognizing that policies of some kind need to be devised, has been plainly at a loss to know how these might come into being. Why else would it have suggested a hierarchy of national and international committees? This strategem, the most obvious, is well calculated to amplify its own eloquent statement of foreboding about the consequences of the misuse of antibiotics, but unlikely to make much headway with dealing with the causes of misuse. These, in turn, are deeply embedded in the ways of societies blessed with the chance of using

antibiotics — the freedom of physicians to prescribe, the freedom of other professional people (farmers, for example) to use chemical products in ways that they think proper and profitable and the general restraint on democratic government from excessive interference with what people do (or do not do). Dr Levy's group, for all the best reasons, finds itself up against the kind of social ethos that made the discovery of antibiotics possible. That, it may be thought, is the irony of their (and our) dilemma. How is it to be resolved?

The dismal science would suggest a simple remedy, not entirely to be scorned. If some uses of a potential social benefit are judged democratically to be prejudicial for society at large, why not put a tax on them? The snag is that the demand for antibiotics is greatest in those societies in which the prices of supposedly lifesaving drugs have the least negative effect on the demand for them; elsewhere, where the "need" is perhaps greatest, the "demand" (muted by the capacity to pay or even by ignorance) is least. An alternative solution may be found in the common ethics of the medical profession, which increasingly requires that physicians should balance the benefit that a single patient will receive from a certain treatment against the more general benefit if the resource in question is dispensed in some other way — the allocation of a kidney machine to one person rather than another, for example. In the use of antibiotics, the counterpoise of individual benefit is, however, non-use for the greater good. Logically, the problem is the inverse of that in which physicians subject their patients to the small risk of damage from the use of a vaccine (coupled with the benefit of immunity against the corresponding infection) for the sake of the wider benefit. The trouble is that non-use is certain to be regarded, in democratic societies, as deliberate and thus reprehensible neglect. How, in the last resort — as it may be — does a physician explain that the administration of an antibiotic likely to be beneficial must be withheld for fear that resistance will be encouraged?

Physicians properly concerned about problems of antibiotic resistance must manage more adequately to come to terms with the societies in which they work. It would, of course, in many other fields than this, be splendidly simple if individuals would put their expectation of personal benefit second to the communal benefit, even in matters of life or death. In the short run, however, that is exceedingly unlikely. And there is no immediate prospect that there will quickly arise such a persuasive analyst of the machinery of society that some compromise will be found between the doctrines of the two greatest analysts so far - Adam Smith (who can be bowdlerized as holding that the communal good is the sum of individuals' estimates of their individual benefit) and Karl Marx (whose essential argument, grossly oversimplified, is that the greater good will benefit most but not all individuals). So what should physicians do about antibiotics? The hierarchy of committees now proposed, potentially useful, should be given positive rather than negative terms of reference. The objective should be somehow to suggest things to do rather than not to do - handlists for physicians to help them to make empirical decisions, programmes for the more effective control of infection in hospitals, plans for helping forward the development of new kinds of antibiotics (for research people are even more adaptable than bacteria) and, above all, studies of how a proper balance between use and non-use is to be struck in modern democratic society. Nobody will pretend that these tasks are simple. In the long run, however, nobody will listen seriously to Dr Levy's group unless it tackles these broader subjects.

London bites private bullet publicly

University cuts spending across the board

The University of London last week let its constituent colleges know how much they will have to spend next year, and how many students they will be permitted to educate three years from now, in 1983-84. The target reductions of the student population are in line with the gloomier forecasts; most colleges are disappointed with their financial allocation for the academic year immediately ahead.

Some, however, are relieved. Earlier this year, Chelsea College, one of the science-based smaller colleges in the university, was singled out by the second interim report of the Committee on Academic Organization commissioned by the university for oblivion or dismemberment; Chelsea is therefore understandably relieved that the now threatened reduction of its student numbers three years hence is a mere 14 per cent.

Other colleges, previously thought to be set fair, have been downcast. Queen Elizabeth College, for example, applauded in the most recent interium report of the Committee on Academic Organization (under Sir Peter Swinnerton-Dyer) has been asked to plan for a student body reduced by a third three years from now. The reduction is even more drastic than that decred by the University Grants Committee for the University of Aston in Birmingham.

Under the university's plan for the cutting of the cake for the coming academic year, the larger colleges do best. University College London, singled out for its high unit costs, by the Swinnerton-Dyer Committee, will have to stomach only a five per cent cut in student numbers in the next five years.

In its letter to schools of the university last week, the Court said that it had decided to take full account of the recently decreed increase of fees for part-time students, but had made a special but interim provision for Birkbeck College (most of whose students are part-time). On overseas students (or the lack thereof) the Court said that it could not undertake to help those branches of the university hard-hit by recent changes.

The Court's letter contains an element of second guessing in the statement that it has "devised arithmetical values for the various UGC verbal indications about Arts and Sciences". The upshot of the Court's calculation seems to be that, after allowing for the buoyant future spelled out for Imperial College, the population of science

students in the rest of the university will decrease by 11.3 per cent in the next three years, compared with the reduction of 5.7 per cent expected in other academic fields.

The university court seems, nevertheless, to have been perplexed by some of the consequences of its arithmetic. Given the substantial reduction of student numbers in science foreseen, and the grants committee's recommendation (to the university as a whole) that special attention should be given to veterinary science, mathematics and engineering, the Court records that it enquired whether a reduction was required in biology.

Apparently it "emerged" that the committee had not intended that the

subject group of "biological sciences" should "suffer such a large reduction" but that the committee was in favour of a reduction of the numbers of students following degree courses combining two science subjects, "a category not specifically mentioned in the UGC letters".

On balance, the Court has planned for a real reduction of 5 per cent in its resources in the coming academic year. Given previous commitments to medical and other professional schools in the university, the Court says that the generality of its dependants will have to make do with 7.5 per cent less in the coming academic year.

Soviet Union urges space demilitarization

The Soviet Union has proposed a new treaty banning the deployment of weapons in outer space. The terms of the treaty, set out in a letter from the Soviet Foreign Minister, Andrei Gromyko, to the Secretary General of the United Nations, Dr Kurt Waldheim, differs from the 1967 treaty on outer space in two important respects. It covers all weapons, and is envisaged as an instrument of the United Nations, to be available for signature by "all states" with the instruments of ratification deposited with the United Nations Secretary General and not, as in 1967 with individual sponsoring governments. Accordingly, in the opening clauses of the draft, the text, refers to 'member states'' and switches to "signatories" only in details dealing with the technicalities of ratification, amendment and possible withdrawal. According to TASS, the essential articles of the draft read as follows:

- 1.1 The member states undertake not to put into orbit around the Earth objects bearing weapons of any kind, not to install such weapons on celestial bodies, and not to deploy any weapons in outer space in any other way including on piloted spacecraft of multiple use both of the existing type and also of other types which may be developed in the future by member states.
- 1.2 Each member state... undertakes not to help, encourage and incite any state, group of states or international organization to engage in activities running counter to the provisions of paragraph 1 of the present article.
- 2. The member states shall use spacecraft in strict accordance with international law including the UN Charter in the interests of maintaining international peace and security, and for the development of international cooperation and mutual understanding.
- 3. Each member state shall be bound not to destroy, damage or disturb the normal functioning or alter the flight trajectory of space vehicles of other signatories.
- 4. For the purpose of ensuring confidence in the observation of the provisions of the present treaty, each member state shall use

the national technical monitoring facilities at its disposal in such a way as to comply with the generally accepted principles of international law.

The document is, of course, only a draft, and with the United States toying with orbital laser weapons seems unlikely to achieve anything concrete in the near future. The US shuttle, however, and its possible military implications have been causing considerable concern to Soviet military experts. The past few months have seen numerous comparisons in the Soviet media between the "militarist" capabilities of the shuttle and the essentially peaceable nature of the Soviet Salvut orbital stations. Such concern seems, at least in part, to lie behind Mr Gromyko's letter to Dr Waldheim, which notes that the risk of the militarization of outer space has "recently

If the treaty should materialize, several ambiguities in Mr Gromyko's draft would doubtless have to be resolved. Thus article 3, as it stands, seems to leave open two justifications for damaging or disturbing a spacecraft in flight — that it was not (or was erroneously thought not to be) the property of a member state, or that it was allegedly launched in breach of article 1.1. Again, the draft contains no clear definition of a 'weapon''. In its strictest sense, article 1.1 would preclude even the carrying of a hand gun or life-preserver by, say, senior officers of a large space station. Such a station, too, might well wish to deploy small rockets with explosive warheads to destroy artificial debris or meteors on a dangerous approach course. Would such rockets be classed as "weapons" if they were "accidentally" launched against the spacecraft of another signatory? Moreover, just as in 1967, the treaty makes no provision for inspection, simply calling (article 4.3) for consultations and exchange of information "where necessary". Without such provisions, any such treaty would be little more than an Vera Rich ethical gesture.

UK biotechnology

Fast start

Britain's "official" biotechnology company, Celltech Limited, seems not yet to have decided how to respond to Stanford University's advertisement of terms for non-exclusive licences to exploit the Boyer-Cohen patent. Part of the difficulty is that it is not yet clear how far companies outside the United States, part of whose business will come from the licensing of their own patent rights to other non-American companies, will be affected by the patent. But there is also an element of waiting and seeing. So far there have been no direct talks with Stanford.

In any case, the company is probably too busy. Since Celltech moved into its new headquarters in Slough (20 miles west of London) just after Christmas, the staff has grown more quickly than originally foreseen, to just about sixty. The company seems pleased with the eagerness of scientists to join up, mostly from industrial companies but with a substantial proportion of people from the universities.

One of the newest and most significant recruits is, however, a sales invoice clerk. Although the company's prospectus has made no bones about the time that will be needed to put biotechnology on the commercial map, it does plan to sell what it can as soon as it can. One of the first products will be an anti-interferon monoclonal antibody, principally for use by the manufacturers of interferon. A laboratory for the preparation of this and other monoclonal antibodies is being assembled. But the company is anxious that this part of its business should not be thought of as more than a kind of sideline.

Understandably, the company is reluctant to say where its chief efforts will go, but gives the impression that it will not be investing a major effort in the production of interferon of any kind. At some stage, though, it seems clear that Celltech will be in the industrial enzyme business. For the time being, the practical aim seems to be to establish itself well in the basic techniques of genetic manipulation, among which nucleic acid chemistry and the techniques of gene expression are conspicuous.

The nucleic acid chemists have built themselves an automatic nucleotide assembly machine rather than buying one off the peg, chiefly for the flexibility that results. They claim that their machine will add nucleotides (up to a score or so) at the rate of one every half an hour, which means the production of one full-length oligonucleotide during each overnight run.

Celltech's fast start means that its initial capital of £12 million will be used up more quickly than originally foreseen. The plan now is to recruit up to 150 people by September 1983, half of them scientists.

The shareholders in the company (among whom the National Enterprise

Board is the largest, with 44 per cent) have agreed to the accelerated investment plan, and will take a close look at its successor in just over two years time. The officers of the company say that they are heartened by the long-term view taken by the shareholders, which also include the Prudential Assurance Company, the Midland Bank, British and Commonwealth Shipping and Technical Development Capital. This, the guess is, should give Celltech a good chance when, in two or three years, there is likely to be a shakeout in the biotechnology field.

The company has no fixed ideas about the way in which it will sell its products, but plans not to enter the marketing of manufactured products nor manufacturing as such (which does not exclude the setting up of manufacturing companies in partnership with others). Enquiries from industry have been flowing in, some of which may lead to research contracts.

On the relationship between Celltech and the Medical Research Council, on whose developments the company has first refusal, Celltech says that developments not of direct interest may be licensed to others, in which respect the company will act as a kind of broker. It expects, however, to make direct use of most of the ideas suggested by the Medical Research Council, which, nevertheless, retains the right to bypass Celltech if too much time passes.

US graduate salaries

Engineers ahead

Washington

Statistics for 1980 recently released by the US Department of Education show a significant increase in the number of bachelors and first professional degrees in science and engineering awarded by US colleges and universities for the first time since the early 1970s.

The main reason for the increase is a growth of 11.5 per cent in the number of engineering graduates, continuing a trend that started in 1976. Since then, the rise has been balanced by a drop of about 4 per cent a year in the number of graduates in the social sciences and psychology — there was a drop of only 1.2 per cent in 1980.

The continued growth in engineering graduates reflects the relatively high salaries that even first-degree graduates are being offered by industry. Figures published by the College Placement

Council two weeks ago showed that 1981 graduates are entering jobs with starting salaries between 10 and 14 per cent higher than a year ago.

The average starting salary for petroleum engineers with a bachelor's level degree was \$26,652, and for chemical engineers \$24,360. This compares with starting salaries for economists with similar qualifications of \$16,440, and for other social scientists of \$13,992.

The high salaries being offered by industry also seem to be discouraging engineering graduates from staying on to complete higher degrees, leading to difficulties in higher education institutions of attracting qualified teaching and research staff. In comparison with the 11.5 per cent increase in bachelors' and first degrees awarded in engineering, the number of masters degrees in the same subjects rose by only 4.3 per cent, and the number of doctors' degrees remained virtually constant.

Concern about the implications of these figures has prompted Congress to oppose President Reagan's plans to eliminate the National Science Foundation's support for graduate fellowships. Committees in both the House and the Senate have agreed to restore funds for the fellowship programme, which makes about 500 new awards a year, although the precise level of funding for the foundation's overall education efforts has still to be negotiated between the two legislative bodies.

Another problem now emerging is a shortage of qualified science teachers. States have experienced a rapid decline in the number of science teachers turned out by their colleges of education; in physics, for example, the supply of new secondary school teachers has virtually dried up at a time when warnings are being voiced in Washington about the dangers of a growing "scientific illiteracy" among the general population.

According to Dr James Rutherford, who has been appointed director of a new science education programme within the American Association for the Advancement of Science, many schools are reporting that their science teachers are being approached with tempting job offers from private companies — often mathematics and physics teachers are offered jobs in computer sciences at salaries considerably higher than they receive for teaching.

David Dickson

Degrees awarded in 1980 (figures from National Centre for Education Statistics)

	Bachelors' professiona		Master	s' degrees	Doctors' degrees		
Total	1,009,879	(+0.9%)	299,492	(-0.9%)	32,758	()	
Science and engineering	292,271	(+1.2%)	54.463	()	16,495 (+ 0.8%)	
Science and mathematics	117,906	(-1.1%)	,	(-1.2%)		+1.0%)	
Social sciences and psychology	114,469	(-1.2%)	15,552	(-2.7%)		+0.8%)	
Engineering	59,903 (+11.5%)		(+4.3%)		+0.1%)	

Values in parentheses are per cent changes from 1979 figures; a dash indicates no change.

Indian health care

Keeping it simple

Lucknow

If India's ambitious target of "health for all" by the year 2000 is to be approached, then the present curative hospital-based, urban-centred health service may have to give way to simpler low-cost alternatives, especially in rural areas.

Some advances have been made in developing "appropriate technology" for health care. Low-cost audiovisual aids and teaching methods for various levels of health worker have been developed under the aegis of the Indian Council of Medical Research. The Central Scientific Instruments Organization has developed a cycle-ambulance with a stretcher for the patient and a seat for the attendant; a clinical thermometer with temperature ranges indicated as coloured bands for easy use by illiterate health volunteers; a simple apparatus for vasectomy, now ready for human trial; a fetal stethoscope which can detect a fetal heart beat at week 12 of pregnancy; a miniature cardioscope and a colorimeter. The Vector Control Centre, Pondicherry, has demonstrated that certain fishes such as Aplocheilus blochii and Gambusia affinis, can be used as effective controls of mosquitoes, on whose larvae they feed.

For maternal and child health care by traditional birth attendants, called dai, a simple dai kit has been developed at the Postgraduate Institute of Medical Education and Research, Chandigarh. The kit should reduce the risks of tetanus, by achieving better asepsis, and helps the birth attendants to spot mothers and infants at risk—again using colour-coding principles on measuring sticks and weighing scales.

A much-needed boost to rural health care should come with the recent decision of the Indian government to set up a national committee on appropriate technology for health care. The Indian Council of Medical Research is to provide technical support for the national health programme—and at a national workshop held recently by the council the value of community health volunteers and multipurpose health workers was underlined.

Zaka Imam

Blood products

Surplus sale

Products extracted from donations to the British National Blood Transfusion Service, and for which the National Health Service has no use, may in future be sold to pharmaceutical companies, the government has decided. Although some companies have occasionally bought products from the transfusion service in small quantities, usually for research purposes, there have been no formal arrangements for buying in bulk. Thus British companies have had to buy from abroad.

The decision to sell off surplus blood products has emerged from a lengthy wrangle over the use of products that are now discarded, expected to be exacerbated as the transfusion service puts into operation a plan to make more blood plasma available for fractionation. There has also been a long discussion about the revamping and expansion of the Blood Products Laboratory, which carries out all fractionation of donated blood. The laboratory suffers from some of the problems of the former Lister Institute of Preventive Medicine, whose site it shares. The institute was closed in 1978 after it began to lose money and could not afford to modernize its facilities.

An earlier government suggestion that the Blood Products Laboratory be handed over to commercial management fell flat after many organizations, including blood donor groups, trades unions and Labour Members of Parliament, objected to the rise of blood donated by volunteers for overtly commercial purposes. As a compromise, the government has decided to keep the laboratory firmly under the wing of the Department of Health and to sell products surplus to the National Health Service's requirements. Any profits will be ploughed back into the health service, although it is not yet clear whether they will be spent on updating the laboratory.

The success of the scheme will depend on whether the laboratory can supply companies with products they need. Companies are negotiating with the health department, which has said only that outdated red cells and plasma fractions containing nonspecific antibodies will be available. Products for which the health service's demand exceeds supply, however, will not be for sale. They include factor 8, the blood clotting agent used for treating haemophiliacs, and albumin.

Future sales will, however, depend on the plan to make more blood plasma available for fractionation, by substituting concentrates of red cells for whole blood in transfusions and on the laboratory's ability to take on the extra work. The aim is that by the end of 1982, the laboratory will have enough plasma to produce two-fifths, compared with one-fifth at present, of the national demand for factor 8, in which it is hoped that Britain will ultimately be self-sufficient. That will mean that other products, such as factor 9, immunoglobulin and albumin, will be produced in excess and will be available for sale.

One company now discussing its needs with the Department of Health is Beechams Pharmaceuticals Limited, which would like to buy plasminogen for clinical trials in the treatment of deep-vein thrombosis. Whether the laboratory will be able and willing to supply such specific products, or will simply make certain plasma fractions available, remains to be seen.

Judy Redfearn

Paying court

Forensic scientists in Britain are rather touchy at the moment, following the suspension of one of their number for allegedly failing to reveal crucial ambiguities in evidence on blood samples, and now the "demotion" of another for writing a research paper which is said to reveal detachment.

This second scientist resigned rather than accept a teaching post — which he regarded as demotion — and then went to an industrial tribunal to claim what he saw to be effectively unfair dismissal. However, the tribunal rejected the scientist's claim, and he is to appeal to the Employment Appeal Tribunal in London on 3 September.

His paper, which appeared in the official journal of the British Academy of Forensic Science - Medicine, Science and the Law — in 1977 (vol. 17 no. 1, p.37), deals with the quantitative toxicology of corpses — addressing the issue of how to estimate a drug or poison dose. The paper uses a simple law of linear diffusion to relate the drug content of various organs (taken from the data sheets of real cases) to dose and time of death as estimated by the police and witnesses. The data are clearly very scattered and the fit loose, and the author concludes "A pessimistic view is that it is impossible to remove the last vestiges of alchemy from [forensic] toxicology because of the nature of the material for analysis. It may be, apart from the fact of death, that one cannot be absolutely certain of anything and there are always contrary authorities to be found".

This conclusion may not have recommended itself to the Forensic Science Service, which as part of the Home Office runs forensic science in the United Kingdom. Other forensic scientists may equally have been unsettled.

Robert Walgate

US uranium

Falling demand

Washington

A report from the US Department of Energy (DoE) has revealed just how depressed the domestic market for uranium has become. This was already evident from the dramatic price reduction in 1980, when uranium oxide fell from \$43 to less than \$30 a pound. Now DoE has shown that, for the first time, the amount of new orders for future supplies placed by US utilities was more than outweighted by cancellations of existing commitments.

The DoE's report, A Survey of United States Uranium Marketing Activity, published last week, is based on information gathered from 60 utilities with nuclear reactor projects, 30 present or potential uranium users and five reactor manufacturers. It says that domestic

buyers contracted with US primary producers for an extra 12,500 tons of $\rm U_3O_8$ in 1980, but that this was offset by a reduction of 25,600 tons for commitments made under previous contracts.

The survey showed that, despite the current depressed prices, most utilities and producers expect the price of uranium to rise sharply over the next decade; future contracts which guarantee a floor price for any 1 year quote an average of \$75 a pound by 1990.

The drop in uranium demand is blamed on the extended licensing period faced by companies building new reactors, a reflection of increasingly stringent safety requirements and on the substantial reduction, in estimates for future demand for electricity as the United States becomes more conservation-conscious, and energy costs continue to rise.

Some business analysts feel that the price of uranium may have bottomed out, at least temporarily. According to calculations by the Californian consulting company Nuclear Exchange Corporation (Nuexco), uranium prices remained virtually constant at \$24 a pound between April and June, and actually increased slightly last month.

However, the slump in prices — and demand — is clearly reflected in difficulties being faced by the uranium mining industry. Some estimate that as many as 4,000 workers, 25 per cent of the workforce, have been laid off.

This decline, too, is reflected in DoE statistics. In another report, soon to be published, the department notes that the uranium mining industry is entering a period of major retrenchment. Having initially planned to spend \$316.5 million on new mining facilities in 1981, for example, the industry now estimates that total spending will be only about \$172 million. Projections for capital spending on mill facilities have dropped even more dramatically, from \$241.9 million to \$99.3 million.

According to DoE officials, several processes are keeping the price of uranium oxide down. One is that many utilities, faced with high interest rates, are selling stockpiled uranium back to producers. Another is that, even though uranium mining has been cut back, production figures are still going up as companies work through stockpiles of ore. Nuexco had predicted a drop of 2.5 million pounds between 1979 and 1980 to about 34.5 million pounds — still about double the amount consumed by the nuclear industry.

In fact, however, production increased by 13 per cent to 42.5 million pounds. Some wastes which have already passed through the mill are being rerun, a way of obtaining more uranium oxide without having to pay for any extra mining. Some industry analysts feel that this will continue, so that even if demand picks up it will not be reflected by an immediate boost for the mining industry.

David Dickson

German cancer research

Politics ousts science

Brussels

The scandal at the German Cancer Research Institute has grown into a national issue. The scientific director has resigned, the administrative director appears to have been removed, the oppositions parties (CDU/CSU) have attacked the government and the institute itself is now involved in internecine strife.

The national research centre in Heidelberg is a union of eight institutions centrally administered by the Bundes Ministerium für Forschung und Technologie (BMFT), with a 1981 budget of DM 79 million (\$1,817 million). The latest scandal began with the appointment of Dr Hans Neurath, born in Vienna, but previously professor of biochemistry at the University of Washington, as the centre's scientific director. The post had been vacant since 1976 and the chief administrator, Bodo Spiekermann, was relieved to have found someone with the qualifications and drive to attempt the much needed reorganization.

The other directors of the centre were not consulted about the appointment and soon raised a storm about the professor's demand that the institute pay for a villa in addition to his salary of DM 167,000 a year. The attack soon broadened to Neurath's qualifications and it turned out that he had in fact previously only been scientific advisor to the University of Seattle.

The centre's director for toxicology and chemotherapy, Professor Dietrich Schmaehl, declared that Neurath could not possibly be sufficiently knowledgeable about cancer research to take over leadership of the centre. Nothing daunted, Neurath set about appointing outside investigators to assess the value of the research projects in hand with the intention of directing resources to the most promising areas. The multidisciplinary, clinically-orientated approach was rejected, and Neurath, with the support of the ministry's director, Wolfgang Spinke, proposed to cut back on activities such as nuclear medicine and chemotherapy.

The zeal with which Neurath set about reforming the institute inflamed the already poisoned atmosphere, the battle amongst the divided researchers spilled over into the press and by May this year it had become a political issue, with the opposition, CDU/CSU, siding with Neurath's opponents and the government defending its appointee who by then had been in charge for a year.

The opposition claimed that the administration of the centre was chaotic and scandalous. By July CDU/CSU was describing the working climate as completely destroyed by internal power struggies complete with the "terrorization of administrative personnel".

On 24 July, Neurath announced that he

had resigned but would explain the reasons for his decision only in September. BMFT also announced that Spiekermann was being withdrawn, and the conflict became an out and out battle between the ministry and the research centre. The ministry now plans to examine the scientific activities of the centre since 1973.

The latest chapter in the saga is the government's reply to the CDU/CSU's accusations in July. It reaffirms BMFT's desire to strengthen pure research rather than to concentrate on already well-explored areas. In addition, the government says it has long been convinced that the quality of the centre's research could be improved and that the routine work carried out runs counter to the aims of the centre. "Internal egoism", it says, has been responsible for the present situation; the attack against Neurath is merely an attempt to divert discussions from the real issues at stake.

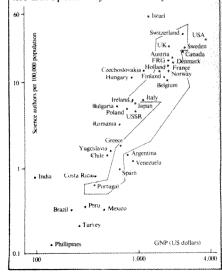
Jasper Becker

Productivity by numbers

India, Eastern Europe and Israel stand out as being unusually scientific in the accompanying graph prepared by sociologist Dr Pedro Gonzalez-Blasco of the University Alcalá de Henares, Madrid.

The plot shows the number of scientists per hundred thousand population against gross national product (GNP) per capita in 1967. The box encloses Western European countries. The main trend is for the proportion of scientists to rise at about the third power of GNP per capita — so that scientists are added more rapidly per unit of additional GNP the higher the GNP. However, this may be read in two ways. Are the countries above the trend more effective at creating scientists? Or are their scientists less effective at creating wealth?

Dr Gonzalez-Blasco's plot forms part of the background to a seminar on the state of physics in certain less developed European countries to be held in Istanbul on 4-5 September under the auspices of the European Physical Society.



CORRESPONI

Badger vaccination

Sir — It was noble of Mr Rees (Nature 9 July, p.104) to come to the defence of the Zuckerman report; however, my criticisms were of the report rather than the Ministry.

Mr Rees misreads my plea that some attention should be paid to vaccination of badgers, as advocacy of vaccination as the answer.

It is well known that BCG is one of the less effective vaccines in man, both in the percentage of the population protected, and in the type of protection afforded (infection is not prevented, it is merely limited). Reports have shown that protection can vary from 78% of the vaccinated (human) population at best to negligible responses at worst. However, protection of only some of the population is epidemiologically significant, and whilst protection may not be absolute it is of proven value in reducing the transmission to further individuals - the significant factor here. The epidemiological basis in controlling a disease is to reduce the number of individuals infected by each diseased animal in its lifetime to less than one. Therefore any means of reducing the number of susceptible individuals contacted by each infected badger will be of significance. (Gassing undoubtedly comes into this category).

Though attempts to vaccinate foxes orally (against rabies) have shown what problems there can be, badgers are well known among zoologists for the ease with which they will take quite considerable quantities of easily prepared oral baits. This has been used to advantage in a variety of ecological studies.

The degree of infection in some areas was also cited as a reason against vaccination, and whilst gassing may be the most effective approach in severely affected areas, it is nevertheless still the case that vaccination alone, if feasible, would have some positive effect. What does not appear to have been considered was the value of vaccination in less infected areas, or even ring-vaccination around those areas to be gassed (migration into cleared areas being a problem).

At this stage all conjecture is limited by the lack of knowledge. However, I must still regret that the report did not more positively specify research aims. I still believe vaccination to be one of these.

M.J. CHAPMAN

Easingwold, York, UK

White or brown?

Sir - Concluding his review of two important books of contemporary scientific and medical interest on dietary fibre and health1, John Yudkin says "Let us by all means eat brown or wholemeal bread if we like it. But let us not delude ourselves that it will make us healthier or prolong our lives". This statement is not justified by the data available on the efficacy of dietary fibre in lowering disease risk. There may be useful effects of certain dietary fibres in preventing or ameliorating intestinal

diseases in general, but this aspect is for others more expert to discuss^{2,3}. We now comment on the scientific aspects of fibre use in relation

Countries with a high incidence of atherosclerosis and coronary heart disease usually have a high incidence of colon cancer, and vice versa⁴⁻⁶. For example, Great Britain has a high incidence of both kinds of disease, the Japanese a low incidence. Finland is an exceptional country, exhibiting a high incidence, in fact among the highest in the world, of atherosclerosis and myocardial infarction; however, interestingly, their colon cancer incidence is only slightly higher than that of Japan, but much lower than the other Scandinavian countries^{7,8}. Independent groups in the United States and Europe have investigated the underlying mechanism^{9,10} Detailed inquiry showed that the Finnish people consumed a diet not too different with regard to the amounts of most components, especially lipids and proteins, from those of people in New York or Copenhagen, so, by current concepts their diet and nutrition account for their high incidence of heart disease. However, the Finnish people also consumed rather large amounts of whole grain bread and other sources of cereal fibre. resulting in a stool bulk two to three times larger than those of the populations in New York or Copenhagen. At the level of metabolic epidemiology^{9,10} specifically in relation to secondary bile acids which were demonstrated to be promoters in the carcinogenic process11, the people in Finland and New York showed the same total bile acid levels, as would be expected with similar lipid intakes. However, and importantly, because of the increased stool bulk in Finland, the concentrations of bile acids were much lower similar to the concentration found in Japan. The Japanese level was that low, not because of high fibre intake, but because of low fat intake¹². Thus, the epidemiology was explained by metabolic biochemical approaches, and it was demonstrated through studies in man that cereal fibre accounted for a decreased risk of colon cancer in Finland. Furthermore, parallel studies showed that cereal fibres, particularly bran, could decrease the incidence of colon cancer in animal models^{13,14}. The few instances where this was not so involved feeding the fibre during the period when carcinogen was administered, but not during the important promotion phase 15, or when too high a level of carcinogen was administered, thus overwhelming the promotion phase where cereal fibre exerts its major protective role16.

To be sure, more research is necessary on the effect of diverse fibres, and in fact on the effect of other micronutrients. Unlike Yudkin, we believe that the present data base is adequate to conclude that brown or wholemeal bread with adequate fibre and other whole grain cereals, such as bran-containing breakfast cereals, are not only tasty but, indeed, have every chance of reducing the risk of colon cancer, a major type of cancer in the Western world. It is so easy to implement, and in fact would simply be a return to the situation prevailing before extensive milling of cereals removed much of the valuable fibre. Thus, adverse health effects owing to possible mineral imbalances are unlikely. The

consequences in lowering the risk of colon cancer, possibly other intestinal disease and even early diabetes^{17,18} would be appreciable if not "dramatic". Please, Dr Yudkin, be objective.

> LH WEISBURGER B.S. REDDY

American Health Foundation. Naylor Dana Institute for Disease Prevention, Valhalla, New York, USA

- Yudkin, J. Nature 291, 173-174 (1981)
- Burkitt, D.P. & Trowell, H.C. (eds) Refined Carbohydrate Foods and Diseases (Academic, London, 1975).
- Burkitt, D.P. Am. J. clin. Nutr. 34, 428-431 (1981)
- Blackburn, H. Prev. Med. 8, 612-678 (1979).
 Winawer, S.J., Schottenfeld, D. & Sherlock, P. (eds) Colorectal Cancer: Prevention, Epidemiology, and Screening (Raven, New York, 1980).
- Turpeinen, O. Circulation 59, 1-7 (1979).
 Waterhouse, J., Muir, C., Correa, P. & Powell, J. (eds) Cancer Incidence in Five Continents Vol. III (Int. Union Against Cancer, Berlin, 1976)
- Teppo, L. & Saxen, E. Israel J. med. Sci. 15, 322-328 (1979).
- Reddy, B.S., Hedges, A.R., Laakso, K. & Wynder, E.1. Cancer 42, 2832–2838 (1978).
- IARC Intestinal Microecology Group Lancet ii, 207-211 (1977).
- Reddy, B.S., Cohen, I.A., McCoy, G.D., Hill, P. & Weisburger, J.H. Adv. Cancer Res. 32, 237–245 (1980).

- Weisburger, J. H. Adv. Cancer 1, 67–81 (1979).
 Hirayama, T. Nutr. Cancer 1, 67–81 (1979).
 Reddy, B.S., Mori, H. & Nicolais, M. J. natn. Cancer Inst. 66, 553–557 (1981).
 Nigro, N.D., Bull, A.W., Klopffer, B.A., Pak, M.S. & Campbell, R.I. J. natn. Cancer Inst. 62, 1097–1102
- Bauer, H.G., Asp. N., Oste, R., Dahlquist, A. & Fredlund, P. Cancer Res. 39, 3752–3756 (1979).
- Cruse, J.P., Lewin, M.R. & Clark, C.G. Lancet ii, 1278–1280 (1978).
- Lancet ii, 423-424 (1981).
- Manhire, A., Henry, C.I., Hartog, M. & Heaton, K.W. Lancet i, 1157 (1981).

Yudkin's answer

SIR - My article, to which Weisburger and Reddy refer, was a review of two recent publications, both entitled Medical Aspects of Dietary Fibre. One of these was a report of the Royal College of Physicians, which had this to say about fibre and colon cancer:

"There are reasonable grounds for the statement that, in genetically susceptible persons, large bowel cancer could be favoured by a fibre-depleted diet, but other explanations for the commonness of this cancer in Westernized countries are possible. Definite conclusions must await the identification of the carcinogen(s) and the study of environmental factors on its production.'

As the references mentioned in the report make clear, the authors made at least as exhaustive a study of the literature as did Weisburger and Reddy in their letter; they also include the papers published by these workers.

The RCP authors, however, were clearly not as convinced that this evidence was conclusive as are your correspondents. In suggesting that I am not objective in my account of the role of dietary fibre in preventing colonic cancer, Weisburger and Reddy are really saying that the RCP itself was not objective when writing its report.

JOHN YUDKIN

London NW3, UK

NEWS AND VIEWS

The longest synthetic gene. . . so far

LESS than two years ago, an attempt to synthesize a human interferon gene would have literally been impossible. For it is just two years since nucleotide sequences of interferon genes were first determined by using reverse transcriptase to make DNA from messenger-RNA molecules taken from cells induced to produce interferon. Since then, similar DNA copies of mRNA have made it possible to show that there is a whole family of interferon genes in the human genome - several corresponding to leukocyte interferon (the α variety) and fewer corresponding to fibroblast interferon (called β) and the more elusive immune interferon (y). Inevitably, the amino acid sequence of each of these interferons has not vet been worked out directly, by chemical analysis; instead, the protein structure has mostly been inferred by applying the genetic code to the sequence of nucleotides in the gene. So it is, to say the least, a technical triumph that a group assembled by ICI should now have carried through the total synthesis of a human interferon gene (see Edge et al., p.756).

This gene is the largest so far synthesized, no fewer than 514 nucleotides long. Given that it has been necessary to string together the nucleotides of both the complementary DNA strands, more than 1,000 nucleotides have been assembled in a predetermined way.

The chemistry of the synthesis is more easily described than carried out. The starting point is an initial nucleotide coupled to a polyacrylamide resin. Further nucleotides are added in pairs, by means of a coupling agent in anhydrous pyridine. and the authors say that the time taken for each cycle is a mere hour and a half. At that rate, ambitious people might calculate, it should be possible to assemble 5,000 nucleotides in a year, or a whole genome in ..., well, a few centuries. Calculations such as these show that even the modifications of DNA-synthesizing techniques worked out by the ICI group fall a long way short of assembling whole genomes.

Indeed, as now described, short sections of each strand 15 or more nucleotides in length have to be made separately so as to keep the yield of the synthesis respectably high, and then short pieces of the double helix assembled before being joined

together in the complete gene. That the gene is complete has been shown by its incorporation into a plasmid, its amplification and subsequent analysis. Edge and his colleagues do not say whether their plasmids have been able to direct the synthesis of proteins — and if so, what they are — but something is produced, for a further report on its biological activity is promised. It would be a great surprise if the product were not α -interferon.

The gene now synthesized is not however a naturally occurring gene but an analogue, designed so as to simplify the synthesis, but also to avoid combinations of neighbouring nucleotides that may impede expression of the gene in its unnatural environment of the plasmid. It should make one of the versions of α -interferon and, with luck, should do so more efficiently. But it would be sheer good fortune if the gene now synthesized turned out to be a superabundantly efficient interferon gene. The importance of this development is not the gene itself, but the demonstration of the technique. It is a little like climbing Everest, known to be easier the second time.

But these days, it is possible to buy machines that will automatically assemble nucleotides in some predetermined sequence. What can be so special about the synthesis of this artificial gene? For one thing, the simple assembly of a score or so of nucleotides is only half the battle. Purification is a tedious and even uncertain business. Prudent people make sure that the nucleotide sequence they have made is indeed the one they set out to make by subsequent analysis. But the most important need in the construction of a largish gene is an intelligent strategy for deciding what pieces of the gene to make, and in what order to assemble them. The use of computers to spot sequences in the target gene that might cause trouble during its assembly is also obligatory.

This does not mean that the work now reported is simply a technical tour de force. In the past few years, synthetic pieces of genes have been widely used in several different ways — as probes for finding within some intact genome a piece of genetic material containing some particular sequence, for example. Those

whose business is to insert foreign genes into plasmid DNA increasingly find it safest to resynthesize the ends of the genes back to the nearest convenient restriction site so as to ensure that nucleotides are not lost when the tails needed for ligation to the plasmid DNA are added chemically. Fashioning pieces of DNA expected to have important functions such as those acting as sites for the promotion of replication has also become fashionable and valuable. But for a group with its roots in the pharmaceutical industry, it must be especially pleasing that it is now possible to plan systematically to vary the constitution of a gene for the sake of the variations of protein structure that result. Is there just a trace of nostalgia in the statement that "classical medicinal structure-activity analysis should [now] be possible with large peptides"?

The prospect is exciting. There is already ample evidence, derived from the experience of traditional pharmacology, that natural products can usually be modified to make more efficient drugs. The changes rung on the structure of the naturally occurring antibiotics in the past several decades are a sign of that. The interferons promise to be a nightmare for pharmacologists - whatever their biological effects, unspecificity seems to be their common characteristic. So it is natural that in the past few months, there has been great interest in hybrid molecules of interferon — one end from one and the other from another. Now it should be possible to set about making more subtle modifications, not those that depend on where some restriction site within the gene happens to fall. The result should help throw light on the functions of different parts of the interferon molecule. Yet improving on naturally occurring interferon is bound to be a daunting task. While reliable biological assays of the effectiveness of molecules related to interferon are few and far between, and while the efficiency with which interferon genes embodied in plasmids yield protein product is as low as at present, it would be hoping for too much that the uncertainties that abound in the genetic engineering of medicinal interferon will quickly be cleared

Saturn's rings

MUCH of the interest in Saturn's rings during the impending close encounter of Voyager II will centre on explanations of the structure of the ring system, involving either resonance with the major planetary satellites or the effects of smaller satellites co-moving with the rings. The excitement may obscure the argument due to Joseph E. Avron and Barry Simon (*Phys. Rev. Lett.* 46, 1166; 1981) published earlier this year that the radial structure of the rings of Saturn may simply reflect the instability at certain radii of solutions of the full many-body problem.

The argument is simple, although incomplete. Ignoring all but Saturn and one ring particle, supposed of negligible mass, orbits with all possible periods of revolution between that of the orbit that grazes the equator of the planet and infinity are stable. No more complicated version of the problem is, however exactly soluble. The general equation of motion is, however, $\ddot{\mathbf{r}} = \mathbf{F}(\mathbf{r}(t))$ where the force-function \mathbf{F} , a vector function, itself entails a solution of the many-body problem of Saturn, together with its substantial satellites and, for that matter, the Sun.

Approximations are evidently necessary, one of which is that the true force-function should be represented as the sum of forces within the equatorial plane and those perpendicular to it whereupon it is also sensible to represent the radius vector as the sum of a vector in the plane and another perpendicular to it with the form w(t). To a first approximation, supposing w to be small, the result is the need to solve a Schrodinger equation with (at best) a quasi-periodic potential. This simplest version of the problem of the motion of Saturn's ring particles can, in other words, be approximated to by the still insoluble problem of knowing what states are accessible to an electron in a quasiperiodic electric potential.

The upshot is that many periodic solutions of the simplest equations are unstable and therefore, over a substantial period of time, untenable. But which are they?

Avron and Simon did not pretend, in their paper published at the end of April, to be able to calculate the periods of the orbits that would be unstable (and thus the position of the gaps in Saturn's rings). Instead, they argued that even the simplest approximation to the manybody problem gives rise to Schrodingerlike equations whose solutions are restricted. Some orbits are possible and stable. Others are not. Avron and Simon surmise that between the rings, stable orbits should be distributed in radius as the numbers in a Cantor set — sparsely but not isolated.

Solar spin variation

from David W.Hughes

ONE of the most striking features of normal main-sequence stars is the great difference in the angular momentum per unit mass between the early and late types. There is a sharp drop at spectral type F, as shown in Fig.1. Some braking process must act preferentially on stars of low mass.

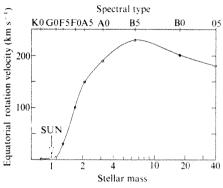


Fig.1 The equatorial rotational velocity of main-sequence stars plotted as a function of spectral type and stellar mass (measured in units of the solar mass).

Schatzman (Ann. Astrophys. 25; 1, 1962) pointed out that braking would occur if the gas emitted by a star was magnetically constrained to co-rotate with the star out to distances that were large compared with the stellar radius. Under these circumstances a small amount of mass loss would yield a proportionally much greater loss of angular momentum, simply due to the effective increase in the moment of inertia of the outflowing gas. The efficiency of the braking depends on the strength of the subsurface convection which is itself closely related to the mass of the main-sequence star. This convection is also responsible for the surface activity, such as sunspots, for the corpuscular emission associated with these spots and for part of the general magnetic field of the

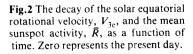
Edward H. Geyer of the Hoher List Observatory, University of Bonn, has studied the braking of solar rotation by magnetic activity and his conclusions have recently been published in *The Moon and the Planets (24, 399; 1981)*. Geyer compares the Sun with the primary component of the eclipsing binary XY Ursae Majoris (UMa). This is a solar-type star, with

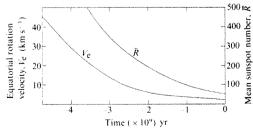
similar mass but about 50 times more magnetic activity than the Sun. The binary has an orbital period of 11.5 hours, the components are close and suffer considerable tidal deformation. The XY UMa primary has a spot cycle of about 3.7 years, in comparison with the solar cycle of 11.04 years. At maximum the XY UMa primary has about 20 per cent of its surface covered in spots. XY UMa is spinning quickly - the braking that would lead to a loss of rotational angular momentum is counteracted by a replenishment from the much larger orbital angular momentum of the secondary by a tidal feedback mechanism.

The rotational angular momentum of a star on the lower main sequence determines its spot activity. As magnetic brakes have been applied to the Sun for at least the past 4.6×10^9 years, the Sun must have been spinning faster and showing more magnetic activity in the past. Geyer assumes that the loss of angular momentum takes place at the same heliographical latitudes (5° to 40° north and south) as the maximum spot activity; this maintains the differential rotation. He also assumes that the mass loss is less than a few per cent of the present mass and that the radius has remained constant. He concludes that the angular velocity and the spot activity both decay exponentially, with a mean lifetime of 1.55×10^9 years. Today the mean sunspot number, \bar{R} , is 51 ± 35 and the equatorial spin velocity is 2.02 km s-1. The values of these quantities in the past are plotted in Fig.2.

The effects of these changes are interesting. In the past, the faster spinning Sun would be more oblate, which would perturb the orbital motion of the inner planets. The greatly enhanced sunspot activity must have influenced the evolution of life on the Earth. The increase in solar particle flux and extreme ultraviolet radiation would disturb the terrestrial magnetic field and the original atmosphere; the production rates of carbon-14 and tritium would be higher than they are today; and the atmosphere would be enriched in ³He.

Geyer concludes that evolution of life and its transference from the protecting water to the exposed land were governed by the solar activity and its decay with time.





David W. Hughes is in the Department of Physics, University of Sheffield.

Cosmochemistry and the origin of life

from Geoffrey Eglinton, Ann Henderson-Sellers and Stephen Moorbath

At a recent NATO conference* on 'Cosmochemistry and the origin of life'. Ponnamperuma (University of Maryland) summed up the overall picture by showing that there is a continuity stretching from space through to the present in terms of the formation of organic molecules and those that are found in present-day living organisms, and held that this is the natural course of events. Viola (Indiana University) started with the 'big bang' and then discussed nucleosynthesis and the abundance of the elements, particularly those essential to life. He also discussed the life cycle of stars and models of the Universe - salutary topics which should be compulsory course work for all scientists at some stage in their careers. Greenberg (University of Leiden) and Irvine (Chalmers University, Sweden) continued with details of the nature. composition and distribution of the interstellar dust and gas medium, and emphasized the enormous reservoir of organic molecules and most basic raw materials of life known to exist in interstellar space. Conference participants thought it unnecessary, at this state of our knowledge, to invoke Panspermia - the seeding of our planet, at any stage in its history, by life from extraterrestrial sources. In this much publicized context the conference generated neither science fiction nor science friction.

NASA's spaceship Enterprise, ably piloted by de Vincenzi (NASA Headquarters), took us on a tour of the Solar System, culminating in an account of the Mars Lander's biological experiments. Initial optimism and excitement soon gave way to the more sober realization that surface oxygen release could be better accounted for by presence of labile, active inorganic oxides in martian soil than by biological activity. Prospects for contemporary life on Mars are bleak, but perhaps in its originally airier and wetter manifestation it might have temporarily harboured the seeds of early life. The rest of the Solar System is decidedly no place for protoplasm!

The conference then rapidly came down to Earth. However improbable it seems, life and mind flourish, and some 4 billion years separate the earliest replicating organic molecules from the deliberations of the present conference. After presenting an inventory of terrestrial surface carbon, Henderson-Sellers (University of Liverpool) noted that, without its hydrosphere and biosphere, the Earth would be very similar to Venus, where carbon dioxide is not tied up in carbonate rocks. She, as well as Ponnamperuma, summarized much of

the evidence that the atmosphere of the early Earth was dominated by carbon dioxide, water, nitrogen and possibly some carbon monoxide, and not by ammonia, methane and hydrogen as was once widely believed. The bulk of the hydrosphere was undoubtedly present from very early prebiotic times. Ample energy sources, including electrical discharges, ultraviolet radiation, radioactivity and volcanic/geothermal heat, were available on the early Earth to foster complex, prebiotic organic syntheses from relatively simple mixtures of volatiles and gases, with clay minerals as a likely base for the earliest biotic processes.

Moorbath (University of Oxford) showed that isotopic dates on early Precambrian sediments containing biological or molecular fossils could frequently be obtained from stratigraphically closely associated igneous rocks, and more rarely from the sediments themselves. Algal stromatolites and other simple cellular structures were known from sediments probably as old as 3.5 billion years, although in some cases there is a worrying stratigraphic gap between the sediment and the dated igneous rock. Whether the Isua metasediments of West Greenland, dated at about 3.8 billion years, contain biogenic markers is still a matter of controversy. Schidlowski (Max-Planck-Institut, Mainz) is convinced from detailed carbon isotope studies that Isua carbonaceous sediments represent a 3.8 billion year-old record of biological activity. There is a continuity of the δ^{13} C record from the present back to Isua times, and yet the Isua rocks also exhibit a marked shift in δ^{13} C which may result from their strong metamorphic overprint. Indeed, some workers claim that the δ^{13} C record of the Isua rocks has been achieved by metamorphism alone. Walters (University of Maryland), after painstakingly pyrolizing 27 Isua graphite samples, observed no trace of biogenicity, with one possible exception. The metamorphism was certainly intense enough to erase any biogenic evidence.

Ochiai (University of Maryland) pointed out that bioinorganic chemistry was essential for understanding chemical evolution. Many trace metals, such as Fe, Cu, Mo, Mn, Co, Zn, Ni, Cr and V, form complexes which catalyse organic reactions considered essential for life processes. He also discussed the problem of the major Precambrian Banded-iron Formations, ranging in age from about 3.8 to 2.2 billion years, and regarded by many as biogenic markers in the sense that their precipitation may have required oxygen released from marine organisms, in the virtual absence of atmospheric oxygen. This 'iron age' was followed at about 1.5 billion years onwards by copper sulphide deposits — the 'copper

age'. Before this, prevailing redox conditions would not permit copper to appear in a dissolved, mobile state. A tentative correlation might be made between the sudden appearance of the copper deposits and the distribution of copper enzymes in present-day organisms. In this view, the copper superoxide dismutase is a later and more efficient development than the manganese and iron superoxide dismutases.

From the organic record in more recent sediments, Eglinton (University of Bristol) produced abundant evidence that molecules can retain a high degree of structural and stereochemical specificity over hundreds of millions of years if they are not exposed to raised temperatures or subject to oxidation or microbial attack. Specific biological marker compounds, or 'chemical fossils', can be interpreted in terms of the palaeoenvironment. The thermal history of a sediment can also be understood in terms of epimerization, aromatization and carbon-carbon bond cleavage reactions observed in samples with different burial histories. Of particular interest is the possibility of relating the molecular lipid record to microbial activity. Thus the archebacteria display unusual membrane lipids which may provide a clue to their activity during the Precambrian.

All participants agreed that there is an urgent need to continue the search for ancient, unmetamorphosed sediments earliest from the accessible Precambrian. so that micropalaeontologists, inorganic and organic geochemists, clay mineralogists, geologists and geochronologists can work closely together towards a progressive characterization, through geological time, of the biological record in the rocks. Recent geological work clearly shows that the earliest known (about 3.8-3.5 billion year) sedimentary and volcanic rocks characterize a depositional environment which is totally compatible with conditions now widely regarded as favourable for the earliest life on Earth. Of course, one big question was forcibly put to us by the students - "when did life begin?". The best bet was 4.0±0.1 billion years ago. The origin of life could be even older, but most people agreed that it was unlikely to be vounger.

Altogether, it was a creative and original Study Institute, which looked as much into the future as into the past. Is it just conceivable that a conference on the same topic is at this moment being held somewhere else up there in the night sky?

Geoffrey Eglinton is in the School of Chemistry, University of Bristol; Ann Henderson-Sellers is in the Department of Geography, University of Liverpool; and Suphen Moorbath is in the Department of Geology, University of Oxford.

^{*}The NATO Advanced Study Institute was held in Maratea, Italy on 1-12 June 1980. The director of the institute was Professor Cyril Ponnamperuma.

Holocene wiggles

from R.W. Fairbridge

THE trouble with time series relating to geophysical and climatic phenomena is that the instrumental data base is often less than 100 years long, whereas many of the suspected planetary steering mechanisms operate on quasi-periodic wavelengths in excess of 103 years. More precise chronostratigraphical methods are needed when looking for correlations in the area of celestial mechanics where hindcasting may often be estimated with a year-to-year accuracy, for at least 104 years. The customary geological dating technique in this age range, radiocarbon, commonly suffers from an accuracy of only ± 250 vears or so.

Some new developments discussed at a recent meeting* promise to provide a yearto-year chronology dating back to beyond 13,000 BP, with a precision of ± 1 year. First, there is the old varve chronology of De Geer. To begin with it suffered from a 'bad press'; people did not feel much confidence in a simple thickness counting. But over the past two decades the Swedish scientists and government have considered it important enough to spend several millions of kronor proving the accuracy of the method (within the error limits of radiocarbon), and re-counting the old series. I. Cato (University of Uppsala) recently completed the count up to AD 1980 and incidentally has shown that it is the annual peak flood (1-2 days) that corresponds to the maximum thickness of the

summer varve. Soon it will be possible to state the peak flood years in south and central Sweden for the past 13 millennia.

Second, the ¹⁸O/¹⁶O analyses of ice cores taken in Greenland and Antarctica are being extended and 'massaged' to generate far more precise time series than at first seemed possible. At the meeting, C. Langway (SUNY, Buffalo) described some of the techniques and W.D. Hibler (Cold Regions Research Laboratory, New Hampshire) demonstrated an approximately 20 year cycle recognized in both ice cores and tree rings since AD 530. W. Dansgaard (University of Copenhagen) recently reported1 some of the ice-core problems and solutions. Absolute (year-toyear) counting now goes back nearly 2 millennia and is rapidly being extended. Hammer, Clausen and Dansgaard² have described an ingenious pH-measuring device that records the acid layers in the ice cores that develop as a result of volcanic gas 'rain-out'. As Clausen (University of Copenhagen) demonstrated at the meeting, it is now possible to identify many historically known volcanic eruptions and thus independently confirm the layer counts. When combined with the oxygen isotopes, this gives a climatic reading.

The third category of Holocene wigglyline time series concerns dendrochronology. In contrast to varves and ice cores that reflect regional climatic trends, tree rings initially reflect only individual trees, but with regional studies and sophisticated analysis this material also furnishes valuable climatic series. In addition, and most importantly, the tree rings provide a fourth wiggly line — the variations in the radiocarbon flux — from which come the cosmic ray and solar wind fluctuations. We now have a picture of solar activity that takes us back nearly 8,000 years³, and this limit can be extended to beyond 13,000 BP as soon as some floating chronologies can be tied in.

The problem of floating time series is a speciality of D. Schove (St David's College, UK). There are numerous varve and dendrochronological series from Europe, North America, South America and Asia that have not been tied in to a known datum. If these long runs can be spliced together we shall get a year-by-year record of climatic events (local, regional and, we hope, global) going back nearly 14,000 years. Schove works on the principle that most of the wiggles represent local climatic 'noise' and may have little global significance, but certain key years disclose very remarkable 'spikes' which recur in distinctive patterns. The methodology works very well with tree rings, varves and, on a longer time scale, geomagnetic reversals.

Simplistic correlation attempts with the 11 year sunspot 'cycle' have often run aground, but analyses of phase changes and interactions with other cyclic effects can be rewarding, as reported by J. Harlin⁴. Schove⁵ has considered the fact that the quasibiennial cycle (26 months). when superimposed on a powerful annual signal, may eventually lead to a 3 year jump. Inasmuch as the sunspot maxima fluctuate markedly, the combined effects result in distinctive patterns for any given century. Although the astrophysical and meteorological mechanisms are still shrouded in mystery, the pattern system seems to work, providing the approximate time span is known — and this link is provided by radiocarbon and other geological techniques. Particularly important time series that may soon be tied in by these 'teleconnections' are the Finnish varves, described by M. Saarnisto (Oulu University, Finland), and those of southern Germany by J. Merkt (University of Hannover). Schove has proposed teleconnections in the mid-Holocene between America (Bristlecone), Ireland, the Crimea and Anatolia⁶.

Hints about the long-term variables were to be found in the palaeomagnetic records displayed at the conference by K. Creer (University of Edinburgh). Systematic coring of undisturbed lake beds from different parts of the world is now beginning to bear fruit. Both inclination and declination show systematic fluctuations with a quasi-periodicity of the order of 1,000-3,000 years. A variable rate of westerly secular drift introduces a longitudinal lag in dates, and at the same time warns of the variability in the outer core dynamics.

Inasmuch as both the geomagnetic field and the solar wind (a magnetized plasma

^{*}A conference on 'Holocene Correlation' was held on May 1, 1981 at the Warburg Institute, University College London, under the auspices of the UNESCO-supported International Geological Correlation Programme no.158. The meeting was jointly chaired by Dr. Derek Schove and Professor Rhodes W.



100 years ago

NOTES FROM THE MALAY
ARCHIPELAGO

A correspondent in Java sends us the

The cattle plague has been raging in the west end of Java, Bantam, the Preanger, and Batavia residences — during the west monsoon (now finishing) with redoubled vigour. It has now abated a little (after four years—it may well do so, from want of victims) in these parts, but is extending eastward, its appearance in Krawang being the most alarming. The authorities have decided upon making a double-fence right

across Java at its narrowest part. This means a line from somewhere about Cheribon due south. In the interval — a considerable one — between the two fences, no cattle will be allowed to pass or exist.

There is a bird (native name Jallak) which follows the buffaloes about and perches on their backs. Query, can this bird have anything to do with the spreading of the plague? If so I don't see what Government can do. They can't fence him out.

In all the parts where the cattle-plague has raged the most awful fevers have been the result amongst the native population. In Bantam alone 50,000 died in 1880. In the Preanger and Batavia the death-rate was also very high. There is no doubt whatever that this is due to the imperfect interment of the carcases. The Government says it is due to the wet season; but this is a lame excuse, for why is there no fever elsewhere? In the wet season it is, of course, worse, for the heavy rains cause more miasma.

From Nature 24, 25 August, 399, 1881.

R. W. Fairbridge is Professor in the Department of Geological Sciences, University of Columbia.

flow) modulate the incoming galactic cosmic rays, the radiocarbon flux, monitored by the tree rings, can now be related in part to core dynamics. A new monitoring isotope, beryllium-10, was discussed at the meeting by G.M. Raisbeck (Orsay, France). This isotope is also formed in the atmosphere by cosmic ray collisions and within a year deposited on the Earth's surface. It can be measured most conveniently in ice cores, thereby providing not only a way of cross-checking the 14C data, but also of extending the cosmic radiation record back for perhaps up to 105 years.

R.W. Fairbridge (Columbia University) displayed colour slides of the field evidence illustrating how the cyclicity of highlatitude storminess cycles on the Hudson Bay seems to reflect a 45 year 'Double Hale' solar cyclicity7. He then reviewed new unpublished works that demonstrate solar-planetary periodicities from the Permian period, 250-280 million years ago: first, from an anhydrite series in New Mexico (R.Y. Anderson, University of New Mexico); second, from an evaporite series in the Ural Mountains, USSR (I.

Vulis); and third, from a varve series in Brazil (Rocha-Campos). The astonishing thing is that they all display what seem to be the same periodicities that occur in the Holocene, which, if nothing else, would appear to dash the hopes of velikovskian catastrophists.

- Dansgaard *Nature*, *News & Views* **290**, 360 (1981). Hammer, Clausen & Dansgaard *Nature* **288**, 230 (1980).
- Eddy Scient. Am. (1977). Harlin Phys. Geogr. 1(2), 108.
- Schove Weather 26, 201 (1971).
- Schove Palaeogeogr. Palaeoclimatol. Palaeoecol. 25, 209 (1978); in Moraines and Varves (ed. Schlütter; Balkema,
- 7. Fairbridge & Hillaire-Marcel Nature 268, 413 (1977).

Pre-equilibrium processes in nuclear reactions

from P.E. Hodgson

THE processes that take place when a fast nucleon (10-100 MeV) interacts with a nucleus are quite well understood, but there are many detailed features that require further study. The incident nucleon first collides with a single nucleon or perhaps a cluster of nucleons in the target nucleus. The products of this collision have a high probability of escaping from the nucleus, particularly if the collision takes place on the periphery. This initial interaction takes place rapidly, in the time it takes for the incident particle to traverse the target nucleus, typically about 10-22 seconds.

The particles from this first collision that do not escape from the nucleus undergo further collisions until all their energy is transferred to the compound system. The energy is shared among the nucleons until eventually, by statistical fluctuation, a nucleon or group of nucleons receives enough energy to escape; this continues until the energy falls below the particle emission threshold. The residual nucleus eventually attains its ground state by y and B decay. The statistical processes by which the compound nucleus loses its energy take much longer than the direct process, typically about 10-16 seconds.

The direct and compound processes are quite well understood theoretically, so the corresponding cross-sections can be calculated with some confidence. The simplest direct process, elastic scattering, can be calculated by the optical model, and inelastic scattering by the coupled channels or distorted wave formalism. Nucleon transfer reactions can be treated by the distorted wave Born approximation, and the probabilities of emission of various particles from the decay of the compound nucleus can be calculated using the Hauser-Feshbach theory with the transmission probabilities obtained from optical model analyses of elastic scattering.

The direct and compound processes usually both contribute to each reaction and the total cross-section may be obtained

by adding them incoherently. This may be compared directly with the measured crosssections, after the latter have been energyaveraged to remove the fluctuation in the compound nucleus cross-section.

Such calculations are generally fairly successful at lower energies, but at higher energies additional processes can occur and have to be accounted for. In particular, it is possible for particles to be emitted after the direct process but before the nucleus has attained statistical equilibrium. These preequilibrium or pre-compound particles can be recognized experimentally and their cross-sections have been calculated using several different models.

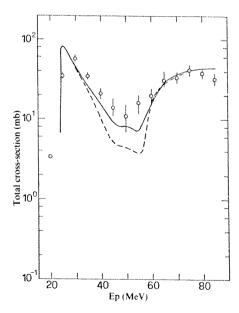


Fig.1 The excitation function for the 50Ti(p,2p2n) 47Sc reaction compared with exciton model calculations. The dashed line shows the result of a calculation without the pre-equilibrium particles and is clearly inconsistent with the data. From ref.3.

P.E. Hodgson is in the Department of Nuclear Physics, University of Oxford.

The particles emitted in the direct, preequilibrium and compound stages are characterized by their differing energy and angular distributions. Direct particles are emitted predominantly in the same direction as the incident particle, with similar energies, whereas compound nucleus particles are emitted in all directions, equal numbers forwards and backwards, and when the final states merge into a continuum have a characteristic maxwellian energy distribution. Preequilibrium particles tend to be emitted forwards, and are generally more energetic than those from the compound nucleus.

To calculate the cross-section for preequilibrium emission, the interaction process is considered to take place in several stages. The first stage can lead to the emission of direct particles, or to the excitation of a particle-hole pair. In the second stage further interactions can occur, which can lead to the excitation of further particle-hole pairs, and so on until finally the compound nucleus is formed. At each stage in the excitation process it is possible for pre-equilibrium particles to be emitted.

This process has been formulated mathematically, enabling the pre-equilibrium cross-sections to be calculated. One type of theory due to Blann¹, Griffin², Gadioli³ and others gives semi-classical expressions for the cross-sections in terms of the basic transition matrix elements that express the probability of going from one stage to the next. Such theories have been used to calculate the cross-sections of many different reactions and a typical comparison with experiment is shown in Fig.1. In this work the cross-section is determined radiochemically, so it is not possible to distinguish between the (p, a) and the (p, b)2p2n) reactions. The former reaction is favoured energetically and is responsible for the first peak, and the latter for the second. The two curves are calculated with and without the pie-equilibrium contribution, and comparison with the experimental data shows the presence of these

More recently, a quantum-mechanical theory has been developed by Feshbach, Kerman and Koonin⁴, and numerical calculations made by Colli-Milazzo and collaborators⁵. This gives both the angular and energy distributions of the emitted particles, and extensive comparisons with

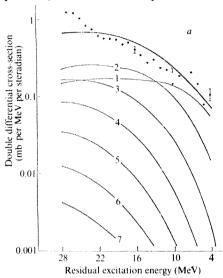
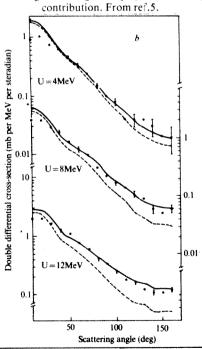


Fig.2 a, Energy spectra for the 48Ca(p,n) reaction at 45 MeV at 90° as a function of the energy of the residual nucleus. The individual curves show the contributions of the various multistep processes. The excess of particles at higher excitation energies, corresponding to lower emission energies, is attributed to multiparticle emission. b, Differential crosssection for the 90Zr(p,n) reaction at 45 MeV showing the single-step (dashed line)



- Blann, M. A. Rev. nuclear Sci. 25, 123 (1975)
- Griffin, J.J. Phys. Rev. Lett. 17, 478 (1976); Phys. Lett.
- Gadioli, E., Gadioli, Erba, E., Hogan, J.J. & Burns, K.I. Phys. Rev. (1981).
- Feshbach, H., Kerman, A. & Koonin, S. Ann. Phys. 125, 429 (1980).
- Colli-Milazzo, L., Bonetti, R., Camnasio, M. & Hodgson, P.E. Phys. Rev. (1981).
 - Tamura & Udagawa, T. Phys. Lett. 71B, 273 (1977); 78B, 189 (1978).

the experimental data for (p,n) reactions from 25 to 45 MeV on nuclei from calcium to tin show good overall agreement. An example of such a comparison is given in Fig.2, showing the contributions of the one-step (direct) and various multi-step (pre-equilibrium) processes.

Calculations of the contributions of multi-step processes to proton inelastic scattering have been made by Tamura and Udagawa6 using an extended version of their distorted wave formalism. This theory is also able to give a good overall account of the measured cross-sections.

There is still much work to be done to justify and establish the accuracy of some of the assumptions and approximations made in these calculations, and it will be important to test the different theories by using them to analyse the same reactions.

Mammal tracks on mountain slopes

from Robert M. May

ONE of the many differences between the Victorian Age and our own is written in the paths in the English Lake District. Today's path from Wasdale Head to Sty Head follows the direct line (and the steepest slope) across the shoulder of Great Gable; few use the nineteenth century path that zig-zags in a more leisurely and scenically varied way along the side of Lingmell Beck. From the head of Langdale one can ascend to Angle Tarn directly by the stoney boredom of Rossett Gill, or more slowly via the sweeping switchbacks of the almost vanished pony track.

Faced with these choices, the behavioural ecologist may be prompted to ask which paths are energetically optimal. Some pertinent laboratory and theoretical studies are due to Taylor, Caldwell and Rowntree (Science 178; 1096, 1972). These authors began by observing that the energy needed to elevate an animal to a given height is proportional to its body weight, whereas the animal's power output available to perform this task tends to be roughly proportional to its surface area; hence the energy costs of moving uphill (compared with moving at the same speed on level ground) are relatively greater for larger animals. Turning to the laboratory, Taylor et al. substantiated this analysis by showing that movement up a 15° incline at 2 kmh⁻¹, compared with running on level ground at the same speed, resulted in a 40 per cent increase in oxygen consumption for a chimpanzee, but only 1-2 per cent for a mouse. Extrapolating their experimental results, they estimated that running vertically at 2 kmh-1 would increase the oxygen consumption of a 30 g mouse by 23 per cent, of an 18 kg chimpanzee by 190 per cent, and of a 1,000 kg horse by 630 per cent.

This work helps explain why squirrels can run up trees at much the same speed as they run from tree to tree, and why fell

Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

runners are lean. But it sheds no direct light on whether largish animals should ascend hills directly, or obliquely in zig-zags. Indeed, an analysis along the lines of Taylor et al. suggests that the energy involved (in addition to baseline metabolic costs) in getting an animal of mass m to the top of a mountain of height h is simply mgh, independent of the angle of attack. A more gradual ascent, at an angle of ascent θ relative to the horizontal, involves a relatively smaller cost of moving up the slope (by a factor $\sin \theta$), but takes longer to reach the top (by a factor $1/\sin\theta$); the two effects cancel each other out.

Reichman and Aitchison (Am. Nat. 117; 416, 1981) have recently made a most interesting attempt to find out how animals actually do ascend or traverse hillsides in nature. To this end, they measured trails left in the snow on hillsides in the southwestern United States, by various mammals. By choosing fresh snow that covered existing trails, these authors hoped to find trails that had been made at the discretion of the individual animals; records were compiled only for situations where the animal appeared to be moving freely up a slope, rather than wandering from one feeding spot to another, or fleeing pursuit. Each mammal was identified by its footprints, and the average weight for that species assigned to it. In this way, Reichman and Aitchison compiled data for some 130 individual animals, of 22 different weights ranging from a 0.025 kg mouse to a 680 kg buffalo.

Reichman and Aitchison defined the 'trail angle' to be the angle between the actual trail on the hillside and the horizontal; thus an animal proceeding directly up the mountain at the angle of steepest ascent has a trail angle of 90°, while an animal moving around the hillside at a constant elevation has a trail angle of 0°. This trail angle has a slight, but statistically very significant, propensity to be smaller for heavier animals. The negative correlation is even more striking if one plots the maximum trail angle observed for any one body size against the weight of the animal. As Reichman and Aitchison observe, "it seems intuitive that the trail angle used by animals should decrease with increased body size, and the field data support this outcome".

A clearer test of the basic ideas, however, is provided by examining the correlation between trail angle and the slope of the mountain itself. The analysis outlined above suggests that, to a first approximation, the energy costs are the same whether one ascends obliquely or directly. This would lead to the expectation that the trail angle is uncorrelated with the slope of the mountain. Alternatively, for animals in a hurry to get to their destination, we might often expect a direct ascent, and hence trail angles of 90° irrespective of the mountain slope. This latter expectation may be modified for large animals on steep slopes, where the metabolic costs of sustaining uphill motion may become prohibitively large; that is, there may be a maximum slope, $\theta_{max}(m)$, beyond which an animal of mass m cannot sustain uphill motion. Such a θ_{max} will decrease with increasing body weight; on an almost vertical hillside, mice and squirrels may scamper up directly (trail angle of 90°), but heavier animals are likely to ascend obliquely at a trail angle of about θ_{max} . Of course, questions of local topography and, above all, the purpose of the journey would complicate all this.

On this basis, I would expect that, when Reichman and Aitchison's data (embracing all body weights) were plotted in the form of trail angle versus mountain slope, there would either be no correlation at all, or else a propensity for trail angles to be random at low mountain slopes, and for steep trail angles to be rarer at high mountain slopes. In fact, such a plot reveals a puzzling tendency for trail angle to be approximately proportional to mountain slope. Remarkably, essentially no animals — not even the mice — are ascending shallow hills directly (at trail angles of 90°).

It seems likely that the animals making these tracks were, by and large, simply wandering across or skirting the shallow-sloped mountains. (Though why they should be doing so almost exclusively at low trail angles remains a mystery to me.) On the steeper hillsides the tracks may be telling us more about energetics of moving up a slope, but even here it could be that the geometry of the tracks depends mainly on why the animal was going from A to B, rather than on anything else.

In short, the study by Reichman and Aitchison is a very original and thought-provoking one, but I think the next steps will need to include consideration of the behavioural ecology of the animals in question. If we seek to plot our paths through the hills according to principles of optimality rather than sheer delight, the current literature remains unhelpful.

HLA genes, immunoglobulin genes and human disease

from Ken Welsh

THE human lymphocyte antigens (HLA) were recently described by one of my colleagues as simple equivalents of the ABO system found on all cells except red cells. In fact the HLA system comprises a group of several hundred genes located on the short arm of chromosome 6 but the only gene products of this region well recognized in the human HLA-A.-B.-C.-D and -DR and complement components C2, C4 and Bf. All these are polymorphic to a greater or lesser extent and a range of products of this region is expressed on most lymphoid cells.

Almost ten years ago at an International Transplantation meeting in San Francisco, participants were given large badges inscribed with the words "Are you my HLA type?". At that time the close association between HLA-B27 and ankylosing spondylitis (AS) had just been rumoured1,2 and prevailing evidence suggested that good HLA matching of donor and recipient would solve the major problems of transplant rejection. The badge implied not only hope for transplantation but also that by suitable selection of partner, major diseases could be avoided in the next generation. Now, although hyperacute rejection has been virtually eliminated, we are no closer to predicting acute or chronic rejection on the basis of HLA type and the closest HLA and disease association is still that between HLA-B27 and AS. But the years of consolidation and continual searching are beginning to be rewarded; at the recent European Dialysis and Transplantation meeting in Paris we heard how revisitation of red cell types and skin testing techniques coupled with precise HLA cross-matching and HLA-DR typing have again given us hope for success in transplantation.

On the question of HLA and disease, a paper published in this week's issue of Nature (p.768) by Uno and colleagues may provide the necessary break in the flood wall and enable us accurately to identify individuals at risk from certain major diseases. Several cracks had already appeared in the wall, so a break is not unexpected.

The first came with the finding that a combination of HLA type and acetylator status can identify with very high reliability those women who will develop auto-immune complications if treated for hypertension with the drug Hydrallazine³. HLA-DR4-positive, slow acetylator females always develop the complication;

Ken Welsh is in the Tissue Typing Laboratory at Guy's Hospital, London.

DR4-positive, slow acetylator males will escape unless given high doses of the drug. About 8 per cent of hypertensive patients treated with Hydrallazine develop a lupuslike complication. In the normal population about a quarter are DR4 positive, and half are females and half are slow acetylators, giving us an expected complication rate of about 6 per cent. Occasional DR4-positive males on high drug doses, DR4-positive rapid acetylator females and DR4-negative slow acetylator females develop the complication and account for the small discrepancy. This discovery shows how a combination of apparently unrelated genetic 'markers' can be used for accurate identification of a population at risk.

Two other recent discoveries bear directly on the work of Uno et al. The first was that the relative risk of developing chronic active hepatitis was 11.7 for those people having HLA -B8/DR3 and 2.3 for those having a particular immunoglobulin allotype4 - a genetic marker expressed on antibodies. As in the Hydrallazine lupus story, a combination of the two markers is associated with a significantly greater risk, in this case 40-fold greater, than if neither marker is present. The second discovery5 was that relapse in hyperthyroid Graves' disease can be predicted by a combined analysis of HLA -DR3 status and the level of thyrotrophin receptor antibody at the time of drug withdrawal. The prediction in this case was accurate in 62 out of 65

An alternative approach to identifying individuals at risk would be to use a combination of genetic marker and family studies. For example, if incidence of a disease is 1 in a 100 in the population and the disease is associated with HLA-B27 (which is present in about 10 per cent of the population), then even if all the people who get the disease are B27 positive it still means that only 1 in 10 B27-positive people are at risk. However, if the disease is already present in a family, this risk rises very dramatically for B27 family members.

Uno and co-workers have combined these two approaches in their study of Japanese families having more than two first-degree relatives affected by Grave's disease. In the data presented, 32 out of 33 children suffering from this disease carried a particular HLA and IgG-linked gene combination. The single child having only the IgG-linked susceptibility could be explained by a recombination between the HLA marker gene and a hypothetical HLA gene actually responsible for the disease. A more complete HLA typing involving SB

antigens, complement allotyping and additional enzyme markers would clarify the point.

Although in the face of the evidence presented one tends to think of tissue typing at birth (to predict the occurrence of autoimmune disease) or perhaps even before one goes to the computer dating service, there are practical scientific reasons for being cautious. For example, Uno et al. selected only 15 of the 30 families studied for inclusion without saving how or why this selection was made. Second, IgG allotype frequencies are very different in Caucasoid and Japanese populations. Third, Graves' disease is very strongly associated with B8/DR3 in Caucasoids but with DR5 and DR8 in Japanese populations. A study of Japanese families living in America might show whether these differences are due to a different environmental factor interacting with different IgG or HLA haplotypes in the two populations or to putative susceptibility genes going with different allotypes in the two populations.

Theoretically the reasons for expecting HLA and immunoglobulin-gene linked associations with immune response in general and autoimmune disease in particular are overwhelming: HLA-DR antigens (or antigens in the same chromosome area) are necessary for antigen handling and presentation by a lymphoid cell subset; markers in this region (by analogy with the mouse) are important for interaction of T cells during a response; immunoglobulin genes are also involved in T-cell recognition and control; HLA-A,-B and -C antigens are important at the effector arm of the cellular response; and finally, C2, C4, Bf and immunoglobulin are involved at the effector arm of the humoral response. Thus, HLA and immunoglobulin genes are active throughout the immune response from recognition to control and it is slight differences in an individual allele's ability to function, or more exactly a summation of slight differences, which we are measuring as disease susceptibility. Theoretically therefore we expect that use of HLA and immunoglobulin allotyping data together with other genetic markers and environmental factors should allow autoimmune diseases to be predicted exactly. However, there is still a considerable amount of analysis of both HLA-region genes and IgG-region genes to be done in order to achieve this goal in the general population.

In Japanese families, the occurrence of Graves' disease can be exactly predicted on the basis of HLA and immunoglobulin allotypes, but it is too early to start wearing "Are you my HLA type?" badges outside Japan. Nevertheless, elimination of autoimmune diseases by genetic counselling is now much closer to being realized: if two independent susceptibility genes are necessary for disease onset, then it is possible to ensure that the genes will not occur together in the next generation.

Positronium and positronium ions

from T.C. Griffith

POSITRONS and positronium, the bound state of an electron and a positron, have in recent years been used in a rapidly increasing range of experiments in atomic physics, solid-state and surface physics and positronium chemistry. Positronium has also played an important part in fundamental experiments designed to check the validity of certain aspects of the theory of quantum electrodynamics (QED). It is therefore pertinent that the recent detection of a new entity, the positronium negative ion, Ps- (e-e+e-), initially predicted to exist as a bound state by Wheeler1 in 1946, should be greeted as an important historical landmark which could greatly extend the activities of positron physicists. The careful experiment performed by Allen Mills2 at the Bell Laboratories, New Jersey clearly demonstrates that Ps can be produced in the laboratory under controlled conditions.

Ps⁻ is the analogue of H⁻ ions produced by slow proton bombardment of thin foils and the method used by Mills incorporates the same ideas. In his experiment, a nearly monoenergetic beam of low-energy positrons, guided by a longitudinal magnetic field, strikes a thin film of carbon in ultra-high-vacuum conditions. The energy of the positrons is adjusted such that the beam is partially transmitted and application of a suitable potential to a grid located at the exit side of the carbon film will reflect the transmitted positrons back to the carbon whilst accelerating the weak beam of Ps- into a field-free drift region beyond the grid. A lithium-drifted germanium detector is used to measure the energy spectrum of the resulting annihilation photons and a y-ray peak is found whose energy depends on the accelerating potential applied to the grid. It was demonstrated that this peak can be attributed to a Doppler-shifted annihilation y resulting from the 2y decay

As well as being of interest in its own right, Ps-is also important because it might be used to produce beams of positronium of well defined energies. This could be achieved by accelerating the Ps- to a given energy and then photoionizing it to give positronium which could, perhaps, be used in scattering experiments. Another suggested application is to use the annihilation of Ps in flight to provide a tunable source of y rays of precisely known relative energy shift. The intrinsic properties of the Ps-such as its lifetime (~ 2 nanoseconds), its 2y angular correlation, its (1y/2y) branching ratio, its magnetic moment and measurement of its photoionization cross-section are obvious candidates for extensive studies.

The glamour of detecting the Ps- ions should not be allowed to overshadow the deeper significance of this experiment. The major triumph underlying this work is the dramatic technical advances associated with the production of intense beams of slow positrons of well defined energies achieved at Bell Laboratories and at Brandeis University by Karl Canter and coworkers3. Technology has now reached such a level that within a few years lowenergy positron beams of intensities of 109 positrons per second, or even several orders of magnitude higher, can be envisaged. Positron emitters created in situ in a nuclear reactor followed by carefully designed moderators and beam transport systems may be used for this purpose. If allowed to strike clean metal surfaces under ultra-high-vacuum conditions these positrons can be converted, with almost 100 per cent efficiency, into low-energy positronium atoms. We might therefore expect intense and highly localized sources of positronium atoms which can be used to study the excited states of positronium using laser beams in experiments, similar to those performed for atomic hydrogen, that will provide further high-precision tests of

Even more exotic possibilities exist. Quantum chromodynamics predict that orthopositronium can decay into a new elementary particle, the axion, and a y ray. The axion must have a mass of less than 1 MeV for this to be possible and if this were the case, then an intense positronium source from a low-energy positron beam could well find application in a search for the axion.

Brewerton, D.A. et al. Lancet i, 904 (1973).

Schlosstein, L., Terasaki, P.I. & Bluestone, R. New Engl. J. Med. 288, 704 (19737.

Batchelor J.R. et al. Lancet i, 1107 (1980).

Whittingham, S. et al. Clin. exp. Immun. 43, 80 (1981).

McGregor, A.M. et al. Lancet i, 1101 (1980).

T.C. Griffith is in the Department of Physics and Astronomy at University College London.

Wheeler, J.A., Ann. N.Y. Acad. Sci. 48, 219 (1946).
 Mills, A.P. Jr Phys. Rev. Lett. 46, 717 (1981).

^{3.} Canter, K.F. Review to be published in Can. J. Phys.

Two Voyagers to Saturn

The pages which follow may be taken as a celebration of the impending encounter of Voyager 2 with Saturn and are a pointer to the richness of data likely to be gathered in the next few days and weeks. Although there may be some to whom the work of these two remarkable spacecraft will be proof of how even more remarkable would have been the Grand Tour of the Solar System planned in the 1960s but executed only in the form of the two Voyagers, to most people the encounters with Jupiter and Saturn will seem exciting enough for the time being. Yet there are Uranus and Neptune to come.

This group of scientific articles includes some of the first detailed attempts to make sense of last year's Voyager 1 observations of Saturn—and Nature acknowledges its debt to Dr G. E. Hunt of University College, London for having helped to recruit these articles and to give shape to the ground they cover. The scope is necessarily restricted, for there is hardly a branch of planetary astronomy which has not been changed in some way by the data from Voyager 1—and which is not about to be changed again.

How Voyager 2 has been reprogrammed

from Edward C. Stone

In the past two years, the Pioneer 111,2 and the Voyager 13,4 encounters with Saturn have revealed a remarkable richness and diversity of physical phenomena. As a result of these discoveries and of continued Earth-based studies, major changes have been made in the observations planned for Voyager 2, providing additional opportunities for discovery and understanding of the Saturn system. These opportunities are further enhanced by the significantly different path that Voyager 2 must take through the system, passing 101,000 km above Saturn's cloud tops on 26 August as it continues on to Uranus. Of necessity, the most exciting discovery will be unexpected; but some of the opportunities for new observations are described below for each of the four major areas of investigation—the atmosphere, rings, satellites and magnetosphere.

Atmosphere

The study of the dynamics of Saturn's atmosphere is of interest because Saturn, like Jupiter, is a giant rapidly-rotating body composed mainly of hydrogen and helium, encircled by bands of clouds of frozen ammonia with large-scale wind patterns distinctly different from those of the Earth's atmosphere. On Jupiter, there are opposing eastward and westward jet streams at the edges of adjacent cloud bands, with a maximum eastward equatorial velocity of $\sim 450 \text{ km h}^{-1}$. On Saturn, Voyager 1 found equatorial winds four times faster and found that the pattern of opposing jet streams occurred only above ~35° latitude.

Voyager 2 will not only yield a monthlong time-lapse movie of the wind that the bright broad B-ring appears to consist of hundreds of ringlets separated by narrow gaps, each one of which may be cleared by small, kilometre-sized satellites. The Voyager 2 photopolarimeter will observe the star Delta Scorpii through the shadowed region of the rings for 2 h 18 min, during which time the star will

move radially outward behind the rings, its apparent intensity varying as it moves from behind a ringlet into the adjacent gap. This stellar occultation should determine the location, width and spacing

patterns, but will also provide a highresolution view of the pattern of opposing jet streams in the north polar region. The winds in the atmosphere above the clouds exhibit a different pattern which will be determined from a high-resolution thermal map of the upper atmosphere. Other studies include a deep probing of the temperature and pressure of the atmosphere by the radio beam as the spacecraft disappears behind the planet, where images of the dark side of Saturn will be taken to search for lightning. Since Voyager 1 discovered UV auroral emissions at 80° latitude and similar emissions near the equator, Voyager 2 will also perform a systematic latitude search for such emis-

Rings

The rings, the second major area of study, presented most of the surprises from Voyager 1, and the Voyager 2 observations programme has, as a result, been almost completely revised and enlarged. The Voyager 2 observations will be further improved by the increased solar illumination of the north face of the rings as the Saturn year continues towards northern summer and by a much closer approach to the rings than on Voyager 1. Unlike both Voyager 1 and Pioneer 11, Voyager 2 will view the lighted face of the rings at closest approach.

One of the Voyager 1 discoveries was

of the ringlets with a precision of 100–300 m. A second, shorter stellar occultation observation of Beta Tauri will provide five times more precise measurements of the braided F-ring and the outer edge of the main ring system. Near closest approach, the highest-resolution images should reveal any structure down to the sub-kilometre scale.

The dynamics of the braided F-ring will be studied in a sequence of observations keyed to assessing the importance of various physical processes in its formation. The fine submicrometre-sized material in the braids is radially confined by two shepherding satellites (1980 S26 and 1980 S27) on either side of the F-ring. Images of the F-ring near these two satellites may indicate whether they are perturbing the motion of the ring particles in such a way as to cause the braided appearance while repeated images of the ring should indicate whether the braided structure is stable or varies on a short time scale. Images of the portion of the ring emerging from Saturn's shadow may provide clues to the influence of electrostatic charging of the small ring particles on the braided structure, since the electrostatic charge on the particles will probably change with the cessation of photoelectron emission normally occurring in the sunlight.

The 'spokes' in the B-ring are also not understood, although they appear to form sporadically in Saturn's shadow and then almost to dissipate before the completion of a single orbit about Saturn. Like the F-ring braids, the spokes consist of fine particles, possibly elevated in a cloud above the ring plane. As Voyager 2 crosses the ring plane, three images will be taken of the rings edge-on, in search for any elevated material. Before closest approach, a 13.5-h time-lapse movie will be taken to study the dynamical properties of individual spokes as they are distorted by the greater angular velocity of the spoke material nearer Saturn.

Edward C. Stone is the Chief Scientist of the Voyager Project at the Jet Propulsion Laboratory and Professor of Physics at the California Institute of Technology, Pasadena.

Table 1 Voyager 2 closest approach distances to Saturn's satellites (23-26 August 1981)

Name	Diameter (km)	Orbital radius $(R_s)^*$	Closest approach distance (km)†
1980 S28	40×20	2.28	287,170
1980 S27	220	2.31	246,590
1980 S26	200	2.35	107,000
1980 S3	90×40	2.51	147,010
1980 S1	100×90	2.51	222,760
Mimas	390	3.08	309,990
Enceladus	500	3.95	87,140
1980 S25	30-40	4.88	284,400
Tethys	1,050	4.88	93,000
1980 S13	30-40	4.88	153,520
1980 S6	~160	6.26	318,200
Dione	1,120	6.26	502,250
Rhea	1,530	8.74	645,280
Titan	5.140	20.25	665,960
Hyperion	290	24.6	470,840
lapetus	1.140	59	909,070
Phoebe	~160	215	2,075,640

- * Mean distance of the satellite from the centre of Saturn in units of Saturn radii (1 $R_s = 60,330$ km).
- † From a distance of 100,000 km, the geometrical resolution of the imaging system is 2 km per line pair.

The closer approach to the rings also makes possible further study of the electrostatic discharges detected by Voyager 1. Although the origin of the discharges has not been directly determined, they occur episodically every 10 h 10 min, suggesting an association with a satellite with this orbital period embedded within the B-ring. Additional high time-resolution samples of electromagnetic wave activity will be acquired over the 10-40-MHz frequency range, including one when the spacecraft crosses the ring plane.

Satellites

Another major area of study is the saturnian satellites, of which 17 are now known. Of these, only Titan is a major satellite, similar in size to the planet Mercury. Titan, with a dense atmosphere which includes nitrogen and methane in which organic photochemistry is occurring, was a primary target for Voyager 1. Voyager 2 will observe Titan from a much greater distance (see Table 1), returning information on the light-scattering properties of the photochemical haze and possibly on the winds.

Seven of the satellites are intermediate in size, composed mainly of water ice, with diameters ranging from about 300 to 1,500 km. Of these, Voyager 1 observed Mimas, Dione and Rhea with high enough resolution to show that their icy surfaces are heavily cratered from impacts, as was expected. Unexpected, however, were regional systems of fractures and sparsely cratered plains which are evidence of significant crustal evolution. Voyager 2 will extend the high-resolution coverage to Tethys, which has a ~750-km-long valley, and to Enceladus, which is highly reflective and appears to be relatively devoid of impact craters. The absence of craters on Enceladus would require a source of energy large enough to cause significant modification of the surface. To investigate Enceladus's thermal properties, the rate of cooling of the satellite as it moves into Saturn's shadow will be

determined from IR temperature measurements.

The second secon

Less is known about Hyperion and Iapetus, the other two intermediate satellites. Voyager 2 will fly closer to both than did Voyager 1, returning images with enough resolution to determine whether there are large valleys as on Tethys or whether there is any other evidence of crustal evolution.

As indicated in Table 1, there are at least nine other smaller satellites with estimated diameters ranging from about 30 to 220 km. Voyager 1 determined approximate shapes for only 1980 S28, which is just outside the main rings, and the two satellites, 1980 S1 and 1980 S3, which share essentially a common orbit. All three were found to be distinctly nonspherical. Voyager 2 will determine the shape of all the remaining known satellites, including the F-ring shepherding satellites (1980 S26 and 1980 S27), the two satellites recently discovered in ground-based images⁵⁻⁷ which share a which share a common orbit with Tethys (1980 S13 and 1980 S25) and 1980 S6, which shares an orbit with Dione. There will also be a search for new satellites, both outside and embedded within the ring system. Photometrically accurate measurements of the amount and degree of polarization of light scattered from the different satellites will provide additional information on the texture and gross composition of their surfaces.

Plasma

The fourth major area of study is that of the planetary magnetic field, plasma, trapped energetic particles and wave emissions comprising Saturn's magnetosphere. Periodic bursts of radio emissions in the 100-500-KHz frequency range provided the first accurate determination of the period of rotation of Saturn's magnetic field, which is believed to be the period of rotation of Saturn's interior regions where the magnetic field originates. This period, which is 10 h 39.4 min, will be more accurately determined with the longer time-base provided by Voyager 2, while measurements of Saturn's magnetic field may identify the longitudinal asymmetry resulting in the localization of the radio emission to specific saturnian longitudes. Voyager 2 will also provide additional observations of the 2.7-day modulation of the radio emissions thought possibly to result from Dione's interaction with the magnetosphere.

The effects of Mimas and Enceladus on the magnetosphere will be directly measured as Voyager 2 passes just inside Mimas's orbit. Measurements deep within the magnetosphere will better define the structure of the equatorial disk of plasma and the extent to which the icy satellites are sources or absorbers of magnetospheric particles. From Voyager 1, it is known that Titan's atmosphere is the source of at least some of the particles.

Voyager 2 may also provide a rare opportunity for observing the effects of Jupiter's magnetotail on Saturn's magnetosphere. There is recent evidence from Voyager 2 that Jupiter's magnetotail, which may resemble a tattered wind sock flapping in the solar wind, extends at least 4.5×10^8 km outwards from Jupiter. Because, during the past few months, Saturn has been almost radially aligned with Jupiter, it is likely that Saturn has been occasionally immersed in the extended jovian magnetotail. Such an immersion could at least temporarily result in variations in the size, shape and other characteristics of Saturn's magnetosphere, leading to the possibility that there are significant differences between the Voyager 2 observations and those of Pioneer 11 and Voyager 1.

Outcome

Intensive observations of the saturnian system, which began on 5 June, will continue until 28 September, when Voyager 2 will return to the cruise mode and, like Voyager 1, continue exploring the interplanetary medium at increasing distances from the Sun. In late 1985, Voyager 2 will return to the encounter mode as it approaches a flyby of Uranus on 24 January 1986, and heads towards an encounter with Neptune on 24 August 1989. Beyond Neptune, both spacecraft will continue exploring the outer limits of the region dominated by the solar wind, where low-energy galactic cosmic rays may be first observed and beyond which lies the interstellar medium. Thus, there is potential for a continued and significant scientific return from the Voyager spacecraft well beyond that originally planned for the mission to Jupiter and Saturn.

- Science 207, 400-453 (1980).
- Science 212, 159-243 (1981). Science 212, 159-243 (1981). Nature 292, 675-755 (1981).
- Seidelmann, P. K. et al. Preprint (1981)
- Veillet, C. IAU Circ. No. 3593, 3608 (1981).
- 7. Larson, S. M. Icarus (in the press).

Thermal structure and dynamics of Saturn and Jupiter

J. A. Pirraglia⁺, B. J. Conrath⁺, M. D. Allison[†] & P. J. Gierasch[‡]

* NASA/Goddard Space Flight Center, Code 693.2, Greenbelt, Maryland 20771, USA
† Department of Space Physics and Astronomy, Rice University, Houston, Texas 7701, USA
‡ Laboratory for Planetary Studies, Cornell University, Ithaca, New York 14853, USA

High resolution Voyager IRIS measurements for Saturn and Jupiter are assembled in meridional cross-sections of the retrieved upper tropospheric temperatures. The calculated thermal wind shear in the upper troposphere is highly correlated on both planets with the cloud top winds derived from imaging data. In contrast, temperatures below ~ 300 mbar are not simply related to the zonal jet structure. The upper tropospheric temperatures seem to have been more consistently correlated with cloud top winds than with major albedo features at the time of the Voyager encounters.

THE atmospheres of Saturn and Jupiter are the most energetic observed in the Solar System. Both planets are encircled by high velocity super-rotating equatorial currents and an attendant system of axially-symmetric westward and eastward jet streams at higher latitudes. While differing in their axial tilt, gravitational potential, emission temperature and visual contrast, Saturn and Jupiter are approximately similar in size, rotational period, and gross composition. Despite these similarities major differences in their dynamical structures are observed. Smith et al. report that the equatorial prograde velocities measured relative to the radio period are much larger and wider in latitudinal extent on Saturn than on Jupiter. Also, with one exception, anticyclonic shear on Jupiter is generally well correlated with brighter cloud bands or zones while on Saturn no such correlation is evident.

A preliminary analysis² of the saturnian atmosphere by the Voyager IR instrument (IRIS)³ has revealed substantial latitudinal structure in upper tropospheric temperatures. This structure is further investigated here by constructing a meridional temperature cross-section and, for comparison, similar cross-sections were obtained using IRIS data for Jupiter. Correlation of the structure of the upper troposphere with the cloud level dynamics is explored for both planets by comparing the computed thermal wind shear with the imaging measurements of zonal wind velocites.

Zonal thermal cross-sections

A meridional thermal cross-section for Saturn's northern hemisphere is shown in Fig. 1a: temperature profiles retrieved between 730 and 150 mbar from 82 individual spectra taken with roughly uniform distribution in latitude but at various longitudes were assembled. For these measurements the IRIS field of view projected onto the planet varied between 2° and 5° in latitude. The vertical resolution for the retrieved temperature is $\sim \pm 0.5$ scale heights and the error in temperature due to instrumental noise for each spectral measurement is $\sim \pm 0.5$ K. Continuous high resolution latitudinal coverage was available only in the northern hemisphere.

Thermal cross-sections between 140 and 500 mbar for Jupiter's northern and southern hemispheres, assembled from 150 and 166 Voyager 1 spectral measurements respectively, are shown in Fig. 1 b, c. The field of view projected onto the planet, vertical resolution, and thermal noise level for these data are comparable with those for Saturn.

The pointing data for these measurements were provided by the Voyager supplementary experimenter data record (SEDR)⁴. In some cases it has been possible to check this pointing information with reference to simultaneously shuttered Voyager imaging records. In these cases agreement between the imaging and the SEDR has been within 1° latitude. Although such verification is impossible for spectral measurements taken on the dark sides of the planets, the estimated residual 3σ pointing error is expected to be within 1°-3° of latitude, depending on the projection of the field of view onto the planets. Latitudinal correlations between the thermal and visual observations may therefore be sought within these error bounds.

The gross structures exhibited in the cross-sections for both Saturn and Jupiter show horizontal scales of variation comparable with the width of the zonal jet streams at mid-to-high-latitudes. The direct visual comparison of latitudinal thermal variation in these cross-sections with both albedo and wind velocity is more difficult. The Saturn cross-section (Fig. 1α) shows no simple correlation with the visual albedo variation measured by Voyager imaging¹, but some correlation is observed between the visual albedo and the structure of Fig. 1 b, c for Jupiter at upper levels near 150 mbar. Higher albedo is in most places associated with lower temperatures.

The upper tropospheric structure for Jupiter seems to be in rough agreement with the IR measurements of Pioneer 11 reported by Orton et al.⁵. A region of special interest is that

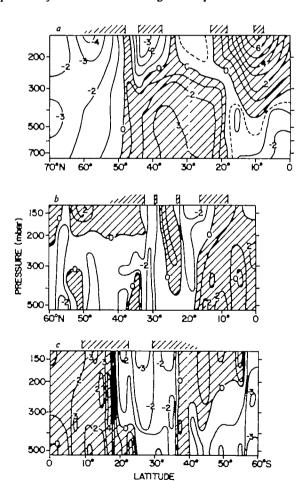


Fig. 1 Zonal cross section of deviations from the latitudinal mean retrieved temperature (K) in. a, Saturn's northern hemisphere as measured by Voyager 1 IRIS, November 1980. b, Jupiter's northern hemisphere, measured in March 1979. c, Jupiter's southern hemisphere, measured in March 1979. The latitudes of major albedo features are indicated schematically.

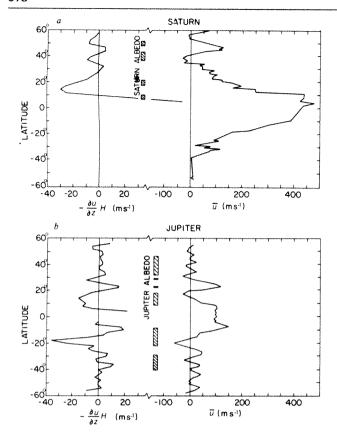


Fig. 2 a, Comparison between the thermal wind shear at 150 mbar and the cloud top zonal wind velocities on Saturn. b, Comparison between the thermal wind shear at 140 mbar and the cloud top zonal wind velocities on Jupiter obtained from Voyager 1 IRIS measurements. For convenience the negative of the wind shear multiplied by one atmospheric scale height is displayed. The major albede contrast is also indicated.

between 24° and 30° N (historically the North Temperate Belt or NTB). According to Peek⁶ this region is one of the most variable on the planet. The region was dark during the Pioneer 10 and 11 encounters and the 20 µm (upper tropospheric) temperatures were correspondingly high. Figure 1b shows that this region was still warm at 150 mbar during the Voyager 1 encounter; it was also warm during the Voyager 2 observations. The appearance of this region during the Voyager encounters, however, showed a high albedo typical of a zone¹. According to the Earth-based observations by R. F. Beebe (personal communication) the belt-like region began to cloud over in 1977-78 so that the zone-like appearance had been present for <2 yrs before the Voyager encounters. This interval is, however, well within the radiative response time of 10⁸ s for this level of Jupiter's atmosphere⁷, and the upper tropospheric temperatures may not vet have responded to the reduction of IR flux at the cloud level.

The thermal cross-sections for both planets show substantial differences in the horizontal structure between levels above and below 300 mbar. These differences may reflect inaccuracies in the temperature retrievals at low levels due to the opacity effects of latitudinally variable aerosol concentrations. If the retrieved temperatures are correct at all levels, however, then the presence of some as yet unknown driving mechanism is indicated. Possible candidates include aerosol heating in the upper troposphere, radiative screening effects associated with clouds, adiabatic heating and cooling due to vertical motion⁸, and eddy or wave generated fluxes of heat and momentum.

Thermal wind shears

The observed latitudinal gradients in temperature on Saturn and Jupiter require a dynamical response, and the velocity and length scales inferred from the Voyager imaging measurements are consistent with geostrophic balance. Therefore, with the additional assumption of hydrostatic equilibrium, the vertical

shear of the eastward zonal flow u is given by the thermal wind equation

$$\frac{\partial u}{\partial z} = -\frac{R}{2\Omega a H \sin \lambda} \frac{\partial T}{\partial \lambda}$$

where the derivative of temperature with respect to latitude λ is taken along an isobaric surface, and R is the gas constant. The planetary rotational frequency is $\Omega=1.64\times10^{-4}~\rm s^{-1}$ and $1.76\times10^{-4}~\rm s^{-1}$ for Saturn and Jupiter respectively. The respective planetary radii are given by $a=6.03\times10^4~\rm km$ and $7.14\times10^4~\rm km$. The scale height H is $\sim38~\rm km$ for Saturn and 22 km for Jupiter at upper tropospheric levels. Because of the limited spatial resolution of the IR measurements and the uncertainty in the ammonia condensation level, it is impossible to match reliably an integrated wind shear to the zonal velocities at the cloud deck. Nevertheless, an estimate of the thermal wind shear in the upper troposphere for the observed zonal temperatures on the two planets is of diagnostic value in determining the extent of the coupling between the upper level dynamics and the observed zonal winds.

Figure 2 shows the thermal wind shear derived from the temperature retrievals at 150 mbar for Saturn and 140 mbar for Jupiter. These computations used the same data sets as those used for the cross-sections in Fig. 1. For Saturn the meridional derivative was computed for every 2.5° latitude as a finite difference over 5° between temperatures given by a smooth fit to the data. For Jupiter the finite difference interval was 4° at every 2° of latitude. The imaging measurements of the zonal velocity \bar{u} at the cloud tops are plotted to the right of the wind shears for comparison. The cloud top zonal winds for Saturn were taken from ref. 1 while those for Jupiter were adapted from ref. 9. The approximate latitudinal positions of the visual albedo variations at the cloud deck are also indicated in Fig. 2 with dark markings for 'belts', interspersed with the lighter 'zones'.

Wherever the sign of $-(\partial u/\partial z)H$ is the same as that for \bar{u} a reduction of the cloud top velocity with height is implied. Different scales have been used for $-(\partial u/\partial z)H$ and \bar{u} to aid their visual comparison. Because of the finite field of view for the measurements computed shear magnitudes are best regarded as lower limits for gradients of thermal fields which may vary significantly over scales too small to be completely resolved.

A comparison of the results for the two planets implies that the dynamical length scale, as indicated by the latitudinal change in the sign of the shear, is slightly larger for Saturn than for Jupiter. In general, there is a good correlation between the positions of most of the observed jets and the wind shear extrema. The shear is in the sense required to reduce the strength of the zonal velocities at cloud level with height, but the magnitude of the shear is such that several scale heights would be required for a significant modification of wind speeds comparable with those observed at the cloud tops.

On Jupiter, alignment between the peaks of the jets and extrema of the thermal wind shear is in most places within the $\pm 1-3^{\circ}$ of residual pointing uncertainty. The agreement seems to be especially good at the strong prograde jet at 23° N and throughout the alternating system of jets between 15 and 40° S. Although it is not certain how close to the equator the computed thermal wind shears are valid, they have been plotted within $\pm 4^{\circ}$ of the equator and some reduction in the strength of the equatorial jet with height is indicated.

The thermal wind shear at 18° S on Jupiter seems to be stronger than at any other mid-to high-latitude location. The retrograde jet at 20° S as measured by the Voyager imaging is unusually broad. The apparent enhancement in the wind shear at this location may therefore simply reflect the better spatial resolution in the thermal measurement relative to the jet width.

Note that the region between 24 and 30°N on Jupiter retains a strong correlation between the thermal wind shear and the wind field at the cloud tops. This is a consequence of the relatively warm upper troposphere even through this normally belt-like region had clouded over before the Voyager 1 encounter. Thus, the upper tropospheric temperatures seem to have been more

strongly correlated with the cloud level jets than with the large scale albedo features at the time of the Voyager observations.

The computed thermal wind shear for Saturn is closely correlated in position with the retrograde jet centred near 38° N and with the prograde jet near 46° N, again in the sense required to reduce the cloud top velocities with height. The magnitude of the thermal wind shear relative to the measured \bar{u} , however, is much larger for the retrograde jet. There is also some correlation between the vertical shear and the weak retrograde jet centred near 55° and at least a correlation in sign for the prograde cloud motions around 32° N. The thermal wind shear between 5° and 10° N indicates some reduction in the strength of the very rapid equatorial jet with height. Between 10 and 30° N the computed thermal wind shear shows a significant departure from the general correlation observed elsewhere. Here the shear is in the sense consistent with a retrograde velocity, but the imaging measurements show this region to be a part of the prograde equatorial jet.

Smith et al. indicate that the Saturn jets at latitudes above 35° show some correlation with the albedo variation. Unlike Jupiter, however, the peaks of the jets seem to coincide with the middle of the bright and dark bands. It is interesting that the extrema of the computed thermal wind shear also show some correlation with the middle of the bright and dark bands, not only at the same high latitudes, but also at 32° N and especially at 15° N where the strong retrograde shear corresponds closely to the middle of a bright region to the south of Saturn's north equatorial belt. Comparison of thermal wind shears with zonal velocities in Saturn's southern hemisphere must await the more

comprehensive Voyager 2 IR measurements.

Conclusions

The thermal wind shear calculated from the IRIS derived temperatures in the upper troposphere is highly correlated with the imaging derived jets at most latitudes on both Jupiter and Saturn and is in the sense required for the wind to decrease with height. An exception is the strong retrograde feature inferred from the thermal data at 15° N on Saturn which does not appear in the imaging data. Contrary to expectation, the thermal structure in the lower troposphere below ~300 mbar is not well correlated with cloud top winds on either planet. This may be the result of a haze or cloud effect not taken into account in the thermal retrievals; however, the possibility that the thermal structure is real cannot be ruled out until particulate contributions to the IR opacity of the lower tropospheres of both planets is better understood.

The thermal wind shear and major albedo features have similar latitudinal scales, but the exact nature of this correlation differs on the two planets. On Saturn, the extrema in the wind shear tend to fall near the centres of the bands while on Jupiter they occur near the interfaces of belts and zones. The observation of the anomalously bright but warm region between 24° and 30° N on Jupiter suggests a better correlation between upper tropospheric temperatures and the jet streams than with the large scale albedo features at the time of the Voyager encounters.

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- Smith, B. et al. Science 212, 163-191 (1981)
- Sonth, B. et al. Science 212, 103–191 (1901)
 Hanel, R. et al. Science 212, 192–200 (1981)
 Hanel, R. et al. Appl. Opt. 13, 1391–1400 (1980)
 Hanel, R. et al. Voyager Infrared Interferometer Spectrometer and Radiometer Documention for Radiocal Data Records for Impact.
- Space Flight Center Greenbelt, 1980)
- Orton, G 8 et al. Iceres (submitted)
 Peek, B M. The Planet Impier (Faber, London, 1958)
 Giorasch, P. J. & Goody, R. M. J. atmer. Sci. 26, 979–980 (1969)
- 8 Courath, B. J. et al. J. geophys. Res. (in the press)
 9. Ingersoll, A. P. et al. J. geophys. Res. (in the press)

Morphology of Saturn's aurora

B. R. Sandel & A. L. Broadfoot

Earth and Space Sciences Institute, University of Southern California, Tucson Laboratories, Tucson, Arizona 85713, USA

Aurorally-excited emissions of atomic and molecular hydrogen come from a narrow circumpolar band near 80° north and south latitude on Saturn. The aurorae, which lie near the edge of the polar cap region, are continuously excited in both the north and south. If the strong variations observed in the auroral intensity are temporal, rather than longitudinal, they may be related to the periodic structure in the Saturn kilometric radiation.

BECAUSE Saturn possesses a dense atmosphere and a strong magnetic field, it is reasonable to expect that aurorae should also be present. Before the encounter of Voyager 1, two lines of evidence suggested the presence of polar aurora on Saturn, but both admitted alternate interpretations. Pioneer 11's ultraviolet photometer showed enhanced emission in its long wavelength channel when the field of view crossed the polar limbs, as compared with the centre of the disk1; this could have been due to auroral excitation or to limb brightening. Spatially-resolved images of Saturn obtained by the International Ultraviolet Explorer (IUE) satellite showed enhancements in $HLy\alpha$ emission which could have come from Saturn's polar atmosphere². At different times the enhancements appeared at both north and south poles, but were sometimes completely absent. The polar enhancement ranged from 300 to 900 R averaged over the resolution element of 6×10 arcs. The source of the emission could not be localized to the atmosphere of Saturn, and it was noted that the observations were consistent with scattering of sunlight by a sporadic cloud of neutral hydrogen associated with Saturn's rings. The first unambiguous evidence for saturnian aurora was provided by the UV spectrometer (UVS) on board

Voyager 1. Aurorally excited emissions were found at HLyα and in the Lyman and Werner bands of H₂ near Saturn's poles. We now expand that brief description³ of the aurora.

The UVS is an objective grating spectrometer covering the wavelength range 530-1,700 Å in 126 contiguous channels^{4,5}. This wavelength region includes the atomic HLy α line that can be excited by resonance scattering or particle impact, and the particle-excited Lyman and Werner bands of molecular hydrogen. During the encounter the UVS was used to map the planet at varying spatial resolutions.

Of the data examined so far, the polar scan sequence shown in Fig. 1 has yielded the best information on the position of the aurora. The path of the slit intersected an auroral oval twice, once on each side of the pole. The presence of the aurora was defined by locally enhanced emissions at HLya and in the H2 bands. In the polar cap region, the auroral emission was reduced by a factor of at least 4, and may have been entirely absent. To determine the width and location of the auroral excitation, the data from the crossing on the near side of the pole have been compared with a simple model of the distribution of auroral emission. This model shows that the auroral emission extends

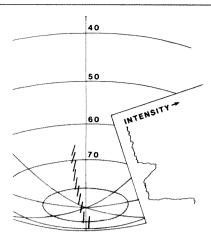


Fig. 1 Geometry of a polar auroral scan. The range is $3.6 R_s$ and the latitude of the spacecraft is -36.6°. Dashes show the position of the 0.1° × 0.86° UVS slit at 1.5-min intervals as the slit scanned across the south pole and onto the dark limb. The stippled area shows the inferred location of the source of auroral emission between 78° S and 81.5° S. The slit crossed the aurora on the near side of the pole at $\lambda_{SLS} = 190^{\circ}$ near the dusk terminator. H₂ band emission along the path of the slit is shown in the inset.

between 78 and 81.5° S. On the far side of the pole, the emission occurs very close to the limb of the planet, and is difficult to locate accurately. Furthermore, on the far side, the uncertainty in latitude introduced by parallax and the uncertain altitude of the auroral emission is much greater. No information on the distribution of the intensity within the aurora is yet available. The average apparent brightnes is ~5 kR based on the intensity of the 1,105-Å H₂ band feature, which should be optically thin⁵. Because the aurora was observed at an angle of 56° from the local zenith, the nadir intensity was $\sim 2.8 \, kR$. Figure 1 shows the auroral oval at constant latitude; this is consistent with, but not required by, the data. We have also anticipated a result derived later—that the aurora is at least roughly continuous in longitude, to extend the auroral oval around the planet in longitude.

In the north, the aurora occurs at nearly the same location, as the nearly dipolar, axisymmetric nature of the magnetic field would imply. Approximately 11 h after closest approach the north polar area was searched for aurora. The geometry was similar to that shown in Fig. 2b, except that the spatial resolution was a factor of 3 better. Two scans near local midnight show that the boreal aurora lies poleward of 76° N, and may not extend below 78° N. These scans do not define the poleward extent of the excitation. We assume that the boreal and austral aurora have the same latitudinal extent, as their equatorward boundaries fall at nearly the same latitude, and the magnetic field has north-south symmetry. The two scans show a 50% increase in H₂ band emission (from 2.5 to 3.7 kR) from the first to the second, 20 min later. As the planet rotated only 13° in this time. and because the field of view was positioned near the 'elongation' of the auroral oval, the longitude range within the slit did not change significantly between scans. The change in measured intensity is probably due to a temporal variation in auroral brightness.

More extensive observations at lower spatial resolution are available for study. The north-south map (NSM) sequence was executed ~36 h before and after closest approach. This observing sequence, shown in Fig. 2, consisted of a series of swaths from north pole to south pole, approximately along the central meridian of the planet. Each swath of the pre-encounter NSM was made up of 12 steps in latitude, each step lasting 48 s. Swaths were repeated at intervals of 12 min, or 7° in rotation phase, during an almost complete rotation period of the planet. Saturn was therefore mapped in latitude (by stepping the scan platform) and in longitude (by taking advantage of the planet's rotation).

The NSM sequences show that aurorae are present near both poles, and that they are probably continuous, at least during the

few days nearest encounter. Because the apparent size of the auroral oval is small, the fact that the slit does not always scan exactly along the central meridian is important. The field of view was well centred on the central meridian at the beginning of the post-encounter NSM, but drifted partially off the aurora to the east during the 10-h sequence. Therefore we cannot derive strong conclusions about time or longitudinal variations over the full span of the observation, but the data clearly demonstrate the presence of aurora near the north pole throughout the NSM sequence. The favourable configuration shown in Fig. 2b was maintained for the first 3 h of the sequence. During this time the brightness of the H₂ bands decreased from its initial level of 20 kR by a factor of ~2. This change can be interpreted as a temporal variation or as a longitudinal variation in the brightness of the aurora. The field of view sampled the auroral oval on both sides of the pole, so the presence of a brighter area near both $\lambda_{SLS} = 25^{\circ}$ and 200° is compatible with the data. (The longitude system used here, λ_{SLS} , is based on a sidereal period of 10 h 39.4 min defined by the Voyager planetary radio astronomy experiment⁷.)

A similar well-defined variation was recorded during the pre-encounter NSM. Part of the data for which we can rule out significant changes in signal level induced by changes in the position of the field of view are shown in Fig. 3. The H₂ band intensity increased by a factor of 5, remained high for 2 h (or \sim 70° in Saturn rotation phase) then returned to its lower level. At earlier and later times, the signal was about the same as the lower level shown in Fig. 3, but at these times the signal could have been modulated to some extent by changes in the position of the field of view. Note that the longitude of the field of view was $\sim 160^{\circ}$ at the peak in H₂ band emission. This differs from the position quoted earlier³ because we have now included the fact

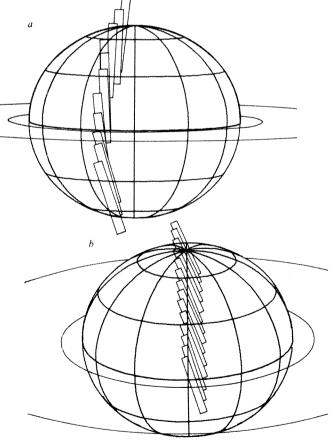


Fig. 2 Views from the spacecraft showing the geometry of the North-South Map sequence. The pre-encounter NSM a was executed when the latitude of the spacecraft was 4.8° N. Both boreal and austral aurorae were visible, although the austral aurora was quite near the limb of the planet. Near the poles, the slit was near 10.00-11.00 LT. For the post-encounter NSM b, the latitude of the spacecraft was 22° N and the latitude of the austral aurora was not included in the scan. The rectangles show the positions of the UVS slit

during a typical swath. Saturn's central meridian is near 3.00 LT.

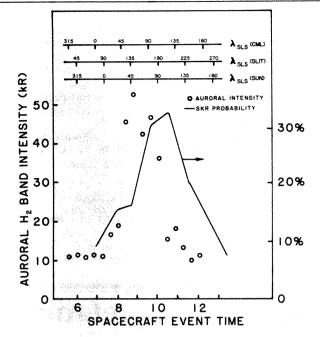


Fig. 3 H_2 band intensity as a function of time during a portion of the pre-encounter NSM. The intensity integrated over the Lyman and Werner bands of H_2 has been determined from the measured intensity of the 1,105 Å feature, which is relatively unaffected by self-absorption. The solid line shows the probability for detection of Saturn kilometric-wave radiation⁸.

that the field of view is not on the central meridian. At the same time, the austral aurora showed a qualitatively similar variation. The unfavourable observing geometry (see Fig. 2b) precludes a quantitative analysis of the austral auroral intensity based on the NSM data. Figure 3 also shows the probability of occurrence of the Saturn kilometric radiation (SKR) taken from Gurnett et al.8. Following the discovery of the SKR by Kaiser et al.9, the probability of observing this radiation was found to be strongly correlated with the longitude of the Sun in the λ_{SLS} system⁸ For the comparison in Fig. 3, the longitude of the Sun was calculated as a function of spacecraft time, and then the relationship between SKR probability and solar longitude was used to plot the SKR probability on the same time scale as the auroral brightness. In spite of a phase difference of $\sim 50^{\circ}$, the correlation between the SKR probability and the auroral intensity apparent in Fig. 3 is good enough to suggest that the two phenomena may be closely linked. A test of this correlation must await the Voyager 2 encounter.

Spectra of the aurora are compared in Fig. 4. The signature of the H_2 Lyman and Werner bands is present between ~ 900 Å and 1,130 Å. The latitude of the spacecraft was different when the two spectra of Saturn's aurora were taken, implying different pathlengths through the atmosphere to the auroral source. Stronger self-absorption by H_2 in the longer atmospheric pathlength of the middle spectrum probably accounts for the gross differences shortward of 1,050 Å in the Saturn spectra (see Fig. 9 of ref. 5). The differences between the auroral spectra of Saturn and Jupiter between 1,100 and 1,200 Å parallel those in the dayglow spectra of the two planets³.

The power required to drive the aurora was estimated earlier³ to be $\sim 2 \times 10^{11}$ W. This was based on an average brightness of 5 kR in the H_2 bands extending over an area of 4.6×10^{18} cm² (the surface area of Saturn between 78° and 81.5° latitude) and the efficiency for electron excitation of the H_2 bands⁵. This re-analysis shows no basis for altering the early estimate, but it does emphasize that the presence of temporal and/or longitudinal variations in the surface brightness of the aurora may bias the apparent average brightness. About two thirds of the auroral information available from the Voyager 1 encounter has been extracted.

Other sources of uncertainty are important. The brightness over all wavelengths in the H_2 Lyman and Werner band systems has been estimated using the measured intensity of the 1,105 Å

feature. This feature is relatively unaffected by self-absorption in long slant-paths through the atmosphere to the auroral source and hence more likely to represent the true emission intensity. The uncertainty in the model relating the total H₂ band emission to the 1,105 Å feature may be as much as a factor of 2 (D. E. Shemansky, personal communication). Further uncertainty is introduced when the apparent brightness of an aurora near the limb of the planet must be used to calculate the surface brightness. Hence we have given more weight to the intensity measurements made at the smallest atmospheric pathlengths in assigning the average auroral brightness of 5 kR. Because of these uncertainties, figures such as auroral power dissipation are uncertain by a factor of at least 4. On the other hand, the relative brightness measured within a given sequence (for which the observing geometry remains constant) depends mainly on the pointing accuracy and should be very good. The location of the aurora, at least at the time of the observation in Fig. 1, is well defined to within at most 2° in latitude. Finally, the emissions in the H_2 bands and at $HLy\alpha$ from a thin layer above the sub-solar limb of Saturn³ are too weak to contaminate these auroral measurements significantly.

The aurora described here are probably related to the bursts of $HLy\alpha$ emission reported by Clarke et al.². The $HLy\alpha$ component of the auroral emissions is generally a factor of ~ 5 fainter than the H_2 band emission. The brightest $HLy\alpha$ emission measured in the UVS data was 9 kR observed during the pre-encounter NSM. Assuming that the radiation reported by Clarke et al. arose from either the northern or the southern aurora defined by the UVS, we estimate that the true surface brightness corresponding to the bursts reported by Clarke et al. ranges between 13 and 40 kR. The brightest levels were observed in only one of the seven observing periods distributed over 6 months; their lowest level bursts detected correspond roughly in intensity to the brightest $HLy\alpha$ emission measured to date by Voyager.

The morphology of Saturn's aurorae resembles that of Earth's aurorae more than that of Jupiter's aurorae. The edge of Saturn's polar cap region lies at a latitude of 78.7°-75° (ref. 6), and thus corresponds closely to the location of the auroral excitation, as in the case of the Earth. This suggests that analogous mechanisms supply energy for the aurora on the two

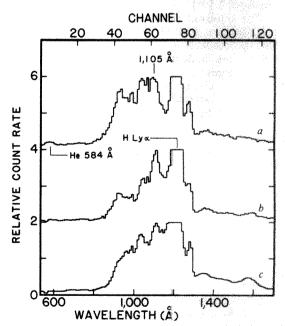


Fig. 4 Auroral spectra. a, Saturn aurora, observed at a local zenith angle 60°. b, Saturn aurora, local zenith angle 76°. c, Jupiter aurora. Aurorally-excited emissions in the Lyman and Werner bands of H₂ are apparent between 900 and 1,130 Å. The spectra have been normalized to equal levels at 1,105 Å. The Saturn spectra differ markedly between 850 Å and 1,050 Å because of different absorption pathlengths. The 1,105 Å feature marked was used as an indicator of the intensity radiated in the H₂ bands. The HLyα line has been truncated.

planets. On the other hand, the equatorward boundary of Jupiter's aurorae matches the magnetic projection of Io's plasma torus onto the atmosphere, suggesting excitation by an interaction between the atmosphere and the plasma torus11. (Aurorae may be present nearer the poles, at the boundaries of the polar caps, as well.) The energy deposited in the Earth's atmosphere by aurorae is correlated with geomagnetic activity, ranging over more than 2 orders of magnitude¹². The average value¹³ is probably near 1.7 erg cm⁻² s⁻¹, which corresponds to about 3×10^{10} W, roughly 1/7 of the power deposited at Saturn. The interaction between the magnetosphere of a planet and the solar wind, the ultimate source of auroral energy on Earth and probably on Saturn, is important in determining the power available to auroral processes. This interaction transfers about

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- Judge, D. L., Wu, F.-M. & Carlson, R. W. Science 207, 431 (1980).
 Clarke, J. T., Moos, H. W., Atreya, S. K. & Lane, A. L. Nature 290, 226 (1981).
 Broadfoot, A. L. et al. Science 212, 206 (1981).
- Broadfoot, A. L. et al. Space Sci. Rev. 21, 183 (1977).
- Broadfoot, A. L. et al. J. geophys. Res. (in the press).
 Ness, N. F. et al. Science 212, 211 (1981).

 10^{11} W into Earth's magnetosphere 14 , about 1/10 of the 10^{12} W injected into the saturnian magnetosphere, calculated using measurements of the saturnian magnetic field⁶ and general scaling relationships for magnetospheric parameters 14. Thus the ratio of power in aurora on the two planets is about the same as the ratio of the power transferred to the magnetosphere from the solar wind. This, and the occurrence of aurora near the edges of the polar caps on both planets, suggest that the aurorae result from similar magnetospheric processes.

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- Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. (in the press).
 Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 239 (1981).
 Kaiser, M. L., Desch, M. D., Warwick, J. W. & Pearce, J. B. Science 209, 1238 (1980).
 Warwick, J. W. et al. Science 212, 239 (1981).

- Sandel, B. R. et al. Science 206, 962 (1979).
 Murphree, J. S. & Anger, C. D. Geophys. Res. Lett. 5, 551 (1978).
- 13. Torr, D. G., Torr, M. R., Hoffman, R. A. & Walker, J. G. C. Geophys Res. Lett. 3, 305 (1976). 14. Siscoe, G. L. *Icarus* 24, 311 (1975).

Saturn ionosphere: theoretical interpretation

S. K. Atreva* & J. H. Waite Jr

* Department of Atmospheric and Oceanic Science, Space Physics Research Laboratory, The University of Michigan, Ann Arbor, Michigan 48109, USA; † Marshall Space Flight Center, Huntsville, Alabama 35812, USA

Voyager 1 high latitude and Pioneer 11 equatorial ionospheric structure indicate a solar EUV-controlled ionosphere with a possible molecular ion in the topside. Vibrationally excited H_2 in the high latitudes may be an important loss mechanism. Dynamical effects are expected to be important for determining the peak density and its location.

THE Voyager 1 coherent dual-frequency radio system (RSS) at wavelengths 3.6 and 13 cm provided the first look at a high latitude (~73 °S) ionospheric profile on Saturn¹. The entry data reveal that near the evening terminator (solar zenith angle, $\xi = 89^{\circ}$), the peak electron density of 2.3×10^{4} electrons cm⁻ occurred at a height of ~2,500 km from the 1 bar pressure level. Earlier observations on Pioneer Saturn measured a peak electron density of 1.1×10^4 cm⁻³ at 1,800 km in the 10 °S latitude range². The observed ionospheric characteristics point to a markedly different ionosphere from that expected on conventional theoretical grounds.

The RSS ionospheric measurements were accompanied by a simultaneous measurement of the neutral atmosphere by means of the Voyager UV spectrometer (UVS). Modelling studies suggest that the observed ionosphere is controlled principally by the ionization of the neutrals caused by the solar EUV.

The UVS solar occultation measurements yield a neutral exospheric temperature of $820 \pm 100 \text{ K}$ at 62,000 km (ref. 3). The large size of the Sun (~500 km) projected on Saturn means that the height level at which the temperature reaches its exospheric value cannot be determined accurately. The exospheric temperature, however, is nearly the same as the topside plasma temperature (750 K)¹.

Preliminary analysis of the UVS stellar occultation data indicates that the temperature is essentially isothermal (~150-200 K) in the mesosphere up to $\sim 750 \text{ km} (H_2 \simeq 5 \times 10^{13} \text{ cm}^{-3})$ and an average temperature gradient of ~0.55 K km⁻¹ prevails above this altitude resulting in an exospheric temperature of -825 K at 2,000 km above the 1-bar pressure level. Indeed, the Saturn stellar occultation transmission characteristics are similar to those of the Jupiter stellar occultation data collected on Voyager 2 (refs 4, 5) when the height scale is corrected for the lower acceleration due to gravity on Saturn. With this information and the hydrostatic law, a working model of the atmospheric density profile at the ionospheric heights can be constructed. An important caveat is that the Voyager ionosphere was measured at high latitudes, while the neutral atmosphere used here is essentially from equatorial to mid-latitude measurements³. The auroral activity on Saturn, however, is confined to the latitude band 78°-81.5 °S which is well outside the latitude region (73 °S) of the ionospheric measurements discussed here. Therefore, no substantial change is expected in the neutral atmosphere at the ionospheric heights from the low to the high latitudes on Saturn.

Knowledge of the atmospheric mixing as represented by the eddy diffusion coefficient, K, is important for determining the distribution of the hydrocarbons which act as an important sink to the major ions, H^+ , H_2^+ and H_3^+ (refs 6, 7). K_h at the homopause may be determined from the atomic hydrogen abundance deduced from the planetary Ly α intensity^{4,8,9}. The UVS data yield 3.3 kR for Saturn Lyα dayglow³ implying 5× $10^{16} \, \mathrm{cm^{-2}} \, \mathrm{H}$ atoms above the homopause, and consequently K_{h} of $(1.5-5.0) \times 10^5$ cm² s⁻¹.

The hydrocarbon density distributions were calculated considering CH4 photolysis with the eddy diffusion coefficient varying inversely as the square root of the atmospheric number density⁷

The important chemical reactions for the Saturn ionosphere were taken from previous work^{6,10}. We consider here lowpressure, low plasma temperature (T_e) approximation for the radiative recombination reaction rate of the major topside ion, H⁺; the new rate is $4 \times 10^{-12} (250/T_e)^{0.7} \text{ cm}^3 \text{ s}^{-1}$ (ref. 11). The calculated ionospheric profile with a comparison with the Pioneer equatorial¹² and Voyager high latitude¹ data are shown in Fig. 1. Protons (H⁺) comprise the major ions in altitudes >750 km. Below 750 km, a transition to H₃⁺ and heavy hydrocarbon ions such as C₂H₅⁺ and CH₅⁺ occurs. Conversion to the higher order hydrocarbon ions, C₃H₅⁺ and C₄H₅⁺, is also probable in the lower ionosphere8.

Although the Pioneer and Voyager data differ in details, there is general agreement about the location and magnitude of the peak, and the extent of the ionosphere. The observed peak electron density in the Voyager data is about a factor of 10 lower than the calculated one. A similar low density jovian ionosphere profile measured by Voyager 1 was interpreted as being due to the reaction of H⁺ with vibrationally excited H₂ (ref. 10). The

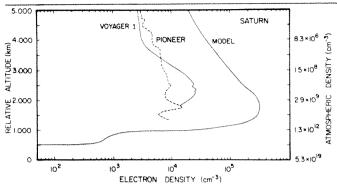


Fig. 1 Model calculations and observations of the Saturn ionosphere. The Pioneer data refer to low latitude (~10 °S), while the Voyager data are for the high latitude (~73 °S)

relatively low exospheric temperature (~800 K) on Saturn results in a small population of H_2 in $v' \ge 4$ vibrational state so that the charge exchange reaction between H⁺ and H₂ $(v' \ge 4)$ would not be expected to be as important on Saturn as on Jupiter. However, the H₂ vibrational levels may be non-thermally populated due to electron precipitation processes. Such non-thermal vibrational distributions were suggested by Cravens¹³ from modelling of jovian electron precipitation processes. Such processes could be important in interpreting the Voyager/Saturn data provided that the effects of auroral precipitation extend beyond the observed latitude band in which significant (~5 kR) H₂-Lyman and Werner band auroral emission have been observed by the Voyager UVS3. The observed H_2 auroral emission imply an energy input of ~ 0.5 erg cm⁻² s⁻¹ (in the 78°-81.5 °N and S latitudes) on Saturn which is about a factor of 10 lower than in the jovian auroral region.

An electron precipitation event composed of 2 keV electrons with a flux of 1.5×10^8 cm⁻² s⁻¹ could reproduce the 3-5 kR of H₂-Lyman and Werner band emission seen by the UVS³. Such an aurora produces a model ionosphere with a peak electron density of 2×10^6 cm⁻³ near 2,400 km when the effects of the charge exchange reaction $(H^+ + H_2 \ (v' \ge 4))$ are omitted. However, if a rate constant of 10^{-9} cm³ s⁻¹ is assumed for the reaction of H⁺ with H₂ ($v' \ge 4$), and a vibrational temperature of \sim 2,000 K, the peak electron density can be brought into rough agreement with the RSS high latitude ionosphere measurements.

Even if the loss of H⁺ by charge exchange with H₂ $(v' \ge 4)$ could explain low peak electron density in the high, non-auroral latitudes, similar low peak electron densities have been measured by the Pioneer 11 spacecraft near 10 °S latitude^{2,12} also, and they apparently need an alternative explanation due to

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- 1. Tyler, G. L. et al. Science 212, 201-206 (1981).
- Kliore, A. J., Lindal, G. F., Patel, I. R., Sweetnam, D. N. & Hotz, H. B. Science 207, 446-449 (1980).
- Broadfoot, A. L. et al. Science 212, 206-211 (1981).
 Atreya, S. K., Donahue, T. M. & Festou, M. C. Astrophys. J. Lett. 247, L43-L47 (1981).
 Festou, M. C. et al. J. geophys. Res. 86, 5715-5725 (1981).
 Atreya, S. K. & Donahue, T. M. Jupiter (ed. Gehrels, T.) 304-318 (The University of
- Arizona Press, 1976).
 7. Waite, J. H. Jr thesis, Univ. Michigan (1981)

the unlikely occurrence of a significant vibrationally excited H₂ population in the Saturn equatorial ionosphere. The 700 R intensity of the non-auroral H2 bands measured by the UVS3 implies a planetwide precipitation of electrons resulting in a relatively low energy deposition rate of 1.3×10⁻² erg cm⁻² s⁻¹ on Saturn. Relatively good agreement between the Pioneer equatorial and the Voyager high latitude Saturn ionospheric data suggests a common H+ loss process missing from current theoretical models. Moreover, the loss of the major ion H⁺ after the charge exchange with vibrationally excited $H_2(v' \ge 4)$ even in the high latitudes is only a remote possibility as the measured ionosphere is $\sim 5^{\circ}-10^{\circ}$ removed from the auroral latitude band.

The most obvious choice for reconciling the calculations and the measurements is an ion-molecule reaction which would convert some of the H+ into relatively short-lived molecular ions. Although methane reacts rapidly with H⁺ to form molecular ions, it is not distributed high in the atmosphere due to the relatively low value of the eddy diffusion coefficient, $K_h \sim$ $10^6 \,\mathrm{cm^2 \, s^{-1}}$. Even an extremely high $K_h \ (\sim 10^9 \,\mathrm{cm^2 \, s^{-1}})$ falls short of the required H⁺ loss rate⁷. An alternative explanation is that water vapour in the rings could provide an important loss to the ionospheric H⁺ (ref. 14). The OH would, however, be concentrated in the region near the rings suggesting a latitudinal variation in the OH + H⁺ loss process, a variation not supported by the Pioneer and Voyager data. Furthermore, the large OH production rates might force unacceptable limits on the longevity of the rings. We also find that the depletion of the peak electron density due to the ring shadow occurs in low latitudes; the effect, however, is minimal (≤10% depletion in the electron density) at the latitudes of the Pioneer observations⁷

There is no adequate common explanation for the low peak electron densities observed at both the low and high Saturn latitudes. Dynamical effects may have an important role in the low latitude ionosphere and auroral effects may be important at high latitudes. However, some as yet unknown H⁺ loss process may be the controlling factor at all Saturn latitudes.

The location of the peak in electron density seems to be ~500 km higher than calculated (Fig. 1); this is, however, questionable as the calculated peak is quite broad. But many theories for reconciling this apparent discrepancy are possible. Calculations indicate that an average diurnal vertical drift velocity of $\sim 14 \,\mathrm{m \, s^{-1}}$ for H⁺, produced by an electrical field of \sim 3 mV m⁻¹, southward neutral winds of \sim 140 m s⁻¹, and/or vertical winds of $\sim 14 \text{ m s}^{-1}$ are capable of raising the peak by 500 km (refs. 7, 15).

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- Hunten, D. M. J. Atmos. Sci. 26, 826-834 (1969)
- Wallace, L. & Hunten, D. M. Astrophys. J. 182, 1013-1031 (1973). Atreya, S. K., Donahue, T. M. & Waite, J. H. Jr Nasure 280, 795-796 (1979).
- Areya, S. K., Dohanue, T. M. & Waite, J. H. Jr. Nature 280, 793-796 (1979).
 Bates, D. R. & Dalgarno, A. Electronic Recombination, Atomic and Molecular Processes (ed. Bates, D. R.) 245 (Academic, New York, 1962).
 Kliore, A. J. et al. J. geophys. Res. 85, 5857-5870 (1980).
 Cravens, T. D. thesis, Harvard Univ. (1974).
- 14. Shimizu, M. Proc. 13th Lunar planet. Symp. (Institute of Space and Aeronautical Science, University of Tokyo, 1980).
- 15. Banks, P. M. & Kockarts, G. in Aeronomy Part B, 170 (Academic, New York, 1973).

C₃H₈ and C₃H₄ in Titan's atmosphere

W. C. Maguire, R. A. Hanel, D. E. Jennings, V. G. Kunde & R. E. Samuelson

Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

Four bands of propane C_3H_8 and two of methyl acetylene C_3H_4 have been identified in the Voyager IR spectrum of Titan. Stratospheric abundances of 2×10^{-5} for C_3H_8 and 3×10^{-8} for C_3H_4 have been determined for the mid-latitude region. A feature at 1,154 cm⁻¹, previously assigned solely to CH₃D, is now identified at least in part due to C₃H₈.

THE Voyager 1 IR instrument (IRIS)1 obtained several hundred spectra of Titan when the angular diameter of the disk was at least five times the angular field of IRIS. Spectra of the centre of

the disk, the polar regions, and both limbs all revealed the signatures of many hydrocarbons and of HCN in emission, indicating the presence of these gases in a warm stratosphere. In

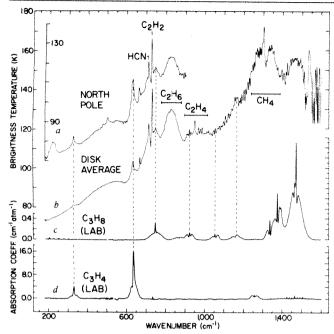


Fig. 1 Comparison of observed Titan spectrum $(4.3 \text{ cm}^{-1} \text{ resolution})$ with laboratory measurements $(2 \text{ cm}^{-1} \text{ resolution})$ at room temperature of C_3H_8 and C_3H_4 . a, An average of 29 spectra from the north polar region of Titan. b, An average of 346 dayside spectra, principally from the centre of the disk but also including data from latitudes as high as 60° N or S. Laboratory spectra of C_3H_8 (c) and C_3H_4 (d) are also shown.

some cases firm identifications and estimates of the abundances were obtained; in other cases, such as for C_3H_8 and C_3H_4 only tentative identifications were made². We provide here additional spectroscopic evidence for the existence of C_3H_8 and C_3H_4 in Titan's stratosphere and give abundance estimates using our laboratory spectra.

Titan and laboratory spectra

Two Titan spectra, containing common strong features, are shown in Fig. 1: the 'disk average' is the average of 346 individual spectra taken near the centre of the disk at a spatial resolution between 1/5 and $\sim 1/50$ of the disk diameter; the average emission angle is $\sim 35^{\circ}$. The signal-to-noise ratio is ~ 430 at 250 cm⁻¹, ~ 130 at 800 cm⁻¹, ~ 16 at 1,000 cm⁻¹, and ~ 35 at 1,500 cm⁻¹. The 'north pole' spectrum is an average of 29 spectra recorded at 68° latitude with an average emission angle of 63.4° . The signal-to-noise ratio is lower in this spectrum, ~ 125 at 250 cm⁻¹ and 35 at 800 cm⁻¹. The behaviour of the continuum emission between 200 and 600 cm⁻¹ is discussed elsewhere³.

The laboratory spectra were recorded at room temperature in two segments: $200-500~\rm cm^{-1}$ and $400-1,600~\rm cm^{-1}$. For C_3H_8 the pressure of the sample was 994 mbar for the low and 404 mbar for the high frequency region. For C_3H_4 , the pressures were 47 mbar for the low and 9.3 mbar for the high frequency region. Sample runs were ratioed with empty cell runs to produce transmission spectra from which absorption coefficients were derived (shown in Fig. 1). Band designations, wavenumbers and band strengths are listed in Table 1 for C_3H_8 and C_3H_4 for the observed $200-1,600~\rm cm^{-1}$ range. Several combination bands at $1,255~\rm cm^{-1}$ of C_3H_4 also appear in Fig. 1 in the laboratory spectrum.

Comparison of Titan and laboratory spectra

The average disk spectrum shows weak but definite spectral features of the C_3H_8 fundamentals at 748, 922, 1,054 and 1,158 cm⁻¹ (dashed lines in Fig. 1). The R branch of C_2H_2 is superimposed on the propane band but the 748 Q-branch of C_3H_8 is clearly present. The weaker bands at 922, 1,054 and 1,158 cm⁻¹ are all visible although in a spectral region where C_2H_4 and other hydrocarbons have features of comparable

strength. We have modelled the bands of CH₄, C₂H₂, C₂H₄, C₂H₆ and CH₃D in the 700–1,400 cm⁻¹ range and found that none of these gases can account for the spectral features now assigned to C₃H₈. Comparison of the spectra also shows that a feature at 1,154 cm⁻¹, which has previously been identified⁴ as due only to CH₃D, is due principally to C₃H₈. The ν_4 Q-branch of CH₃D appears to ride on this feature. The strongest C₃H₈ band in the laboratory spectrum at 1,400 cm⁻¹ shown in Fig. 1 shows no corresponding feature in the Titan spectrum, partly because CH₄ and many other hydrocarbons have strong and overlapping bands in this spectral interval, and partly because the emission originates at about the 6 mbar level where thermal contrast is greatly reduced (Fig. 2).

The disk spectrum and to a greater extent the polar spectrum show the fundamentals of C_3H_4 at $328~cm^{-1}$ and at $633~cm^{-1}$. These features are associated with the same gas because they tend to exhibit similar behaviour with position on the disk, decreasing in strength with increasing distance from the north polar region. They also show identical behaviour in scans across the limb (not shown), rising and falling in unison as each scan proceeds from space to well onto the disk. No signatures of the combination bands at $1,200-1,400~cm^{-1}$ are expected because of their weakness and the above reasons for C_3H_8 .

The identification of C_3H_4 is made firmer by comparing the relative strengths of the features at 328 and 633 cm⁻¹ in the Titan spectra with those in the laboratory spectra. Figure 2 shows most of the mass of gaseous C_3H_4 must be between the condensation point ($T \sim 110$ K; $P \sim 28$ mbar) and a point one pressure scale height above ($T \sim 150$ K; $P \sim 10$ mbar). Thus the temperature of the bulk of C_3H_4 is ~ 130 K. However, the peak brightness temperature observed in the spectrum (Fig. 1) is well below this temperature. Because the Q-branch width is comparable to the IRIS spectral resolution, it follows that the bands are optically thin, and the Q-branch intensity can be approximated by

$$I_{\nu} = \varepsilon_{\nu} B_{\nu} (130 \text{ K}) \tag{1}$$

Table 1 Fundamental frequencies and IR absorption intensities of C_3H_8 and C_3H_4

	Wave number*	Intensity†
Band	(cm^{-1})	$(cm^{-2} atm^{-1} at 300 K)$
Propane		
ν_{22}	(223.0)‡	праводи
ν_9	360.3	0.4 ± 0.4
ν_{21}	748.1	10.1 ± 1.0 §
ν_8	860.3	2.2 ± 0.69
ν_{16}	921.7	4.63 ± 0.65
ν_{15}	1,053.8	4.0 ± 0.45
ν_7	1,157.5	5.04 ± 0.61
ν_{20}	1,191.5}	5.04 ± 0.01
ν_{14}	1,338	10 ± 3
ν_{13}	1,378	24 ± 4
ν_6	1,392}	243.4
ν_5	1,462)	
ν_{12}	1,464	69.9 ± 7.3
ν_{19}	1,472	07.7 ± 7.3
ν_4	1,476)	
lethyl acetylei	ne	
ν_{10}	327	$67.3 \pm 7.0 \parallel$
ν_9	633	282 ± 28
ν_5	930	4.1 ± 2.0
$ u_8$	1,052	2 ± 1
ν_4	1,380	6.1 ± 4.1
ν_7	1,452	72.7 ± 6.5

^{*}From ref. 7.

[†]From ref. 8 for C₃H₈; from ref. 14 for C₃H₄.

[‡]Estimated from combination bands.

Sour measured values. Kondo and Saëki⁸ report 8.9 ± 1.0 for ν_{21} . ||Our measured values. Kondo and Koga¹⁴ report 67.8 ± 4.1 and 355 ± 18 for ν_{10} and ν_{9} , respectively.

where the emissivity of the C_3H_4 layer, ε_{ν} , is the product of the gas density and band strength and B_{ν} is the Planck function at 130 K. The band intensity ratio is therefore given by

$$\frac{I_{633}}{I_{328}} = \frac{S_{633}B_{633}(130 \text{ K})}{S_{328}B_{328}(130 \text{ K})}$$
(2)

where S is the band intensity. The Planck function ratio in equation (2) is $\sim 1/4$, while, from the laboratory spectrum in Fig. 1, $S_{633}/S_{328} \sim 4$ (see Table 1); thus the calculated value for I_{633}/I_{328} is ~ 1 provided the band strength ratio is approximately independent of temperature between 130 and 273 K. On the other hand the brightness temperature spectrum of 68° N (see Fig. 1, ref. 5) indicates that the observed value of I_{633}/I_{328} is ~ 1.2 , depending on the exact location of the continuum: this agreement is consistent within the uncertainties involved.

Abundance determinations

For normal viewing, the solution to the equation of transfer over a narrow spectral range $\Delta \nu$ can be written

$$I_{\Delta\nu} \simeq \int_{P} B_{\Delta\nu} \frac{\partial}{\partial P} \times \left[\int_{\nu'}^{\nu} \phi(\nu - \nu') \exp\left\{ - \int_{P'} \left(\sum_{i} \sum_{i} q_{i} k_{ji} (\nu' - \nu_{i}) \right) \frac{\mathrm{d}P'}{g} \right\} d\nu \right] dP \quad (3)$$

where I is the observed radiance, $\Delta \nu$ is the spectral resolution, ϕ is the instrument convolution function, B is the Planck function, g is the acceleration due to gravity, P is the pressure, and k is the mass absorption coefficient of a gas with a mole fraction q. The summations are over all gases j and all contributing lines i of a single gas. The approximation is very good when $\Delta \nu$ is small because B is essentially constant across such a narrow spectral interval.

Solving for q requires first that the vertical thermal profile of the atmosphere be determined. Second, the vertical distributions and mole fractions of all absorbing gases must be specified; initially the fraction q will be a trial value. Third, the transmission functions of all absorbing gases are calculated. Finally, an iteration is performed on q until equation (3) is satisfied.

We have adopted the shape of the radio occultation profile⁶ between 1,600 and 10 mbar, normalizing it to a surface temperature of 94 K. This profile has been extrapolated to 1 mbar using IRIS data covering the 1,250-1,310 cm⁻¹ range. Methane, the major emitter in this region, is assumed to be uniformly mixed.

Previous abundance determinations² using a line-by-line transmission function program have specified the mixing ratios for C₂H₆, C₂H₂, and HCN. Each gas was assumed to be uniformly mixed down to its condensation level, below which it was reduced to vanishingly small amounts. The cutoff was 50 mbar for C₂H₆ and C₂H₂ and 10 mbar for HCN. To adjust the spectral continuum to the data a stratospheric aerosol was added above 5 mbar, assigned an optical depth of 0.01 at 700 cm⁻¹ and made to increase linearly to 0.05 at 1,400 cm⁻¹. The exact location of the aerosol is unimportant as long as it is optically thin³.

For C_3H_8 , a synthetic spectrum was constructed for the ν_{21} band at 748 cm⁻¹. The band intensity of ν_{21} was measured from the 2 cm⁻¹ resolution laboratory spectra to be 10.1 ± 1.0 cm⁻² atm⁻¹ at 300 K. Other molecular parameters are from refs 7 and 8. Because the distribution of intensities within a 4.3 cm⁻¹ interval is less important to the present analysis than the integrated intensity in this interval, only representative line positions and intensities were used to fit our room-temperature laboratory spectrum. No molecular spectroscopic analysis was performed. Propane was also assumed to be uniformly mixed down to 50 mbar in the model, below which its abundance was set to 0. Equation (3) was iterated until convergence was reached for $q(C_3H_8)$. We infer

$$q(C_3H_8) \sim 2 \times 10^{-5}$$
 (4)

for the disk average which should be accurate to within a factor

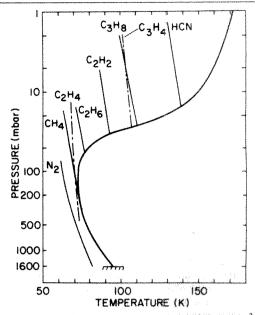


Fig. 2 Vertical temperature profile of Titan's atmosphere $^{3.6}$ and the condensation curves of N_2 and several hydrocarbons with mole fractions listed in Table 1 of ref. 5. Uniform mixing is assumed throughout the stratosphere. HCN will condense at about 15 mbar and C_2H_6 at about 60 mbar. C_2H_4 will not condense at all in the assumed concentration. The condensation products are solid particles for HCN, C_3H_4 , C_2H_2 , C_2H_6 and CH_4 and liquid droplets for C_3H_8 . Very little gas will exist below the condensation point because of the steepness of the temperature profile and vapour pressure curves.

of three. The major sources of error involve the imprecise vertical modelling of the other hydrocarbons and the aerosol, and an uncertain $748 \, \mathrm{cm}^{-1} \, Q$ -branch strength of C_3H_8 . Because the entire $748 \, \mathrm{cm}^{-1}$ band is weak, small errors in the relatively large background continuum lead to large errors in the inferred C_3H_8 mixing ratio. The concentration of C_3H_8 seems to be fairly constant over the disk.

We could now determine the mixing ratio of C_3H_4 from equation (3) if we knew the related values of $k(\nu)$ for this gas. Fortunately the integrated ν_9 band intensity and Q-branch intensity at 633 cm⁻¹ measured from the laboratory spectra is sufficient for obtaining a good approximation to the mixing ratio when C_3H_4 is optically thin throughout this band, as it is for Titan. We can expand the transmission function in equation (3) in a power series and retain only the first two terms. On inverting the orders of integration and summation in equation (3) we have

$$\int_{\nu'} \phi(\nu - \nu') \exp\left[-\int_{P} \sum_{i} \sum_{i} q_{i} k_{ji} (\nu' - \nu_{i}) \frac{\mathrm{d}P}{g}\right] \mathrm{d}\nu'$$

$$= \int_{\nu'} \phi(\nu - \nu') \left[1 - \frac{1}{g} \int_{P} \left(\sum_{i} \sum_{i} q_{i} k_{ji} (\nu' - \nu_{i})\right) \mathrm{d}P\right] \mathrm{d}\nu'$$

$$= 1 - \frac{1}{g} \int_{P} \left[\sum_{i} \sum_{i} q_{i} \left(\int_{\nu'} \phi(\nu - \nu') k_{ji} (\nu' - \nu_{i}) \mathrm{d}\nu'\right)\right] \mathrm{d}P \quad (5)$$

The second term on the right hand side is the optical depth τ_{ν} . On integrating over a spectral interval $\delta \nu$ we obtain

$$\delta \nu \tau_{\delta \nu} = -\frac{1}{g} \int_{P} \left(\sum_{i} \sum_{j} q_{j} k_{ji} \right) dP \tag{6}$$

where k is the integrated strength of an individual line and $\tau_{\delta\nu}$ the optical depth averaged over $\delta\nu$. If we restrict $\delta\nu$ to the width of the Q-branch of a single gas, equation (6) becomes approximately

$$\tau_O = -\frac{S^O}{g} \int_{g} q \, dP \,, \tag{7}$$

where S^O is the Q-branch strength and τ_O is the Q-branch optical depth of the gas. Equation (7) is an excellent approximation as long as there is minimal saturation (that is the gas is

everywhere optically thin), and the strength of the Q-branch is insensitive to temperature. The former is true for the 328 and 633 cm⁻¹ C₃H₄ bands on Titan, while the latter is true for fundamental bands generally, for which the lower state energies are small. The Q-branch strength of C₂H₂, for example, increases from 356 to only 369 cm⁻² atm⁻¹ over the temperature range 300-150 K. If we restrict solutions of q to those for which there is uniform mixing down to some cutoff level, equation (7) becomes

$$\tau_Q = \frac{P}{g} q \frac{S^Q}{\delta \nu} \tag{8}$$

where P is less than the atmospheric pressure at the condensation level, provisionally assumed to be 50 mbar for C₃H₄.

We could substitute equation (8) back into equation (3) and solve for q(C₃H₄) but because the available line-by-line program cannot readily accept a hybrid combination of transmission functions, the following artifice has been adopted. Available line parameters for the 729 cm⁻¹ C₂H₂ Q-branch replace those for the 633 cm⁻¹ C₃H₄ Q-branch. Equation (3) is then iterated and a fictitious mole fraction q^* is determined. The corresponding Q-branch optical thickness derived from equation (8) is correct, however, as long as it is small (no saturation), because it was obtained by fitting the calculations to observed data. Equations (5)-(8) demonstrate that the Qbranch optical thickness is independent of the number, strength, and way in which the individual lines are distributed in the band; q is adjusted accordingly. Hence for optically thin layers qS^Q is an invariant which depends only on the observations and the background opacity. This enables us to obtain the actual C₃H₄

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- Hanel, R. et al. Appl. Opt. 19, 1391-1400 (1980).
- Hanel, R. et al. Science 212, 192-200 (1981
- Samuelson, R. E., Hanel, R. A., Kunde, V. G. & Maguire, W. C. Nature 292, 688-693
- (1981). Gillett, F. C. Astrophys. J. Lett. 201, LA1-L43 (1975). Kunde, V. G. et al. Nature 292, 686-688 (1981). Tyler, G. L. et al. Science 212, 201-206 (1981).

- Gayles, J. N. & King, W. T. Spectr. Acta 21, 543-557 (1965).

abundance from the expression

$$q(C_3H_4) = \frac{S^O(C_2H_2)}{S^O(C_3H_4)}q^*$$
 (9)

where $S^Q(C_3H_4)$ is obtained from our laboratory data, and $q*S^{Q}(C_2H_2)$ is inferred from the strength of the 633 cm⁻¹ C_3H_4

Q-branch in the Titan spectrum. We find

$$q(C_3H_4) \sim 3 \times 10^{-8}$$
 (10)

for the disk average with an uncertainty of a factor of ~ 3 due to attendant uncertainties in modelling the vertical distribution and placement of the continuum, as well as the treatment of the overlapping C₄H₂ Q-branch at 628 cm⁻¹ (ref. 5). The reasons for the enhancement in the north are not known; one possible explanation is that the differences are associated with the north polar hood. The atmospheric hydrocarbon composition of Titan determined from Voyager IRIS data is summarized in Table 1 of ref. 5 for the disk average.

Organic chemistry is significantly different on Titan compared with that on Jupiter or Saturn partly because of a different photo- and high-energy particle radiation chemistry due to a relatively lower abundance of H₂ and higher abundances of N₂ and CH4 on Titan3, and partly because of the presence of an atmospheric sink at Titan's surface 9-14

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- 8. Kondo, S. & Saëki, S. Spectr. Acta 29A, 735-751 (1973).
- Strobel, D. F. Icarus 21, 466-470 (1974).
 Strobel, D. F. J. atmos. Sci. 30, 489-498 (1973)
- Strobel, D. F. Rev. Geophys. Space Phys. 13, 372–382 (1975).
 Capone, L. A., Dubach, J., Whitten, R. C., Prasad, S. S. & Santhanam, K. Icarus 44, 72–84
- Chang, S., Scattergood, T., Aronowitz, S. & Flores, J. Rev. Geophys. Space Phys. 17, 1923-1933 (1979
- 14. Kondo, S. & Koga, Y. J. chem. Phys. 69, 4022-4031 (1978).

C₄H₂, HC₃N and C₂N₂ in Titan's atmosphere

V. G. Kunde, A. C. Aikin, R. A. Hanel, D. E. Jennings, W. C. Maguire & R. E. Samuelson

Goddard Space Flight Center, Code 693.2, Greenbelt, Maryland 20771, USA

The compounds C_4H_2 , HC_3N , and C_2N_2 have been detected in trace amounts in the stratosphere of Titan. The identification of two compounds containing nitrogen, in addition to HCN, provides further evidence for the abundance of free N_2 on Titan.

THE Titan atmosphere is characterized by hydrocarbon and nitrogen-containing organic compounds in an atmosphere which near the surface is predominantly composed of molecular nitrogen, methane, molecular hydrogen and possibly argon¹. Molecules containing oxygen have not been detected. Many organic compounds have now been identified in the stratosphere of Titan from ground-based and spacecraft IR spectroscopic observations. The first hydrocarbon identification was the ground-based observation of methane² (CH₄). Subsequent ground-based measurements identified ethane³ (C₂H₆), acetylene⁴ (C₂H₂) and ethylene⁵ (C₂H₄). Strong IR emissions due to these gases have also been observed with the IR instrument (IRIS)6 on Voyager 1. The IRIS spectrum also indicates the presence of hydrogen cyanide (HCN), methyl acetylene (C₃H₄), and propane^{6,7} (C₃H₈). We now identify three additional organic compounds: diacetylene (C₄H₂), cyanoacetylene (HC₃N), and cyanogen (C₂N₂).

Observations

Voyager 1 took IR measurements of the atmosphere of Titan from ~ 8 h before to ~ 3 h after closest approach. An average of 346 spectra, mostly from the centre of the disk but including data from latitudes as high as $\pm 60^{\circ}$, is shown in Fig. 1. The average emission angle is ~35°. Strong emission features associated with stratospheric gases appear above a background continuum (see Fig. 1 and Table 1 which also indicate weaker emission features due to C₃H₄ and C₃H₈). The high latitude spectrum is an average of 30 individual spectra obtained at 68° N latitude near or on the dark polar hood observed in Voyager images⁸. The average emission angle is ~63°. Several features are evident in the 200-700 cm⁻¹ region of the 68° N spectrum but not in the midlatitude disk average (220, 233, 500, 663 cm⁻¹). Other features are enhanced in the high polar region (328, 628, 633 cm⁻¹). Some enhancement is caused by the higher emission angle of the north polar spectrum compared

with that of the spectra recorded further south. However, in view of the low stratospheric temperatures in the north, the strong enhancement of some spectral features implies a genuine enrichment of these species at high northern latitudes. Differences in temperatures and chemical processes associated with the polar hood may be responsible for the enrichment.

The limb spectrum shown in Fig. 2 is an average of three individual spectra taken at grazing incidence above the north polar cap. The tangent ray defining the centre of the instrument field of view passes ~ 330 km above the surface of Titan at its closest point, while the diameter of the field of view at this point is ~ 190 km. Thus, there is considerable space contamination in the spectrum, which depresses the background continuum relative to the sharp emission features producing clear separation between the 220 and 233 cm⁻¹ features.

Identification of C₄H₂, HC₃N and C₂N₂

After accounting for the emission features of C_3H_4 , HCN, C_2H_2 , C_3H_8 , C_2H_6 , C_2H_4 , and CH₄, several prominent features still remained unidentified in the 200–700 cm⁻¹ region of the IRIS spectra. The sharp appearance of these features (220, 233, 500, 628, 633 cm⁻¹) suggested association with Q branch absorptions of minor stratospheric constituents.

The assignment of these five unidentified spectral features to C₄H₂, HC₃N and C₂N₂ was based on: (1) a correspondence in wavenumber and in the shape of the emission feature with laboratory spectra of the candidate molecules; (2) the apparent agreement of the relative intensities of the features with the corresponding strengths derived from the laboratory spectra if the candidate molecule has more than one feature in the observed spectrum. We only investigated gases which were considered possible constituents of the atmosphere of Titan. A list of potential constituents was assembled from organic chemistry studies involving photochemical and energetic particle bombardment processes⁹⁻¹¹. Spectra in the 200-1,400 cm⁻¹ region were obtained for these potential atmospheric constituents at nearly the same spectral resolution as Voyager IRIS. (The laboratory spectra were obtained at 2 and 4 cm⁻¹ resolution with a Fourier transform spectrometer operated by the Optics Branch, Goddard Space Flight Center. Samples were contained in a 3-cm length cell at pressures ranging from 11 to 140 torr. Sample temperatures were ambient (300 K).)

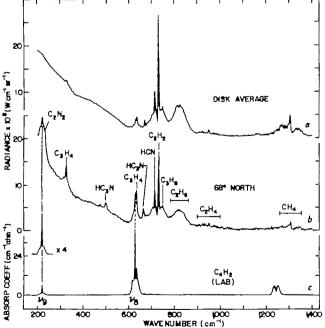


Fig. 1 Comparison of laboratory and Titan radiance spectra. The disk average spectrum (a) represents mainly mudiatitude regions on Titan while the 68° N spectrum (b) is of a region near or over the northern polar cap. Several emission features in the 200-700 cm⁻¹ region are enhanced in the 68° N spectrum. c, A laboratory spectrum for diacetylene (C₆H₂)

Table 1 Organic compounds in Titan's stratosphere inferred from Voyager IRIS

Chemical • family	Gas	Wave number (cm ⁻¹)	Approximate mole fraction
Carbon-hydrogen			
	CH.	130 4	3×10^{-2}
	C ₂ H ₄	822	2×10^{-5}
	C_1H_1	748	2×10 ⁻⁵
	C ₂ H ₂	729	2×10 ⁻⁴
	C,H,	950	4×10^{-7}
	C ₁ H ₄	325,633	3×10-4
	C,H ₂	220,628	10-8-10-7
Carbon-hydrogen-nitrogen			-
, ,	HCN	712	2×10^{-7}
	HC ₁ N	500, 663	10 ⁻⁴ -10 ⁻⁷
Carbon-nitrogen			
-	C_2N_2	233	10 ⁻⁴ -10 ⁻⁷

 C_4H_2 is expected to be present in the upper atmosphere of Titan as a result of methane photolysis and hydrogen escape, leading to C_2H and to the production of C_4H_2 by the reaction $C_2H+C_2H_2$. The two features in the IRIS spectrum at 220 and 628 cm⁻¹ correspond to the Q branches of the ν_p and ν_g fundamental vibration-rotation bands of C_4H_2 , respectively¹². These two features are marked in Fig. 1 but may be seen most distinctly in the high air mass spectrum of Fig. 2. The 220 cm⁻¹ C_4H_2 feature is partially overlapped by the 233 cm⁻¹ C_2N_2 emission and the 628 cm⁻¹ feature by the C_3H_4 emission at 633 cm⁻¹. However, the 4.3 cm^{-1} resolution allows identification of C_4H_2 . The intensity ratio for the two C_4H_2 features in the observed spectrum may be approximated by⁷

$$\frac{I_{220 \text{ cm}^{-1}}}{I_{628 \text{ cm}^{-1}}} = \frac{S_{220}^O}{S_{628}^O} \frac{B_{220}(130 \text{ K})}{B_{628}(130 \text{ K})}$$

where S is the strength of the Q branch of the molecular band, and B is the Planck function at 130 K. Q branch strengths derived from laboratory data yield an intensity ratio of ~ 0.74 , which agrees with the observed intensity ratio of ~ 0.72 for the north limb spectrum (Fig. 2). The observed intensity ratio is therefore consistent with the C_4H_2 identification. The C_4H_2 band at 1,250 cm⁻¹ is too weak to appear in the Titan spectrum.

 HC_3N is expected in Titan's atmosphere from the reaction of CN with C_2H_2 and C_2H_4 , where the CN is produced from the dissociation of HCN and the C_2H_2 from methane photolysis°. As indicated by comparison with the laboratory spectrum in Fig. 2, the HC_3N emissions from the ν_6 and ν_5 fundamental vibration-rotation bands^{13,14} at 500 and 663 cm⁻¹, respectively, are easily identified. The intensity ratio of the two features $(I_{500 \text{ cm}}^{-1}/I_{663 \text{ cm}}^{-1})$ are approximately the same in the observed and the laboratory spectra (\sim 0.8), thus strengthening the HC_3N identification. The HC_3N band at 1,310 cm⁻¹ is too weak to appear in the Titan spectrum.

 C_2N_2 is predicted to occur in the Titan atmosphere through the combination of a CN radical, either with HCN or with another CN radical. The only C_2N_2 emission feature occurring in the IRIS spectrum (Fig. 2) is the strong ν_5 fundamental band ¹³ at 233 cm⁻¹. The detection of C_2N_2 and HC₃N is consistent with experiments carried out for CH₄-N₂ mixtures ¹⁶.

The strong emission features of the three gases in the limb spectrum (Fig. 2) indicate that a large fraction of their mass is in the upper stratosphere (p < 20 mbar, T > 130 K). Additionally large concentrations are not expected to occur in the low temperature environment of the lower stratosphere due to condensation (see Fig. 2 of ref. 7). Thus we can associate the formation of these gases with photochemistry in the upper stratosphere. Crude abundance estimates for these three gases yield mole fractions between 10^{-8} and 10^{-7} , consistent with production by nitrogen ion and methane photolysis photochemistry.

Discussion and conclusion

The organic compounds observed in the atmosphere of Titan are summarized in Table 1, which also indicates the approximate

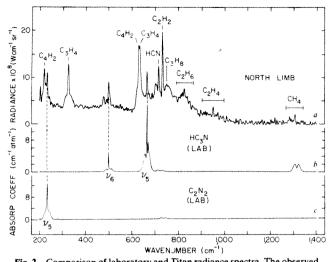


Fig. 2 Comparison of laboratory and Titan radiance spectra. The observed spectrum (a) was obtained off the limb of the planet in the northern polar cap region. The high air mass along the line of sight enhances the weak emission features, while space contamination in the field of view depresses the adjacent continuum. Laboratory spectra for cyanoacetylene (HC₃N) (b) and cyanogen (C2N2) (c) are shown.

mole fraction for each compound identified previously, as determined from analysis of the disk average spectrum. These compounds originate by reactions of methane and nitrogen radicals in a predominantly nitrogen atmosphere. Although Titan's location at 9.35 AU reduces the solar flux by a factor of 87 compared with the flux at the Earth, dissociation of significant quantities of methane still occurs due to Ly α radiation. Titan is located within the Saturn magnetosphere and lacks a shielding magnetic field. This causes energetic electrons and

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- Samuelson, R. E., Hanel, R. A., Kunde, V. G. & Maguire, W. C. Nature 292, 688-693 (1981).
- Kuiper, G. P. Astrophys. J. 100, 378-383 (1944). Gillett, F. C. & Forrest, W. J. Astrophys. J. Lett. 184, L93-L95 (1973).
- Tokunaga, A. Bull. Am. astr. Soc. 12, 669 (1980). Gillett, F. C. Astrophys. J. Lett. 201, L41-L43 (1975).
- Hanel, R. et al. Science 212, 192-200 (1981) Maguire, W. C., Hanel, R. A., Jennings, D. E., Kunde, V. G. & Samuelson, R. E. Nature 292, 683-686 (1981).
- Smith, B. A. et al. Science 212, 163-190 (1981).
- Strobel, D. F. Geophys. Res. Lett. (submitted)

protons to ionize and dissociate the principal atmospheric constituents. Bremsstrahlung extends the effective penetration depth of energetic electrons down to an altitude of 130 km. In addition to charged particles from Saturn's magnetosphere Titan is bombarded by galactic cosmic radiation, consisting mostly of protons in the GeV range, which penetrate to the surface. Particle ionization is expected to lead to neutral fragments and ions, including CH, N, CH₂, N₂⁺, N⁺, CH₄⁺, and CH₃. Subsequent ion-molecule reactions involving methane, molecular hydrogen, and molecular nitrogen lead to complex neutral hydrocarbons. The various energy sources, acting over a long period of time, have formed a complex array of hydrogencarbon-nitrogen compounds.

The wide variety of organic compounds, with fairly high abundances of C₂H₂ and C₃H₈, indicates that the chemistry of Titan is significantly different from that of Jupiter and Saturn. One reason is the low H_2 abundance $(\sim 0.2\%)^1$ which prevents rapid recycling of CH₄ (ref. 18), and yields higher relative levels of the methane photolysis reactive radicals (CH₂, CH₃, C₂H). Also, these and the CN radical from the dissociation of HCN, do not react with the major constituent N2 and thus are available to react with minor hydrocarbon species to form more complex compounds. Additionally, the reduced solar flux leads to an increased lifetime of minor atmospheric constituents against photodissociation.

Many of the Titan constituents have been detected in inter-stellar sources 18-20. Among those with non-zero dipole moments (HCN, HC₃N, C₃H₄, and C₃H₈) only C₃H₈ has not been detected at radio wavelengths for the interstellar sources.

We thank J. Heaney and K. Stewart for operating the laboratory spectrometer, and J. Faris for laboratory assistance. The diacetylene sample was supplied by H. Okabe. Partial support was provided by the NASA Planetary Atmospheres Program.

- Chang, S., Scattergood, T., Aronowitz, S. & Flores, J. Rev. Geophys. Space Phy. 17, 1923–1933 (1979).
- 1923-1933 (1979).
 Allen, M., Pinto, J. P. & Yung, Y. L. Astrophys. J. Lett. (in the press).
 Hardwick, J. L. & Ramsay, D. A. J. molec. Spectrosc. 76, 492-505 (1979).
 Job, V. A. & King, G. W. Can. J. Chem. 41, 3132-3133 (1963).
 Mallinson, P. D. & Fayt, A. Molec. Phys. 32, 473-485 (1976).

- Jones, L. H. J. Molec. Spectrosc. 45, 55-64 (1973). Toupance, G., Raulin, F. & Buvet, R. Orig. Life 6, 83-90 (1975)
- Strobel, D. F. Icarus 21, 466-470 (1974). Lovas, F. J., Snyder, L. E. & Johnson, D. R. Astrophys. J. Suppl. 41, 451-480 (1979).
- Hall, D. N. B. & Ridgway, S. T. Nature 273, 281–282 (1978)
 Betz, A. L. Astrophys. J. Lett. 244, L103–L105 (1981).

Mean molecular weight and hydrogen abundance of Titan's atmosphere

R. E. Samuelson, R. A. Hanel, V. G. Kunde & W. C. Maguire

Goddard Space Flight Center, Code 693.2, Greenbelt, Maryland 20771, USA

The 200-600 cm⁻¹ continuum opacity in the troposphere and lower stratosphere of Titan is inferred from thermal emission spectra from the Voyager 1 IR spectrometer (IRIS). The surface temperature and mean molecular weight are 94 K < $T_{
m G}$ <97 K and 28.3 < M < 29.2 AMU, respectively. The mole fraction of molecular hydrogen is 0.002 ± 0.001 , which is equivalent to an abundance of $\sim 0.2 \pm 0.1$ km amagat.

THERMAL inversion in Titan's atmosphere has been demonstrated by narrow band IR photometry1.2 and spectrophotometric data3 which showed that at least methane, ethane and probably ethene exist in a warm stratosphere overlying a colder background. Danielson et al.4 and Caldwell5 quantitatively explained the major features of the thermal emission spectrum using simple inversion models.

Models with a deeper atmosphere had also been suggested. But even in the model proposed by Hunten⁶, which was remarkably accurate in its thermal and compositional predictions, it was accepted that methane clouds and photochemically

induced smog would obscure the atmosphere below the tropopause. McCarthy et al.7 interpreted their data between 330 and 625 cm⁻¹ as being consistent with an inversion model. An optically opaque base, either the surface or dense clouds, was assumed to be roughly at a temperature of 74 K, below which little, if any, information was available.

This situation changed when Voyager 1 IR data showed limb darkening over the 200-600 cm⁻¹ spectral range⁸. The radiance at small emission angles (the angle at the surface between the direction of viewing and the surface normal) was found to be larger than that for large angles near the limb. This could occur

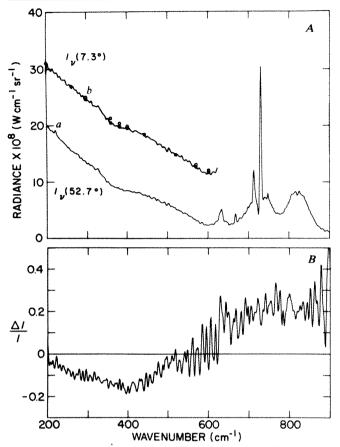


Fig. 1 A. a, average of 29 spectra near the daytime east limb. The average emission angle is 52.7° and the mean latitude is 7.2°N. b, Average of 10 spectra near the sub-spacecraft point. The average emission angle is 7.3° and the mean latitude is 8.3°N. Theoretical fits for a hydrogen mole fraction q = 0.002 (\blacksquare); q = 0 (O). Values are displaced 10 radiance units upwards for clarity. B, northern low-latitude spectral limb function: $\Delta I/I = 1 - I_{\nu}(7.3^{\circ})/I_{\nu}(52.7^{\circ})$.

only if the observed radiation originated principally below the temperature minimum, from the troposphere.

The observed spectral limb function is

$$\frac{\Delta I}{I} = \frac{I_{\nu}(52.7^{\circ}) - I_{\nu}(7.3^{\circ})}{I_{\nu}(52.7^{\circ})}$$
(1)

where $I_{\nu}(\theta)$ is the radiance at wavenumber ν and emission angle θ . $I_{\nu}(7.3^{\circ})$ and $I_{\nu}(52.7^{\circ})$ are averages of 10 and 29 spectra, respectively, and are shown in Fig. 1 with the spectral limb function.

We have found that at low latitudes the limb function is symmetric about the sub-spacecraft meridian, indicating a lack of diurnal and longitudinal variation, although Flasar $et\,al.^9$ find evidence for a slight latitudinal inhomogeneity at $\sim 10^\circ$ south latitude where a weak opacity transition at $200~\rm cm^{-1}$ seems to occur. This coincides approximately with the visual brightness transition found near the equator 10 . Thus the limb function given by equation (1) should be free of horizontal inhomogeneity as the selected data sets are associated with fields of view that are small, closely grouped, and are between 0° and 15° N latitude. Because this latitude range also includes the ingress Earth occultation point, we can incorporate the radio science temperature profile 11 directly into a quantitative interpretation of our data.

Models

The two spectral averages indicated in equation (1) yield independent information, principally because the large difference in emission angles ensures that different effective emission levels in the atmosphere are being sampled. Thus at each wavenumber two independent parameters characterizing the radiative properties of the atmosphere can be inferred. We have chosen for these the optical thicknesses of the stratosphere $\Delta \tau_{\rm S}$ and tropopause $\Delta \tau_{\rm T}$.

We restrict the computational models to the range 200–600 cm⁻¹. Solutions for higher wavenumbers do not converge, probably because of insufficient signal below the tropopause. A simple exponential law of absorption is assumed, which is adequate as long as clouds, hazes and pressure-induced absorption by gases are the only opacity sources. Thus we avoid the spectral ranges 205–255 cm⁻¹, 305–355 cm⁻¹, 460–515 cm⁻¹ and above 605 cm⁻¹, where sharp-line spectral features are seen in the spectra⁸. The formal solution to the equation of transfer

$$I_{\nu} = \int_{0}^{\infty} B_{\nu}(\tau) e^{-\tau/\mu} \frac{\mathrm{d}\tau}{\mu} \tag{2}$$

can be expressed numerically by

$$I_{\nu}(\mu_{k}) = \sum_{i=1}^{n} B_{\nu}(\tau_{i}) \left\{ \exp\left[-\sum_{j=i+1}^{n} \frac{C_{j}}{\mu_{k}} \Delta t_{j}\right] \right\}$$

$$\times \left\{ 1 - \exp\left[-\frac{C_{i}}{\mu_{k}} \Delta t_{i}\right] \right\} (k = 1, 2)$$
(3)

where $B_{\nu}(\tau_i)$ is the Planck function at wavenumber ν (constant across layer i), τ is the optical depth, μ_k the direction cosine of the kth emission angle, n the number of layers (including the ground), and

$$\Delta \tau_i = C_i \Delta t_i (i = 1, \dots, n) \tag{4}$$

is the optical thickness of layer i. The relative vertical distribution of opacity is specified in the model by the reduced optical thickness Δt_i . The C_i in equations (3) and (4) are then solved by an iterative technique. For each wavenumber interval the C_i provides multiplication factors which convert the assumed relative optical thicknesses, Δt_i , into absolute values $\Delta \tau_i$. Because we have only two free parameters available we assign one value to the stratosphere and the other to a layer at the tropopause; the latter is about the level where we expect the upper boundary of methane clouds. The height of the stratospheric layer as well as its vertical extent is varied from case to case. In some cases a third multiplier C=1 is specified in the troposphere to accommodate pressure-induced absorption.

The temperature profiles are patterned after the radio occultation ingress profile (ref. 11 and G. L. Tyler and G. Lindal,

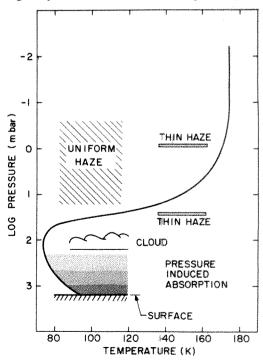
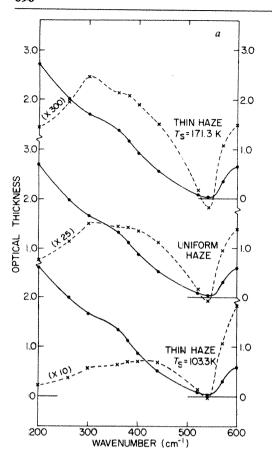


Fig. 2 Temperature profile corresponding to our nominal model. The shape between 10 and 1,600 mbar is taken from data provided by G. L. Tyler and G. Lindal and normalized to a surface temperature $T_{\rm G} = 95$ K. The extrapolation above 10 mbar is forced to be consistent with brightness temperatures observed in the 1,304 cm⁻¹ $\nu_{\rm c}$ band of methane in the IRIS spectra.



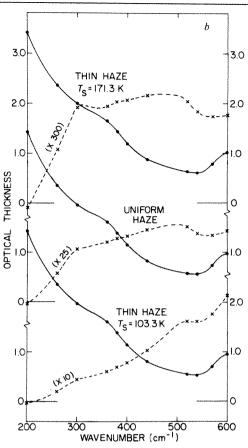


Fig. 3 Optical thicknesses of the stratosphere $\Delta \tau_{\rm S} (- \times - \times - \times)$ and tropopause $\Delta \tau_{\rm T} (- - - \times)$. The three models for $\Delta \tau_{\rm S}$ are expanded by factors of 300, 25, and 10 for clarification. Surface temperature: a, $T_{\rm G} = 94~{\rm K}$; b, $T_{\rm G} = 96.3~{\rm K}$.

personal communication). The shape of this profile is retained, but the magnitude is normalized to different values of the surface temperature $T_{\rm G}$, which is equivalent to assuming different (constant with altitude) mean molecular weights M. Above 10 mbar the profile is extended to 0.01 mbar by extrapolating to 174 K, an asymptotically limiting value consistent with the brightness temperatures at the Q-branch of CH_4 at 1,304 cm⁻¹ in the low-latitude IRIS spectra. Partial checks using the CH_4 P-branch brightness temperatures at 1,260 cm⁻¹ show internal consistency to within \sim 3 K at the 5 mbar level. A nominal working profile with $T_{\rm G}$ = 95 K is shown in Fig. 2.

Figure 2 also shows the various forms of opacity considered in our models. The first set of models consists of one cloud layer at the tropopause and three opacity alternatives in the stratosphere: two thin haze layers located at temperatures of 171.3 K and 103.3 K, and a haze mixed uniformly throughout the stratosphere with an opacity directly proportional to the atmospheric density. Each alternative is computed separately using the radiances measured at both emission angles. The solutions are the total stratospheric and tropopause optical thicknesses $\Delta \tau_{\rm S}$ and $\Delta \tau_{\rm T}$ as functions of wavenumber, and are shown in Fig. 3 for surface temperatures of 94 K and 96.3 K. These temperatures are close to the minimum and maximum permissible values, indicating that the range of possible opacity models is bounded by those shown in Fig. 3.

Thermal opacity

Several conclusions can be inferred from Fig. 3. First, although the wavenumber dependence is not well defined, the stratospheric opacity tends to be small at all wavenumbers for all models. Thus the contribution of emission from the stratosphere is approximately

$$I_{\rm S} = B_{\nu} \Delta \tau_{\rm S} \tag{5}$$

where B_{ν} is the Planck function associated with layer $\Delta \tau_{\rm S}$. (For the uniform haze models an appropriate weighting of the Planck function must be carried out.) Because the Planck function corresponding to stratospheric temperatures is large relative to values corresponding to temperatures in the troposphere (see

Fig. 2), the stratospheric contribution to the measured radiance is not negligible despite small values of $\Delta \tau_s$, but can amount to a large fraction of the total signal. However, without an independent determination of the vertical distribution of the stratospheric haze, it is not possible to separate B_{ν} and $\Delta \tau_s$. Thus, because the wavenumber dependence of B_{ν} varies strongly over the range of stratospheric temperatures, a correspondingly large uncertainty also exists in the wavenumber dependence of $\Delta \tau_s$.

Conversely, the qualitative wavenumber dependence of the tropopause opacity is well defined independently of the way in which the stratosphere is modelled, principally because the stratospheric opacity is too small to impede or modify radiation passing through it from below. From 200 cm⁻¹ to ~540 cm⁻¹ the tropopause opacity decreases monotonically with a reversing trend observed between 540 and 600 cm⁻¹. Beyond 600 cm⁻¹ stable solutions are impossible, apparently because there molecular emission from the stratosphere dominates the spectrum.

The general increase of tropopause opacity with decreasing wavenumber below about $540~\rm cm^{-1}$ has led to two suggestions for the absorption mechanism⁸: pressure-induced absorption by various CH₄ and N₂ combinations, and absorption by methane clouds. Calculations by R. Courtin (personal communication) imply that pressure-induced absorption could supply only a few per cent of the required opacity at the low temperatures prevailing in the Titan troposphere. Other data ^{12,13} suggest CH₄ clouds to be a realistic opacity source. The observed thermal dis-

Table 1 Possible mole fractions	Possible mole fractions of different gases at Titan's surface						
Gas	Mole fraction						
N_2	0.822						
Ar	0.116						
CH₄	0.060						
H ₂	0.002						

The nominal model has a surface temperature of 95 K and a mean molecular weight of 28.6 AMU. The methane fraction of saturation is slightly less than 0.5. Argon ($M=40~\mathrm{AMU}$) is postulated to be present, partly because its presence sexpected on the basis of its cosmic abundance and partly because it is one of the few gases that will not freeze out at the abundance required to balance the mean molecular weight.

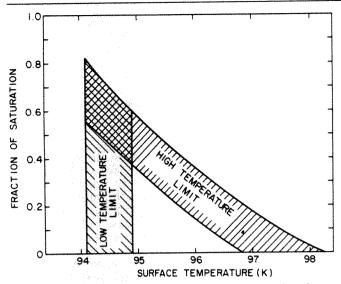


Fig. 4 Minimum and maximum allowable surface temperatures for a given fraction of methane saturation. For a constant methane mole fraction, a fractional saturation of 0.19 at the surface corresponds to complete saturation just below the tropopause. The widths of the bands for the high and low temperature limits give a measure of the uncertainties.

continuity across the equatorial region at 200 cm⁻¹ also implies that clouds contribute at least part of the opacity. Until more extensive laboratory data on liquid and solid CH₄ are considered we can only suggest that methane clouds may provide the dominant opacity in Titan's upper troposphere.

Surface temperature and mean molecular weight

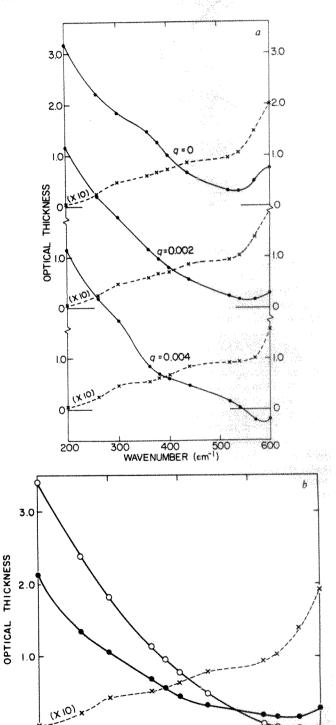
At 540 cm⁻¹, the models in Fig. 3a show a negative stratospheric opacity and a tropopause opacity that is almost zero; hence the total opacity in the atmosphere is effectively zero, and to lower the surface temperature T_G below 94 K would require both opacities to become negative. Because this is physically impossible, 94 K is a lower limit, and the observed emission at 540 cm⁻¹ is dominantly from the surface. If the actual surface temperature were lower, stratospheric emission would have to compensate to yield the measured radiance. This would cause limb brightening, contrary to what is shown in Fig. 1. On the other hand, increasing T_G to 96.3 K causes the calculated stratospheric opacity at 200 cm⁻¹ to become negative (Fig. 3b). This is a consequence of requiring the minimum atmospheric temperature to be higher than the observed brightness temperature of 74 K at 200 cm⁻¹, again a physical impossibility.

Thus the surface temperature seems to be bounded between close limits, although a few corrections need to be made. Some opacity due to hydrogen absorption is required at $540 \, \mathrm{cm}^{-1}$ and our modelling suggests that this can be achieved by raising the minimum surface temperature limit to $94.5 \pm 0.4 \, \mathrm{K}$. Noise in the data and uncertainties in the H_2 absorption coefficients account for the uncertainty in T_G .

The upper limit to the surface temperature is more uncertain. Its assessment depends first on determining an upper limit to the tropopause temperature, and then scaling this to the surface with the aid of the radio occultation profile 11. If the limb darkening of about 0.8 K observed at 200 cm 1 is due partly to multiple scattering in methane clouds, the tropopause temperature must be raised some fraction of this amount. Alternatively, if the 200 cm 1 brightness temperature discontinuity of $\sim 1 \, \mathrm{K}$ across the equator 9 is due to an opacity gradient rather than a horizontal thermal gradient, the tropopause temperature must be reduced accordingly. The upper limit to the temperature at the tropopause becomes 74 K with an estimated uncertainty of $\pm 0.5 \, \mathrm{K}$. This scales to a surface temperature upper limit of $96.3 \pm 0.7 \, \mathrm{K}$.

A further complication is introduced when a variable CH_4 mole fraction below the tropopause is considered, requiring that the mean molecular weight M also be scaled. From published data¹⁴ it is found that the condensation profile for methane

becomes tangent to the temperature profile at $T=75~\rm K$ for a methane mole fraction of $q(\rm CH_4)=0.0266$. Thus, if methane saturates anywhere it will do so at the 75 K level, resulting in a constant mole fraction of 0.0266 above this level and a larger variable fraction below it. Using the T/M value of 2.55 K $(\rm AMU)^{-1}$ at the tropopause¹¹, we find M=29.0 at $T_{\rm T}=74~\rm K$. This leads to a height-independent molecular weight of 29.4 AMU for the remainder of the atmosphere after the contribution from $\rm CH_4$ is subtracted out. On adopting the radio



300

200

400

WAVENUMBER (cm⁻¹)

500

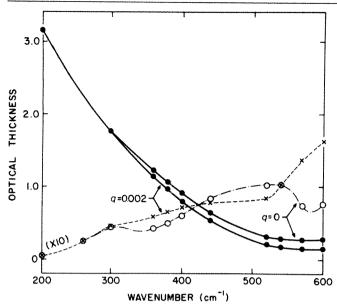


Fig. 6 Modified optical thicknesses. Smooth fits to $\Delta \tau_{\rm T}$ in Fig. 5a for two values of q are shown (——). Corresponding solutions for $\Delta \tau_{\rm S}$ are obtained by forcing a fit to the limb spectrum in Fig. 1 (——×——×, and—·—). The surface temperature is $T_{\rm G} = 95~{\rm K}$.

occultation T/M value of 3.32 K (AMU)⁻¹ for the surface ¹¹ it becomes straightforward to calculate the surface temperature for different mole fractions of methane. As the tropopause temperature of 74 K is an upper limit, the surface temperatures scaled from this value are also upper limits.

The results from these calculations are shown in Fig. 4 as a function of the fraction of methane saturation. Also shown is the lower limit to the surface temperature calculated previously. Both limits are displayed as bands to illustrate the attendant uncertainties. Any combination of surface temperature and fraction of saturation within the enclosed triangle is a legitimate solution. Values for the fraction of saturation below 0.19 are not very likely on physical grounds, however, because they correspond to methane mole fractions less than the tropopause value. The lower troposphere seems to be undersaturated with respect to methane, perhaps substantially so, although the fairly long chain of reasoning involved makes it difficult to assess the associated uncertainties. From Fig. 4, with the fraction of saturation equal to 0.19, the limits for the surface temperature

$$94 K < T_{\rm G} < 97 K \tag{6}$$

from which it follows that the limits for the mean molecular weight are

$$28.3 < M < 29.2 \text{ AMU}$$
 (7)

If the CH_4 fraction of saturation at the surface is >0.19, as is likely, the upper limits in expressions (6) and (7) must be reduced accordingly.

Tyler and Lindal (personal communication) have pointed out that the radio occultation values for T/M (ref. 11) are accurate to two significant figures but probably not to three, adding an uncertainty of about ± 0.8 AMU to the limits of M in equation (7). On the other hand, inferring the surface temperature from one that is measured at the tropopause depends essentially only on the ratio of the respective T/M values. As this ratio is better determined than the individual values of T/M (G. L. Tyler and G. Lindal, personal communications), the upper limits for the surface temperature in Fig. 4 and equation (6) are well established.

Despite the many uncertainties M is probably >28, the value for molecular nitrogen. Taking $T_G = 95$ K and $(T/M)_G = 3.32$, we find M = 28.6. If $q(CH_4) = 0.06$ at the surface (<50% of saturation; see Fig. 4) and the atmosphere is composed only of hydrogen, methane, nitrogen and argon, it is possible to construct the mole fraction for each. The results are listed in Table 1 and show a large amount of argon (~12%), although the

atmosphere remains dominantly nitrogen. Table 1 is only meant to be representative of reasonable conditions. We have no direct evidence that argon exists in the atmosphere, only that some molecular constituent higher in mass than N_2 is probably present, and argon is a plausible choice because of its inert qualities and low condensation temperature. The upper limit of 6% argon inferred by the UV experiment is not inconsistent with our suggested value.

Molecular hydrogen abundance

Superimposed on the general tropopause opacity continuum shown in Fig. 3 are two localized broad features at $360~\rm cm^{-1}$ and $600~\rm cm^{-1}$ which correspond closely to the S(0) and S(1) pressure-induced absorption lines of molecular hydrogen. We have calculated approximate absorption coefficients for the pressure-induced absorption of H_2 due to collisions with N_2 . The calculations are based primarily on the theoretical work of Birnbaum and Cohen¹⁶ for H_2 – H_2 collisions. The conversion to absorption coefficients for H_2 – H_2 collisions was accomplished by scaling the laboratory data of Kiss and Welsh¹⁷ and Kiss *et al.*¹⁸. As both the integrated absorption coefficient and the line half widths were scaled from data obtained at 195 K, there is an estimated uncertainty in the absorption coefficients of ~25% at the temperatures prevailing near the surface of Titan.

We have normalized the temperature profile in Fig. 2 to a surface temperature of 95 K, a realistic value according to Fig. 4. Induced H_2 - N_2 absorption is then added to the other sources of opacity in the radiative transfer calculations. This new opacity source has a pressure-squared dependence, most dominant in the lower troposphere. Above the tropopause the effect was neglected because it is <1% of its value near the surface.

According to the models discussed previously, the tropopause opacity calculations are basically independent of the stratospheric model adopted (see Fig. 3). This remains true when pressure-induced H_2 opacity is added to the troposphere. Figure 5a shows several solutions for the optical thicknesses of the stratosphere and tropopause for the thin haze model with $T_{\rm S}=103.3$ K. Other stratospheric models yield essentially the same results for $\Delta\tau_{\rm T}$. Thus the H_2 mole fraction seems fairly well defined if one imposes the condition that the wavenumber dependence of $\Delta\tau_{\rm T}$ be as smooth as possible. A hydrogen mole fraction $q\sim0.002\pm0.0005$ provides the smoothest fit to $\Delta\tau_{\rm T}$ of the cases examined, although the actual uncertainty in q is larger owing to the 25% uncertainty in the hydrogen absorption coefficients, leading to a final value of

$$q(H_2) = 0.002 \pm 0.001$$
 (8)

The result for q is basically independent of the stratospheric model adopted. Further tests show this to be true for the tropospheric opacity also. One such test, shown in Fig. 5b, entails a model that departs radically from the previous ones. Instead of placing all the tropospheric opacity at the tropopause, substantial pressure-induced absorption associated with a fictitious gas is introduced, which places most of the absorption near the surface. The total optical depth of this absorber is shown as a function of wavenumber in Fig. 5b. The much weaker pressureinduced absorption of H_2 with q = 0.002 is then added, and solutions for $\Delta \tau_{\rm S}$ and $\Delta \tau_{\rm T}$ are obtained as before. Although the quantitative solutions for $\Delta \tau_{\rm T}$ are substantially different from those in Fig. 5a, the shapes of the two curves corresponding to identical values of q are quite similar, showing the same degrees of smoothness. The solutions for $\Delta \tau_{\rm S}$ are nearly identical in both cases. Thus the determination of q seems to be independent of the method by which the tropospheric background opacity is modelled as long as the wavenumber dependence of the latter is smooth.

An estimate of the sensitivity of the solutions to the accuracy of the data can be obtained by replacing $\Delta \tau_{\rm T}$ with a completely smooth curve and forcing perfect fits to the limb radiances I_{ν} (52.7°) (see Fig. 1). Subjectively determined smooth fits of $\Delta \tau_{\rm T}$ from Fig. 5 for q=0 and q=0.002 are shown in Fig. 6. Solutions for $\Delta \tau_{\rm S}$ for q=0.002 and 0 are also shown. These results lead in

turn to the solutions for I_{ν} (7.3°) shown in Fig. 1 for q = 0 and q = 0.002; the observed spectrum at $\theta = 7.3^{\circ}$ is superimposed.

As Fig. 1 demonstrates, the solutions for q = 0.002 fit the data well, whereas those for q = 0 show marked departures across the S(0) line and smaller departures in the vicinity of the S(1) line. In Fig. 6 the q=0 solutions for $\Delta \tau_{\rm S}$ inversely mimic the signatures corresponding in position to the S(0) and S(1) lines. Apparently this is how the model compensates for a lack of H₂ absorption in the troposphere in the process of fitting the limb spectrum in Fig. 1 (see also equation (5)). Conversely the q = 0.002 solutions for $\Delta \tau_s$ in Fig. 6 show no such inclination, implying that the H₂ opacity has been modelled correctly.

The total depth of our model atmosphere is ~90 km amagat, yielding from equation (8) a molecular hydrogen abundance of ~0.2 km amagat. This is a factor 25 lower than Trafton's 19,20

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- Gillett, F. C., Forrest, W. J. & Merrill, H. M. Astrophys. J. Lett. 184, L93 (1973). Low, F. J. & Rieke, G. H. Astrophys. J. Lett. 190, L143-L145 (1974).

- Gillett, F. C. Astrophys. J. Lett. 201, L41–L43 (1975). Danielson, R. E., Caldwell, J. J. & Larach, D. R. Icarus 20, 437 (1973).
- Caldwell, J. Planetary Satellites (ed. Burns, J.) 438 (University of Arizona Press, 1977). Hunten, D. M. NASA Conf. Publ. 2068 127 (1978).
- McCarthy, J. F., Pollack, J. B., Houck, J. R. & Forrest, W. J. Astrophys. J. 263, 201-205
- Hanel, R. et al. Science 212, 192-200 (1981). Flasar, F. M., Samuelson, R. E. & Conrath, B. J. Nature 292, 693-698 (1981).
- 10. Smith, B. A. et al. Science 212, 163-191 (1981)

suggested value of 5±3 km amagat, and a factor 10 lower than his lower limit. We cannot reconcile the two results. However, our value is consistent with the upper limit of 1 km amagat reported by Münch et al.21. It is also compatible with the steady-state mole fraction expected from balancing the production rate of H₂ (due to photochemical and charged particle decomposition of methane) with the H₂ loss rate (due to upward diffusion and subsequent blowoff at the top of the exosphere)6.

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- 11. Tyler, G. L. et al. Science 212, 201-206 (1981).
- Savoie, R. & Fournier, R. P. Chem. phys. Lett. 7, 1 (1970).
 Savoie, R. & Fournier, R. P. Chem. phys. Lett. 7, 1 (1970).
 Obriot, J., Fondère, F., Marteau, P., Vu, H. & Kobashi, K. Chem. phys. Lett. 60, 90 (1978).
 Prydz, R. & Goodwin, R. D. J. chem. Thermodyn. 4, 127-133 (1972).
 Broadfoot, A. L. et al. Science 212, 206-211 (1981).
 Birnbaum, G. & Cohen, E. R. Can. J. Phys. 54, 593-602 (1976).

- Kiss, Z. J. & Welsh, H. L. Can. J. Phys. 37, 1249-1259 (1959).
- 18. Kiss, Z. J., Gush, H. P. & Welsh, H. L. Can. J. Phys. 37, 362-376 (1959).
- Trafton, L. Astrophys. J. 175, 285-293 (1972). Trafton, L. Icarus 24, 443-453 (1975).
- 21. Münch, G., Trauger, J. T. & Roesler, F. L. Astrophys. J. 216, 963-966 (1977).

Titan's atmosphere: temperature and dynamics

F. M. Flasar, R. E. Samuelson & B. J. Conrath

Laboratory for Extraterrestrial Physics, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

In the lower atmosphere of Titan IR brightness temperatures exhibit meridional contrast ≤ 3 K. Seasonal variations are absent because of the large radiative time constant. In the upper stratosphere meridional contrasts are ~ 20 K, consistent with 100 m s⁻¹ cyclostrophic zonal winds, and the radiative time constant is short, implying a large seasonal variation in the temperature and wind field. The absence of longitudinal thermal structure implies that zonally symmetric flows effect the meridional transport of heat. A simple model yields meridional velocities ~0.04 cm s⁻¹ and vertical eddy viscosities $\sim 10^3$ cm² s⁻¹ in the lower troposphere, and meridional velocities ~ 5 cm s⁻¹ in the upper stratosphere.

COVERAGE of Titan by the IR instrument (IRIS) during the Voyager 1 encounter provided information on the global thermal structure of the satellite and its atmosphere at relatively high spatial resolution. Here we present data obtained in three spectral intervals which sample the lower troposphere and surface, tropopause, and the upper stratosphere. We discuss the meridional and longitudinal thermal structure implied by the data. Finally, we estimate radiative time constants at various levels of Titan's atmosphere and examine a class of dynamical flows which are consistent with the data.

Observations

The data were obtained over a 6-h interval when the spacecraft was within 2×10^5 km of the center of Titan and the instrument field of view was less than one-third of the satellite's 2.570 km radius. The season on Titan was close to northern spring equinox. Three spectral regions have been analysed. In the first, centred at 530 cm⁻¹, the total optical thickness of the atmosphere is $\tau \le 0.6$ (ref. 1). The major contributor to the opacity is pressure-induced absorption of molecular hydrogen from H2-N₂ collisions. As this effect is confined to a vertical range close to the ground, the 530 cm⁻¹ brightness temperature should be close to the actual surface temperature. Brightness temperatures near 200 cm⁻¹ correspond approximately to the tropopause temperature¹. The 200 cm⁻¹ opacity has been inferred to be fairly large $(\tau \ge 3)$, confined mainly to clouds, and constrained to a vertical range over which the atmosphere is essentially isothermal. Finally, the 1,304 cm⁻¹ ν_4 Q-branch of

methane is associated with emission from the ~0.3 mbar pressure level.

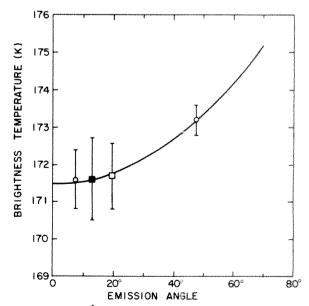


Fig. 1 1,304 cm⁻¹ low latitude limb function. O, Northern latitude daytime data; [], southern daytime data; [], southern nighttime data. Error bars include uncertainties due to noise and calibration.

Table	e 1 1,304-	-1,260 cm ⁻¹ brights	ness temperature	differences
Е	mission			
Latitude	angle	$T_{\rm B}(1,304)$	$T_{\rm B}(1,260)$	$\delta T_{\rm p}({\bf K})$
-55.0°	50.5°	169.8 ± 0.7	153.7 ± 1.7	16.1 ± 1.9
+7.5°	47.5°	173.2 ± 0.4	156.7 ± 1.3	16.5 ± 1.4
+52.6°	50.5°	168.1 ± 0.9	149.1 ± 2.1	19.0 ± 2.3

Vertical temperature gradients produce an emission angle dependence in the observed radiance, which must be removed to infer the horizontal structure in atmospheric temperature. We have therefore normalized the data in each wavenumber region to a common emission angle. Corrections at $200 \, \mathrm{cm}^{-1}$ and $530 \, \mathrm{cm}^{-1}$ were determined from a spectral limb function derived from data¹ restricted to the latitude range $0-15^{\circ}$ N. These corrections have been applied to the data at all latitudes, even though there seems to be a subtle but real meridional change in opacity across the equator. There is no rigorous justification for doing this; however, as the corrections tend to be small ($\leq 0.8 \, \mathrm{K}$ at $200 \, \mathrm{cm}^{-1}$ and $\leq 0.3 \, \mathrm{K}$ at $530 \, \mathrm{cm}^{-1}$) any corresponding systematic error should also be small.

The larger emission angle corrections at 1,304 cm⁻¹ are more reliable. Horizontal opacity inhomogeneities (due to clouds, for example) are not as likely near the 0.3 mbar level as they are at lower altitudes. Moreover, the difference between the brightness temperatures at 1,260 cm⁻¹ and 1,304 cm⁻¹, divided by the difference in altitudes at which the respective CH4 weighting functions peak, gives a crude approximation of dT/dz at each geographical location. Figure 1 depicts the emission angle corrections derived for low latitudes. Table 1 shows the brightness temperature differences $T_{\rm B}(1,304) - T_{\rm B}(1,260)$ at three widely separate latitudes where enough spectra are available for sufficient signal at 1,260 cm⁻¹. The emission angles are very similar, and the values of $\delta T_{\rm B}$ are identical to within the noise of the data. As CH₄ should be uniformly mixed at these levels, the altitude difference between weighting functions should be independent of geographical location. Thus the emission angle corrections derived for low latitudes should also be valid at high latitudes.

Other uncertainties and corrections exist. The statistical uncertainty due to noise was generally about $\pm 1\,\mathrm{K}$ at $1,304\,\mathrm{cm}^{-1}$; at $200\,\mathrm{cm}^{-1}$ and $530\,\mathrm{cm}^{-1}$ it was smaller, $\pm 0.1\,\mathrm{K}$, because it was possible to average over fairly wide spectral intervals $(60-80\,\mathrm{cm}^{-1})$ and because the IRIS signal-to-noise ratio was higher at these wavenumbers. Finally, all nightside data were corrected for a time-dependent calibration drift of the instrument. This drift occurred principally from the heating of the secondary mirror of the instrument which occurred when it became exposed to direct sunlight. The uncertainty in these calibration corrections is $<0.1\,\mathrm{K}$ at $1,304\,\mathrm{cm}^{-1}$ and $\sim0.4\,\mathrm{K}$ and $0.5\,\mathrm{K}$ at $200\,\mathrm{cm}^{-1}$ and $530\,\mathrm{cm}^{-1}$, respectively.

Results

The data have been examined for evidence of longitudinal structure. At several latitudes there were day/night observations separated by $\sim 180^\circ$ in longitude. At low latitudes longitudinal coverage was more extensive, though not complete; over limited spans of longitude, scales down to 30° could be resolved. In general fewer spectra could be averaged in this analysis than in the study of meridional structure, and the

uncertainties exceeded the formal errors quoted above. Within these uncertainties, no longitudinal or hour angle dependence of brightness temperature was discernible. Reasonable upper limits on temperature variation are 1 K at 200 cm⁻¹ and 530 cm⁻¹ and 3 K at 1,304 cm⁻¹.

Meridional variations in temperature are better defined. Table 2 summarizes all the data used. The number of spectra used in obtaining the mean brightness temperatures T_B are listed and the mean latitude and emission angle α of the data set are given. Also indicated is the emission angle correction $\Delta T_{\rm B}(\alpha)$ required to normalize $T_{\rm B}$ to $\alpha = 52.7^{\circ}$. Finally, brightness temperatures corrected for emission angle are listed. The associated uncertainties are the sum of uncertainties due to noise, emission angle corrections, and nightside calibration corrections. Because the first represents a random error while the last two are systematic, we considered the sum of their magnitudes rather than a vector sum in estimating the total error. The corrected brightness temperatures are also shown in Fig. 2. Vertical bars represent the uncertainties listed in Table 2, while horizontal bars indicate the latitude range, defined by the centres of the relevant fields of view, over which the data set comprising each point extend.

The meridional variation in brightness temperature at 200 cm⁻¹ is small. Brightness temperatures in the northern hemisphere are ~1 K higher than those at southern latitudes; a sharp transition occurs near 10° S. The meridional contrast in atmospheric temperature must also be small. The principal source of opacity at 200 cm⁻¹ has been inferred to be a system of methane clouds, situated mainly at or just below the tropopause $(\sim 100 \text{ mbar})$ and ~ 3 in total optical thickness over northern low latitudes. No configuration of clouds with these opacities could mask meridional contrasts larger than a few degrees, as the temperature lapse rate is small near the tropopause. The small difference in brightness temperature between northern and southern latitudes may be an opacity rather than a thermal effect. As the abundance of stratospheric methane is controlled by saturation just below the tropopause^{1,2}, the methane cloud system will not extend very far into the stratosphere. An increase to $\tau \sim 4$ over southern latitudes would easily account for the ~1 K brightness temperature reduction. The correlation between our data and the enhanced visual brightness in the southern hemisphere found by Smith et al.3 also suggests methane clouds. The opacity 'boundary' at ~10° S latitude seems to correlate fairly well with that discovered by Smith et al.3 within about $\pm 5^{\circ}$ of the equator.

At $530~\rm cm^{-1}$, where the effective emission level is close to the ground¹, there is a real temperature variation with latitude. Pressure-induced H_2 absorption is the major opacity source while that from clouds is relatively small, implying that the effective emission level is perhaps $\sim 1,500~\rm mbar$. The thermal variation with latitude is roughly symmetric about the equator. At low latitudes the daytime temperatures, which have smaller errors, are slightly warmer in the north than in the south. This may very well reflect a higher atmospheric opacity in the south, possibly from clouds at higher altitudes. This would be consistent with the north/south opacity difference inferred at $200~\rm cm^{-1}$. When extrapolated, the data suggest a total equator-to-pole surface temperature variation of about 3 K.

In contrast the meridional variation of the 1,304 cm⁻¹

Table 2 200, 530, and 1,304 cm⁻¹ latitudinal temperature distributions

		Latitud	Latitude (deg)		Emission angle $\alpha(\deg)$									
	N 7 -	200 cm ⁻¹		200 cm ⁻¹		,	$T_{\boldsymbol{B}}(\boldsymbol{K})$			$\Delta T_{\mathrm{B}}(\alpha)$			$T_{\mathbf{B}}(\mathbf{corr})$	
Day/night	No. spectra		1,304 cm		1,304 cm ⁻¹	200 cm ⁻¹	530 cm ⁻¹	1,304 cm ⁻¹	200 cm ⁻¹	530 cm ⁻¹	1,304 cm ⁻¹	200 cm ⁻¹	530 cm ⁻¹	1,304 cm ⁻¹
N	13	-59.6	-55.0	54.6	50.5	72.8	91.3	169.8	+0.2	0	-0.2	73.0 ± 0.5	91.3 ± 0.6	169.6 ± 0.7
N	8	-48.3	-45.0	45.8	42.0	73.3	91.3	173.5	-0.1	-0.1	+0.4	73.2 ± 0.5	91.2 ± 0.6	173.9 ± 0.8
D	4	~29.6	-27.5	47.1	43.5	73.1	92.2	174.6	-0.5 ± 0.2	-0.1	+0.4	72.6 ± 0.3	92.1 ± 0.2	175.0 ± 1.0
D	6	-10.0	-9.5	21.0	19.5	74.1	92.8	171.7	-0.6 ± 0.1	-0.3	+1.4	73.5 ± 0.2	92.5 ± 0.1	173.1 ± 0.9
N	5	-8.7	-8.7	13.7	12.8	74.8	92.6	171.6	-0.7	-0.4	+1.6	74.1 ± 0.5	92.2 ± 0.6	173.2 ± 1.1
D	29	+7.2	+7.5	52.7	47.5	74.0	93.1	173.2	0	0	0	74.0 ± 0	93.1 ± 0	173.2 ± 0.4
Ď	10	+8.3	+8.4	7.3	7.3	74.8	93.5	171.6	-0.8	-0.4	+1.6	74.0 ± 0	93.1 ± 0.1	173.2 ± 0.8
Ď	Š	+33.9	+32.0	30.9	29.8	74.5	93.1	171.6	-0.5 ± 0.2	-0.3	+1.0	74.0 ± 0.2	92.8 ± 0.1	172.6 ± 1.1
N	6	+35.7	+33.5	45.8	42.0	74.2	91.9	171.2	-0.2 ± 0.1	-0.1	+0.4	74.0 ± 0.6	91.8 ± 0.6	171.6 ± 1.1
D	10	+57.1	+52.6	55.0	50.5	73.6	91.3	168.3	+0.1	0	-0.2	73.7 ± 0.1	91.3 ± 0.1	168.1 ± 0.9
Ď	6	+67.4	+61.4	66.9	59.2	73.7	90.8	164.8	$+0.7\pm0.4$	$+0.3\pm0.1$	-1.0	74.4 ± 0.5	91.1 ± 0.3	163.8 ± 1.4

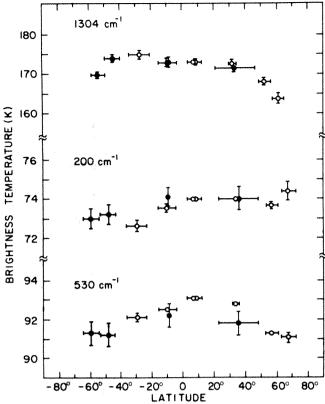


Fig. 2 1,304 cm⁻¹, 530 cm⁻¹, and 200 cm⁻¹ latitudinal distributions of brightness temperature. The 1,304 cm⁻¹ distribution is normalized to an emission angle $\alpha = 47.5^{\circ}$, while the other two are normalised to $\alpha = 52.7^{\circ}$. O, Daytime data; \bullet , night-time data. Vertical bars include uncertainties due to noise, calibration, and emission angle corrections. Horizontal bars denote the latitude range over which data comprising each point extend.

brightness temperature is much larger. Although the extrapolation is large, the poles are apparently 20 K colder than the equatorial region. There is a hemispheric asymmetry, the southern hemisphere being 3 K warmer at mid latitudes than the northern hemisphere. The structure is probably thermal in origin with little or no contribution from meridional opacity variations along constant pressure surfaces. After correcting for an emission angle of 47.5° (to which the data are normalized) the effective emission angle is less than that for the lower wavenumbers ($\alpha = 52.7^{\circ}$), because of its functional dependence on altitude in an extended spherical atmosphere. This also accounts for the slight compression of the $1,304~\rm cm^{-1}$ data along the latitude axis relative to the data at the lower wavenumbers.

Discussion

The observed thermal structure of an atmosphere represents the radiative-dynamical response to an imposed thermal driving, such as solar heating. An important measure of the inertia of an atmosphere to this driving is its radiative relaxation, or response time τ_R , (ref. 4). Smith et al.³ have estimated the radiative response time of Titan's lower troposphere to be $\sim 4 \times 10^9$ s, or 140 yr. This is much longer than a season on Titan. If $\Omega_s = 2\pi/(\text{Saturn's orbital period} = 29.5 \text{ yr})$, $\Omega_s \tau_R = 30$. Such a large value implies almost no seasonal variation in temperature. At each latitude, temperatures should respond to the annual mean insolation which is symmetric about the equator. The symmetry evident in the brightness temperatures at 530 cm⁻¹ is consistent with this.

Estimates of the radiative response time near the tropopause, to which the $200~{\rm cm}^{-1}$ brightness temperatures in Fig. 2 pertain, are not presently feasible. Most of the tropopause emission is at wavenumbers below $200~{\rm cm}^{-1}$ for which we have no data. The low meridional contrast in brightness temperature at $200~{\rm cm}^{-1}$ suggests little seasonal variation in temperature. Either the radiative time constant at the tropopause is large, $\Omega_s \tau_R \gg 1$, or the heating at this level is predominantly from absorption of

radiation emitted lower in the atmosphere where $\tau_{\rm R}$ is large and seasonal effects are small.

Radiative time constants can be estimated for Titan's stratosphere. The contribution from gaseous emission is taken into account using the formulation of Harshvardhan and Cess⁵, which assumes direct cooling to space. The cooling rate N (s⁻¹) at a pressure level P is

$$N = -\frac{\pi g}{C_p P} \Delta \nu \frac{\partial \tau_{\Delta \nu}}{\partial \ln P} \frac{\mathrm{d} B_{\nu_0}}{\mathrm{d} T} \tag{1}$$

where $\tau_{\Delta\nu}$ is the transmittance averaged over a wavenumber interval $\Delta\nu$ (cm⁻¹), B_{ν_0} is the Planck function (erg s⁻¹ cm⁻¹ sr⁻¹) at ν_0 , g is the local gravitational acceleration, T is temperature, and C_p is the specific heat. Transmittances have been obtained from a line by line spectral integration for CH₄ (ν_0 = 1,304 cm⁻¹, $\Delta\nu$ = 200 cm⁻¹), C₂H₆ (ν_0 = 820 cm⁻¹, $\Delta\nu$ = 85 cm⁻¹), and C₂H₂ (ν_0 = 729 cm⁻¹, $\Delta\nu$ = 95 cm⁻¹) (V. Kunde personal communication). Each gas was assumed to be distributed uniformly with height in the stratosphere with an abundance inferred from its observed IR emission². Radiative response times were obtained by summing over the three species (Table 3).

Radiative response times in the upper stratosphere (0.3 mbar < P < 10 mbar) are short relative to Titan's seasons but still long compared with its day. Note that the times are smaller than the preliminary estimates given by Hanel et al.². The longer radiative time constants in the lower stratosphere mainly result from the decrease in T (and hence $dB_{\rm so}/dT$) with depth in this region^{2.6}. The assumption of a uniform vertical distribution is valid only if there is rapid vertical mixing. If C_2H_6 and C_2H_2 are end products of the photo-dissociation of CH_4 at high levels of the atmosphere $(P \ll 0.1 \text{ mbar})^7$, less efficient mixing would imply that the concentrations of C_2H_6 and C_2H_2 increase with height. This would effect a decrease in response times in the upper stratosphere, and an increase at lower levels, relative to those listed in Table 3.

It has been concluded that some of the emission at 200–600 cm⁻¹ originates in the stratosphere, probably from aerosols. As the stratosphere is optically thin in this wavenumber range, the level of emission cannot be unambiguously determined. However, the deduced wavelength dependence of the aerosol emissivity is more like that expected for small particles if the emission is from the lower stratosphere ($P \sim 10-30$ mbar). If the aerosol emission is confined to a slab one scale height thick with emissivity ε_{ν} and temperature T, the radiative response time of the slab is given by

$$\tau_{\rm R}^{-1} = \frac{2\pi g}{C_{\rm p} P} \int_{200 \, {\rm cm}^{-1}}^{600 \, {\rm cm}^{-1}} \varepsilon_{\nu} \frac{{\rm d} B_{\nu}}{{\rm d} T} {\rm d} \nu \tag{2}$$

Table 3 lists τ_R at two pressure levels. The aerosol component reduces τ_R significantly below that computed for gaseous emission alone. This suggests that aerosols are the major source of cooling in the lower stratosphere.

In the upper stratosphere near 1 mbar $\Omega_s \tau_R \ll 1$, and there should be no lag in the thermal response. As only a small fraction of incident solar radiation is absorbed at higher altitudes, the meridional distribution of solar heating and temperature at solstice will be flat in the summer hemisphere, with a slight maximum at the pole, provided that the sources of thermal and solar opacity are uniformly distributed with latitude^{8,9}. The winter hemisphere will be colder with sharper gradients in temperature. At the equinoxes the temperature distribution

Table 3 Radiative response times in Titan's stratosphere

		Gaseous radiative cooling rate $N(s^{-1})$			$ au_{i}$	₂ (s)	$\Omega_{ m e} au_{ m R}$	
P(mbai	$T(\mathbf{K})$	CH₄	C ₂ H ₆	C_2H_2	Gas	Aerosol	Gas	Aerosol
0.3	173	2×10^{-8}	3×10^{-8}	2×10^{-8}	2×10^{7}	*******	0.1	word.
1	170	1×10^{-8}	7×10 ⁹	1×10^{-8}	3×10^{7}		0.2	-mann
10	149	1×10^{-9}	4×10^{-9}	4×10^{-9}	1×10^8	4×10^{7}	0.7	0.2
30	104	3×10^{-12}	2×10 ⁻¹⁰	5×10^{-10}	1×10°	7×10^7	9.0	0.5

should be symmetric about the equator, being warmest there and coldest at the poles. The meridional distribution of the 1,304 cm⁻¹ brightness temperature at northern spring equinox (Fig. 2) roughly accords with the latter case, but the \sim 3 K hemispheric asymmetry evident at mid-latitudes is puzzling. Meridional variations in the infrared flux emitted deeper in the atmosphere where $\Omega_s \tau_R \ge 1$ cannot explain the asymmetry, as the flux is emitted at lower temperatures and does not contribute significantly to the radiative heating near 1 mbar. A hemispheric asymmetry in opacity is possible. On Earth, for example, seasonal variations in the meridional distribution of ozone account for the seasonal behaviour of stratospheric temperatures10. Although variations in aerosols and gaseous absorbers such as C₂H₂ and C₂H₆ are possible on Titan, we cannot unambiguously account for the observed asymmetry.

Figure 3 represents schematically $\Omega_s \tau_R$ with pressure level. Because of the uncertainties in the location of the stratospheric aerosol emission and in the radiative time constant at the tropopause, the transition from $\Omega_s \tau_R \ll 1$ high in the stratosphere to $\Omega_s \tau_R \gg 1$ lower down is not defined very precisely.

The meridional temperature gradients depicted in Fig. 2 imply a zonal thermal wind, u. If the surface drag is sufficiently strong, $u(z=0)\approx 0$, and

$$\frac{u^2(z)}{a}\tan \Lambda + 2\Omega \sin \Lambda \ u(z) = -\frac{1}{a} \int_0^z \frac{g}{T} \, \partial_{\Lambda} T \, dz \qquad (3)$$

where Λ is latitude, z is height, a = 2,570 km is the radius of Titan⁶, $\Omega = 4.5 \times 10^6$ s⁻¹ is its frequency of rotation, and T is atmospheric temperature. If we assume that the meridional temperature has the form $T(z, \Lambda) = T_0(z) + \Delta T(z) \cos \Lambda$ and $\Delta T = 3$ K throughout the troposphere, then at the tropopause $(z \approx 60 \text{ km}, P \approx 100 \text{ mbar})$ we infer an eastward velocity of $u \approx 36 \text{ m s}^{-1}$ at 45° latitude. This is approximately three times the surface rotational speed at Titan's equator. The larger horizontal contrasts in temperature in the stratosphere imply even stronger zonal winds. We assume that the thermal contrast between the equator and pole increases linearly with lnP from 3 K at 100 mbar to 20 K at 0.5 mbar. At 45°, equation (3) yields $u(0.5 \text{ mbar}) \approx 110 \text{ m s}^{-1}$. The estimate of the stratospheric zonal winds is uncertain insofar as we have no direct knowledge of the meridional temperature structure in the lower stratosphere. Nonetheless, it is hard to avoid the conclusion that there are global cyclostrophic flows $(u/\Omega a \gg 1)$ in Titan's atmosphere. The occurrence of such flows in the atmosphere of Venus is already well established11. However, unlike Venus, Titan has a large obliquity. The temperatures in its upper stratosphere, where $\Omega_{s}\tau_{R}$ is small compared with one, should exhibit a large seasonal variation. From equation (3), we expect a concomitant variation in the zonal winds at high altitudes.

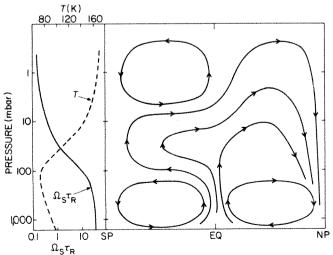


Fig. 3 Schematic diagram of the meridional flow in Titan's atmosphere at northern spring equinox. Mean vertical profiles of temperature and radiative relaxation time are indicated at the left.

Table 4 Radiative and dynamical characteristics of Titan's atmosphere

	$\frac{\Delta\theta}{(\mathbf{K})}$	D (km)	δθ (K)	$ au_{\mathbf{R}}$ (s)	V (cm s ⁻¹)	a/V (s)	(cm ² s ⁻¹)
Lower troposphere $(P \sim 1.000 \text{ mbar})$	3	28	22	4×10^9	0.04	7×10^9	10^{3}
Upper stratosphere $(P \sim 1 \text{ mbar})$	20	42	60	3×10^7	5	6×10^7	

The condition that u(0) be small compared with the zonal winds at higher levels requires that the time for surface drag to deplete the relative zonal momentum of the atmosphere be small compared with the other dynamical time scales of the problem¹². We assume a surface stress of the form $C_D u(0)^2$, where $C_D = 3 \times 10^{-3}$ is a dimensionless drag coefficient¹³. For vertical scales ($D \approx 28$ km) and zonal velocities characteristic of the lower troposphere, the surface drag time is only¹² $D/C_D u(D) \sim 10^6$ s. We will show that the meridional turnover time is much larger, $\sim 10^{10}$ s (Table 4).

The long radiative time scales in Titan's atmosphere imply that there should be no diurnal response to solar heating and this is consistent with the absence of discernible longitudinal structure in the data. The absence of this structure, however, also implies that baroclinic eddies do not have an important role in Titan's atmosphere. Previously, Leovy and Pollack¹³ concluded that baroclinic eddies are probably not effective in transporting heat on Titan. Assuming a vertical structure close to adiabatic, they found that the preferred scale of the eddies exceeded the radius of the satellite. Voyager observations, however, indicate a temperature lapse rate which is much more stable^{2,6}. This increases the preferred scale of the eddies and strengthens Leovy and Pollack's original conclusion that zonally symmetric flow constitutes the favoured mode of meridional heat transport.

Following Leovy and Pollack¹³, we consider zonally symmetric, thermodynamically direct, meridional circulations of horizontal dimension $\sim a$. There are several reasons for considering only thermally direct flows. First, the thermal contrasts forced by differential solar heating will induce a meridional transport of heat which will reduce these contrasts for the atmosphere as a whole. Second, the observed temperatures in the lower troposphere and upper stratosphere, the two regions of the atmosphere which we can treat quantitatively, are consistent with thermally direct flows. The global temperature contrasts are in the same sense as those which would obtain from purely radiative effects but reduced in magnitude. Finally, a thermally direct circulation is the simplest, and allows straightforward calculation, provided that the meridional contrasts in temperature and radiative time constants are known. Given the gaps in our knowledge of Titan's atmosphere, consideration of more complex flows is unwarranted.

We use here the Boussinesq approximation, although this limitation does not affect our conclusions. Consider an idealized meridional cell confined to altitudes z_0 and $z_0 + D$, and latitudes 0 and $\pi/2$. Let the symbol $\delta()$ denote the vertical contrast in a given quantity $(\delta(\cdot) = (\cdot)_{z=z_0+D} - (\cdot)_{z=z_0})$ averaged over latitudes $(0, \pi/2)$; Let $\Delta(\cdot)$ denote the contrast over latitude $(\Delta(\cdot) = (\cdot)^2)$ $()_{\Lambda=0}-()_{\Lambda=\pi/2})$ averaged over the depth D. The thermodynamic heat equation can be cast into the form²⁴: $\partial_t \Delta \theta + \frac{V}{a} \delta \theta \sim \frac{\Delta \theta_e - \Delta \theta}{\tau_R}$

$$\partial_t \Delta \theta + \frac{V}{a} \delta \theta \sim \frac{\Delta \theta_e - \Delta \theta}{\tau_P}$$
 (4)

where θ denotes potential temperature, and factors of the order of unity have been neglected. The term on the right-hand side represents radiative heating and cooling in terms of a linear relaxation back to the radiative equilibrium state, θ_e . The second term on the left-hand side represents the poleward transport of sensible heat and potential energy by the meridional cell. V is a mass-weighted meridional velocity averaged over the upper or lower leg of the cell. When $\Delta\theta > 0$, there is upwelling at low latitudes and poleward motion in the upper leg of the cell. The condition $\delta\theta > 0$ ensures that net transport of energy is poleward¹⁴, and the circulation acts to reduce $\Delta\theta$ ($\partial_t\Delta\theta < 0$). We will use equation (4) to estimate the magnitude of the meridional circulation at various altitudes in Titan's atmosphere.

In the troposphere $(z_0 = 0)$ the long radiative response time and absence of detectable seasonal variation imply a steady circulation, $\partial_t \equiv 0$, symmetric about the equator. As the lower troposphere contains most of the atmospheric mass, we choose D = 1.5 scale heights ≈ 28 km. Over this interval $\delta\theta \approx 22$ K (refs 2, 4). As the inclination of Titan's rotational axis to the ecliptic is presumably 27°, the annual mean insolation of the polar regions is half that at equatorial latitudes¹⁵. A crude estimate of $\Delta\theta_c$ follows from

$$\left(\frac{\theta_{\rm e}(\Lambda=0)}{\theta_{\rm e}\left(\Lambda=\frac{\pi}{2}\right)}\right)^4 \sim 2.$$

With $\theta_{\rm e}(\Lambda=0)\simeq 95$ K, $\Delta\theta_{\rm e}\approx 15$ K. The temperature contrast observed in the troposphere (Fig. 2) is much less, $\Delta\theta\sim 3$ K. With these estimates, equation (4) implies $V\sim 0.04$ cm s⁻¹, equivalent to a dynamical turnover time, a/V, $\sim 7\times 10^9$ s ~ 200 yr (Table 4).

Two potentially important transports have been neglected in this discussion of the Hadley circulation. The first is the equatorward transport by the circulation of latent heat associated with methane condensation. The second is the poleward transport of sensible heat by deep reservoirs of liquid methane which may exist on the surface of Titan. The magnitude of these transports relative to the atmospheric transport of sensible heat and potential energy is difficult to estimate. On Earth, where water is the condensate, they are all comparable, at least over those latitudes where the Hadley circulation dominates ¹⁶. In this complex situation, the net poleward transport of energy on Earth is still comparable to the atmospheric transport of sensible heat and potential energy alone. This suggests that our estimates remain correct to order of magnitude.

In the upper stratosphere the radiative response times are short. Since $\partial_t \sim \Omega_S \ll \tau_R^{-1}$, the time derivative in equation (4) can be neglected: the upper stratosphere is in radiative-dynamical balance with the current value of the seasonal forcing. For the estimate we assume a vertical scale of one scale height. At the equinoxes, radiative equilibrium implies $\Delta\theta_e \sim 10^2$ K. Using the thermal contrast $\Delta\theta \sim 20$ K inferred from the 1,304 cm⁻¹ data, we estimate $V \sim 5$ cm s⁻¹ (Table 4).

The transition from $\Omega_s \tau_R \ll 1$ in the upper stratosphere to

 $\Omega_s \tau_R \gg 1$ occurs in the lower stratosphere and tropopause region, as noted earlier. In the lower stratosphere the thermal response will lag the solar forcing; if $\Omega_s \tau_R \sim 3$ the lag is close to its asymptotic value of one season¹⁷. At the northern spring equinox, temperatures within the lower stratosphere should be warmer in the southern hemisphere than in the north. Such a thermal asymmetry would induce a pole to pole circulation with rising motion in the southern hemisphere. If such a circulation penetrated into the tropopause region, where the seasonally varying radiative heating is probably smaller, adiabatic cooling could conceivably be sufficiently strong to produce lower temperatures and enhanced condensation in the southern hemisphere, as suggested by the observations at 200 cm⁻¹. On the basis of the visible hemispheric asymmetry, Smith et al.³ suggested such a circulation for the lower troposphere. However, the magnitudes of radiative response times (Tables 3 and 4) and the global hemispheric symmetry observed at 530 cm⁻¹ (Fig. 2) suggest that such a cross equatorial circulation is more likely situated in the lower stratosphere and tropopause region.

Figure 3 illustrates one possible model of the meridional flow in Titan's troposphere and stratosphere at northern spring equinox; it assumes a uniform distribution of opacity with latitude. The topology indicated has to be speculative, because we lack sufficient information on the vertical structure of the meridional contrasts in temperature. This cannot be achieved until the vertical distribution of IR opacity is known, particularly in the lower stratosphere and troposphere. Note, however, that the vertical mass flux in the ascending leg of the meridional cell in the upper stratosphere is 0.1 that in the lower troposphere. Thus the streamlines in the stratospheric circulation possibly

close in the lower troposphere, as apparently occurs on $Earth^{18,19}$.

Although eddy transport of heat may not be important on Titan, eddies are crucial in the maintenance of its zonal momentum. The zonal velocity of $36 \,\mathrm{m\,s^{-1}}$ at 45° latitude, computed earlier, corresponds to an angular momentum per unit mass of $3\Omega a^2$. In the upper stratosphere the corresponding angular momentum per unit mass is $2\frac{1}{2}$ times larger. The angular momentum per unit mass in a zonally symmetric circulation controlled by down-gradient angular momentum fluxes is limited to Ωa^2 (refs 12, 20). Atmospheres with excess angular momentum therefore require other transport mechanisms, such as lateral mixing of vorticity or angular velocity by barotropic eddies²¹⁻²³. Upward transport of zonal momentum by vertically propagating waves is another possibility²³, although the lack of longitudinal structure in the observations precludes most conceivable planetary-scale waves on Titan.

The vertical transport of angular momentum by a zonally symmetric meridional cell must be balanced in a steady state by vertical eddy transport. The angular momentum equation, averaged over a horizontal surface from $\Lambda=0$ to $\pi/2$, is 21

$$\partial_{t} \int_{0}^{\pi/2} d\Lambda \cos \Lambda M + \int_{0}^{\pi/2} d\Lambda \cos \Lambda \partial_{z} (WM)$$

$$= \int_{0}^{\pi/2} d\Lambda \cos \Lambda \partial_{z} (\kappa \partial_{z} M)$$
(5)

In equation (5) M denotes the angular momentum per unit mass, W is vertical velocity, and κ is an eddy diffusion coefficient for vertical mixing of momentum. We have assumed the horizontal fluxes to be zero at $\Lambda=0$, $\pi/2$. Consider the lower troposphere which contains the bulk of the atmospheric mass and momentum; here the flow is steady $(\partial_{\kappa}=0)$. The requirement that there be no net exchange of angular momentum between the lower troposphere and surface implies that the vertical flux of angular momentum across a horizontal surface vanish, permitting equation (5) to be written as

$$W\Delta M \sim 2\frac{\kappa \delta M}{D} \tag{6}$$

The quantities ΔM and δM denote, respectively, the horizontal and vertical contrasts in angular momentum. An atmosphere with $\partial_z u > 0(\delta M > 0)$ requires that the equatorial angular momentum exceed that at polar latitudes $(\Delta M > 0)^{21}$. Knowing the meridional velocities from thermodynamic considerations (Table 4) permits an estimate of κ . Since $u(D) \sim \Omega a$ and $u(0) \approx 0$, in the lower atmosphere of Titan $\delta M \sim \langle M \rangle$, a tropospheric average. A Hadley cell which conserves angular momentum in its (upper) poleward branch will have $\Delta M \cong 0$. On the other hand, if eddy mixing of vorticity or angular velocity across latitudes is sufficiently vigorous, $\Delta M \sim \langle M \rangle$. Thus we expect $\Delta M/\delta M \leqslant 1$ and from equation (6)

$$\kappa \leq DW \sim \frac{D^2}{a} V \sim 10^3 \text{ cm}^2 \text{ s}^{-1}$$

From analogous considerations, Gierasch²¹ inferred a low value of vertical viscosity in the atmosphere of Venus, $\kappa \sim 10^4 \, \mathrm{cm}^{-1}$. Note that equation (6), with the added condition $\Delta M/\delta M \lesssim 1$, requires that the time for diffusion of momentum vertically through the troposphere, D^2/κ , not exceed the dynamic turnover time, a/V.

Leovy and Pollack¹³ proposed a symmetric model of Titan's tropospheric circulation and deduced meridional velocities much larger and thermal contrasts smaller than those presented here. Although their model differs from ours in several specific aspects, the most important difference lies in their treatment of internal friction. In not distinguishing between u(0) and u(D), they in effect assumed a Rayleigh friction drag in the angular momentum equation. They therefore implicitly assumed $\kappa \sim C_D u(D) D \sim 10^7 \, \text{cm}^2 \, \text{s}^{-1}$; well in excess of our derived upper limit. From equation (6) this resulted in larger meridional velocities, $V \sim 1 \, \text{m s}^{-1}$, and smaller thermal contrasts. The

anticipated seasonal variation in the zonal wind field of the stratosphere presents a more complex situation, and we will not attempt to analyse the requisite angular momentum transports.

Conclusions

IR brightness temperature at 200 cm⁻¹, 530 cm⁻¹, and 1,304 cm⁻¹ exhibit small meridional contrasts (≤3 K) in the troposphere and tropopause region and larger contrasts (~20 K) in the upper stratosphere. Radiative relaxation times estimated for the troposphere and stratosphere are large compared with the length of Titan's day. In the lower troposphere they are also large compared with the length of a season, $\Omega_s \tau_R \gg 1$, implying a lack of seasonal variation in temperature. In the upper stratosphere, $\Omega_s \tau_R \ll 1$, and seasonal variations in temperature should be large. The location of the transition region, where $\Omega_s \tau_R \approx 1$, is not well determined.

observations suggests that baroclinic waves and eddies are absent in Titan's atmosphere. Zonally symmetric flows are the

The absence of detectable longitudinal structure in the

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- 1. Samuelson, R. E., Hanel, R. A., Kunde, V. G. & Maguire, W. A. Nature 292, 688-693
- 2. Hanel, R. et al. Science 212, 192-200 (1981)
- Smith, B. A. et al. Science 212, 162-190 (1981).
 Gierasch, P. J., Goody, R. M. & Stone, P. H. Geophys. Fluid Dyn. 1, 1-18 (1970).
- Harshvardhan & Cess, R. D. Tellus 28, 1-9 (1976).
- Tyler, G. et al. Science 212, 201-206 (1981).
- Strobel, D. F. in Atmospheres of Earth and the Planets (ed. McCormac, B. M.) 401-408 (Reidel, Dordrecht, 1975).
- 8. Hess, S. L. Introduction to Theoretical Meteorology (Holt, Rinehart and Winston, New York,
- Carlson, B. E., Caldwell, J. & Cess, R. D. J. atmos. Sci. 37, 1883-1885 (1980).
- Prabhakara, C., Rodgers, E. B., Conrath, B. J., Hanel, R. A. & Kunde, V. G. J. geophys. Res. 81, 6391-6399 (1976).

preferred mode of meridional heat transport and thermally driven planetary-scale circulations yield characteristic meridional velocities of 0.04 cm s⁻¹ in the lower troposphere and 5 cm s⁻¹ in the upper stratosphere.

The observed meridional temperature contrasts imply zonal winds which are cyclostrophic, reaching ~100 m s⁻¹ in the upper stratosphere. The anticipated seasonal variation in the stratospheric temperature field implies a concomitant variation in the zonal winds at high altitudes. The inferred cyclostrophic flow has an angular momentum per unit mass which, at least in the stratosphere, greatly exceeds Ωa^2 . In the lower troposphere, an assumed balance in the vertical transport of angular momentum by zonally symmetric meridional flow and down gradient diffusion implies a vertical viscosity of only 10³ cm² s⁻

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- 11. Schubert, G. et al. J. geophys. Res. 85, 8007-8025 (1980).
- 12. Held, I. M. & Hou, A. Y. J. atmos. Sci. 32, 1038-1044 (1975).

- Leovy, C. B. & Pollack, J. B. Icarus 19, 195-201 (1973).
 Stone, P. J. atmos. Sci. 32, 1005-1016 (1975).
 Brinkman, A. W. & McGregor, J. Icarus 38, 479-482 (1979).
 Lorenz, E. N. The Nature and Theory of the General Circulation of the Atmosphere World Meteorological Organization (1967). 17. Cess, R. D. & Caldwell, J. Icarus 38, 349-357 (1979).
- Geller, M. A. J. atmos. terr. Phys. 41, 683-705 (1979)
 Dunkerton, T. J. atmos. Sci. 35, 2325-2333 (1978).
- Schneider, E. K. J. atmos. Sci. 34, 280-296 (1977).
 Gierasch, P. J. J. atmos. Sci. 32, 1038-1044 (1975)
- Rossow, W. B. & Williams, G. P. J. atmos. Sci. 36, 377-389 (1979).
 Leovy, C. B. J. atmos. Sci. 30, 1218-1220 (1973).
- 24. Stone, P. H. J. atmos. Sci. 31, 1681-1690 (1974).

Implications of Titan's north-south brightness asymmetry

Lawrence A. Sromovsky*, Verner E. Suomi*, James B. Pollack*, Robert J. Krauss*, Sanjay S. Limaye*, Tobias Owen*, Henry E. Revercomb* & Carl Sagan§

> * Space Science and Engineering Center, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA † NASA Ames Research Center, Moffett Field, California 94035, USA ‡ Department of Earth and Space Sciences, Suny at Stony Brook, Stony Brook, New York 11790, USA § Laboratory for Planetary Studies, Cornell University, Ithaca, New York 14853, USA

Voyager 1 images of Titan, when normalized to remove limb darkening, reveal an axially symmetric brightness pattern with significant north-south asymmetry. This interhemispheric contrast seems to be a response to seasonal solar heating variations resulting from Titan's inclined spin axis. The contrast significantly lags the solar forcing, indicating that its production involves the atmosphere well below the unit optical depth level. The contrast has a significant effect on Titan's disk-integrated brightness as seen from Earth, and probably accounts for most of the observed long term variation, with solar UV variations accounting for the remainder.

EARTH-based observations^{1,2} show that between 1972 and 1976 Titan's disk-integrated brightness increased by ~9% in the blue (b) and ~5.5% in the yellow (y) to a maximum during 1976-77, and decreased through 1978. This variation and a similar but smaller variation in Neptune's brightness can be correlated with the solar cycle^{2.3}, suggesting solar variability as the common cause. As photochemical reactions are thought to produce the submicrometre aerosols in both atmospheres, we expect the variation in solar UV output during the solar cycle^{4,5} to affect the amount or physical properties of the aerosols. Pollack et al.6 showed that Titan's brightness changes during 1972-76 could be produced by changes in mean particle size, visible absorption coefficient, or optical depth, and that the fractional decrease in the particle production rate required to explain the increase in albedo was roughly consistent with the estimated fractional change in solar UV output.

A new mechanism for explaining at least part of the Earth based photometry of Titan is suggested by the Voyager 1 observations of November 1980 that showed Titan to be shrouded in an apparently unbroken cloud cover with several large-scale zonal features and a surprising north-south asymmetry in brightness7. As Titan's spin axis is inclined 27° to the ecliptic (assuming Titan is tidally locked to Saturn), the angle between Titan's axis and the line of sight to the Earth varies during Saturn's (and Titan's) 29.5-yr orbit around the Sun. The apparent tipping motion, combined with the brightness asymmetry could result in a long-term periodic variation of Titan's disk-integrated brightness. If the brightness asymmetry also varies in response to seasonal changes in solar heating of the deepest layers of the atmosphere, then the contrast would follow the seasonal heating asymmetry with a phase shift of nearly a full season and would reach an extreme value near Titan's equinoxes (near the time of the Voyager 1 encounter). Using a highly simplified model to combine the effects of the suggested seasonal changes in contrast and the time-dependent viewing conditions, the predicted brightness variation is found

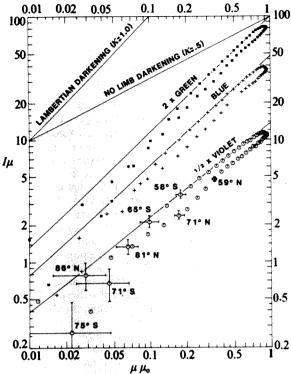


Fig. 1 Voyager 1 intensity measurements in violet, blue, and green filters, along Titan's 150° E meridian. Lines drawn for each colour show the Minnaert fits to intensities between 31° and 52° S. Intensities (in digital counts) have been corrected for dark noise but not converted to absolute units. The error bars labelled by latitude show the effects of an intensity uncertainty of ± 0.5 count and an uncertainty in μ and μ_0 corresponding to a ± 1 pixel error in the image location of Titan's centre. The identification of image line and element coordinates with Titan latitude and longitude, image navigation, is based on predicted spacecraft location and attitude information supplemented by a precise determination of camera pointing derived from the location of Titan's centre within the image.

to be in phase with the Earth-based photometry and within a factor of 2 of the observed magnitude change⁷.

The essential differences between the solar cycle and seasonal components are the driving forces and the response times. The former is driven by the changing UV output of the Sun, and brightness changes in the same direction would be expected at all latitudes at the same time, with a delay of the order of six months relative to the UV forcing function⁸. The seasonal component is driven by the tipping of Titan's axis relative to the Sun, causing simultaneous brightness changes in different directions in northern and southern hemispheres, with a time lag of nearly a full season (~7 yr). In both cases, the properties of the aerosol particles are probably modulated and in turn produce the observed brightness changes.

We now use Voyager 1 and Pioneer 11 observations, with recent Earth-based photometry, to place firmer bounds on the phase shift of Titan's contrast relative to the seasonal forcing. Describing the contrast in terms of a relative normal albedo, we calculate the time dependence of Titan's disk-integrated brightness and find a significant seasonal contribution. Finally, we use the spectral and phase angle dependence to place constraints on the mechanism which produces the contrast.

Titan's brightness contrast at the time of Voyager 1

We selected nearly simultaneous Voyager narrow angle frames in violet (0.37–0.45 $\mu m)$, blue (0.43–0.53 $\mu m)$, and green (0.52–0.60 $\mu m)$ filters (A. Collins, P. Kupferman and E. Danielson, personal communication), pictures 1305S1–003, 1289S1–003, and 1287S1–003 respectively. These were taken at 2.38× 10^6 km from Titan, at a phase angle of 29.6°, with a resolution of 44 km per line pair at the subspacecraft point. The Voyager blue and green intensities roughly correspond to Lockwood's measurements with the b filter (0.45–0.48 μm) and y filter (0.54–0.56 μm) respectively³. Relative intensities were obtained from raw digital counts by subtracting an empirically determined dark noise correction accurate to $\sim \pm 0.5$ count.

To define limb darkening characteristics each image was scanned along the 150° E meridian, using a 5×5 pixel area average stepped at $\sim3^\circ$ intervals (Fig. 1). These scans pass between the subspacecraft point at $134\,^\circ\text{E}$ and the subsolar point at 162° E, providing nearly maximal variation in scattering geometry and intensity. Figure 1 shows the dependence of intensity I (in digital counts) on μ (cosine of the observer zenith angle) and μ_0 (cosine of the solar zenith angle) as $I\mu$ versus $\mu\mu_0$ on logarithmic scales so that Minnaert scattering behaviour, for which

 $I\mu = I_0(\mu\mu_0)^k \tag{1}$

is a straight line with slope equal to the limb darkening exponent k, and intercept (at $\mu\mu_0=1$) equal to the maximum intensity I_0 . Each scan follows two nearly linear traces with similar slopes, moving along one line in the northern hemisphere and a second line, with an intercept $\sim 25\%$ greater, in the southern hemisphere. A transition occurs near the equator. Because roughly the same limb darkening slope applies over most of the latitude range for each colour, we can interpret brightness differences as differences in the intercept I_0 (proportional to the normal albedo).

We define a latitude dependent relative normal albedo (Fig. 2) as the ratio of the observed intensities to the intensities predicted from a Minnaert function fit to the 150° E scan data between 31° and 52° S for each colour. The fitted slopes are 0.773, 0.887, and 0.942 for violet, blue, and green respectively. Four major cloud zones are apparent in Fig. 2: a north mid-latitude zone between about 10° and 55° N, a north polar zone between 55° and 90° N, a south mid-latitude zone between about 20° and 60° S, and an equatorial transition zone between about 20° S and 10° N. The large variations poleward of 60° S and 80° N cannot be interpreted as true variations in normal albedo as they occur where the determination of the viewing geometry is very sensitive to navigation errors (Fig. 1) and where the Minnaert function does not accurately describe Titan's limb darkening. The normal albedo of the south mid-latitude zone is ~25% greater than that of the north mid-latitude zone, while the albedo of the north polar zone is ~7% smaller. The greatest normal albedo contrast between the south and north midlatitude zones occurs in the blue, and the smallest in the green.

The large-scale spatial structure of the normal albedo, as well as subtler cloud features are displayed as normalized images (Fig. 3) in which the intensity is proportional to relative normal albedo. The images clearly show the major cloud zones, and indicate a slightly brighter band at 45° N as identified by Smith et al.⁷. The features in the normalized images are primarily zonal (axially symmetric). The north polar zone is dark in all three colours, but darkest in violet. The bright band just south of this zone is most pronounced in the green and nearly invisible in the

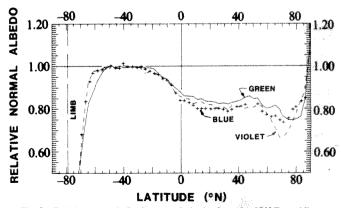


Fig. 2 Relative normal albedo versus latitude along the 150° E meridian. For each colour the relative normal albedo is obtained by dividing observed intensities by predictions based on Minnaert fits to the intensities between 31 and 52° S. The increase of normal albedo north of 70° N is not real: a decrease similar to that near 70° S is found when μ and μ_0 in the polar zone are corrected for the larger height of the clouds there. The decrease near the limb in the south is similar to that found near the limb at other latitudes and is probably a failure of the Minnaert fit to describe the true limb darkening at large viewing angles.

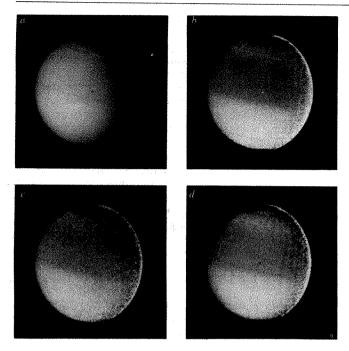


Fig. 3 Voyager 1 narrow angle images of Titan at 29.6° phase angle: unnormalized blue image (a) and normalized images in blue (b), violet (c), and green (d). In all the normalized images intensities are linear in relative normal albedo (see text) but stretched to enhance the visibility of the relatively small contrast range. A similar linear stretch has been applied to the unnormalized image (a). Its different appearance is primarily due to the presence of limb darkening. The latitude-longitude grid overlay has a 45° spacing. The equator intersects the 135° E meridian near the centre of the image (+, Titan's centre). The bright terminator in the normalized images probably results from the haze layer above the main cloud deck⁷, which could cause the observed intensities to fall off more slowly than the predicted intensities, yielding an apparent albedo increase.

violet. Minor, but definite, longitudinal variations also occur. The north polar zone boundary, for example, is slightly inclined relative to latitude lines. The green image also shows an inclined bright feature within the polar zone which may be useful as a tracer of atmospheric motions. Those small departures from axial symmetry have an insignificant effect on Titan's diskintegrated brightness and are ignored.

Phase angle dependence

We assume that the normal albedo contrast is the same at 0° phase angle (corresponding to Earth-based observing geometry) as it is at the 29.6° phase angle applicable to the Voyager 1 data shown in Figs 1–3. To test this assumption we analysed a Voyager 1 blue filter image taken at a phase angle of 9.3° (picture 1379S1–014), using the same procedures as in the analysis of the 29.6° phase angle images. No discernible change in mid-latitude contrast was found between these phase angles. The small size of the 9.3° phase angle image (30 picture elements across Titan's diameter) precludes a definitive statement about polar region changes.

Time dependence of Titan's contrast

The possible time dependence of Titan's brightness contrast can be constrained by comparing the Voyager 1 and Pioneer 11 observations (1.17 yr apart). We used a blue filtered image made by the Pioneer 11 imaging photopolarimeter (IPP) at a phase angle of 27° and at a surface resolution of ~ 170 km at the subspacecraft point (M. G. Tomasko, personal communication). The spectral range of the IPP blue filter $(0.39-0.50~\mu\text{m})$ covers both the blue and violet bands of the Voyager camera system. Because we see little spectral dependence of contrast in this spectral range, and as the albedo of Titan is higher in the blue than in the violet, we expect no significant spectral error in comparing the IPP image with the Voyager blue image.

Using the same procedures as above, a central meridian scan of the IPP image was used to define the relative normal albedo as a function of latitude. The large quantization noise in the IPP

data was reduced by averaging over two latitude belts in which the normal albedo seemed to be constant: $15^{\circ}-35^{\circ}$ S and $30^{\circ}-60^{\circ}$ N. We found a south/north normal albedo ratio of 1.26 ± 0.03 for the Pioneer measurements, compared with 1.25 ± 0.01 for Voyager. This result is consistent with either a fixed contrast or a slowly varying contrast near an extremum. We reject the former possibility because it implies brightness variations grossly inconsistent with the Earth-based photometry. The Pioneer 11/V oyager 1 comparison also suggests that the normal albedo boundary (the point at which the albedo is midway between the mid-latitude values) has moved from about 5° N to 5° S, apparently opposite to the subsolar point. But this tentative result is not included in our seasonal model calculations.

If the contrast slowly varies with a seasonal period (29.5 yr), then the direct observations by Voyager 1 and Pioneer 11 place a strong constraint on the phase shift between the solar forcing due to spin axis tipping and the contrast response. If both the forcing and the contrast response vary sinusoidally, the implied phase shift is $-84^{\circ} \pm 20^{\circ}$, where -90° corresponds to a time delay of one season, and the implied maximum south/north ratio of normal albedos is $1.26^{+0.05}_{-0.01}$. The relatively large uncertainty in phase is due to the small time interval between Voyager and Pioneer and the large quantization noise in the Pioneer results. Present results are consistent with maximum contrast near the time of the Voyager 1 encounter and allow for a large enough phase lag (near 90°) to place the contrast-induced brightness variation in phase with the Earth-based photometry.

If the normal albedo response to the solar irradiance level can be described by a single time constant, then the phase shift is determined by $\tan(\phi) = -2\pi\tau/T$, where ϕ is the phase shift. T is the orbital period of Saturn, and τ is the time constant. As $\phi \le -90^{\circ}$ is not physically possible, the time constants consistent with the phase shifts allowed by the Pioneer 11/Voyager 1 comparison fall in the range $9.6 \text{ yr} \le \tau \le \infty$, with the central phase shift of 84° corresponding to a time constant of 44.7 yr. This range of time constants is not consistent with a direct interaction between solar irradiance and aerosol properties at the unit optical depth level (at a pressure of ~1 mbar), because the thermal radiative time constant at this level and the time to reach equilibrium particle properties⁸ are both <1 yr. Large time constants can be obtained, however, if deeper levels of the atmosphere are involved in the contrast producing mechanism. For the entire atmosphere down to the 1.6-bar level a time constant of 138 yr (ref. 7) $\phi = -88^{\circ}$ is possible. A time constant of 9.6 yr only requires involvement down to the 100 mbar level.

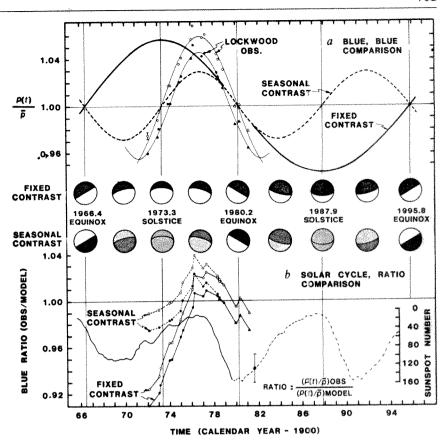
Predicted geometric albedo time dependence

We now evaluate the time dependence of Titan's diskintegrated brightness expected for two models consistent with the Pioneer 11/Voyager 1 comparison: fixed contrast and seasonal contrast with a large phase shift (-90° for this example). Because the Earth-based observations of Titan's diskintegrated brightness^{1,2} have been adjusted to fixed heliocentric distance and zero phase angle (180° scattering angle), they are proportional to Titan's geometric albedo (the ratio of diskintegrated brightness to that of a flat perfect Lambertian reflector of the same diameter). We calculate the relative geometric albedo p(t) by integrating over the illuminated hemisphere:

$$p(t) = (1/\pi) \iint a(\theta, t) [\mu_0(\theta, \theta_s(t), \phi)]^{2k} \sin(\theta) d\theta d\phi$$
 (2)

where $a(\theta, t)$ is the relative normal albedo at co-latitude θ and time t, θ_s is the co-latitude of the subsolar (and subEarth) point, and ϕ is the longitude relative to the subsolar longitude. The time dependence of the subsolar point latitude on Titan is obtained by assuming Titan's spin axis is parallel to Saturn's. We approximate the latitude dependence of the normal albedo using a 16-zone step function with boundaries spaced $\sim 10^\circ$ in latitude. Average relative albedo values for each zone are calculated for each colour from the results shown in Fig. 2. Because of the navigational and Minnaert fit uncertainties we

Fig. 4 a, Relative time variation of Titan's blue geometric albedo according to fixed contrast (heavy solid line) and seasonal contrast (heavy dashed line) models. Relative time variations derived from Lockwood's Earth-based photometry are shown for two different estimates of the mean brightness, each based on fitting an offset sine function of fixed period to the observations. One case uses the 11-yr solar cycle period; the second uses the current 13.8-vr period of the predicted seasonal brightness variation (the ellipticity of Saturn's orbit causes a deviation from the mean 14.7 period). Both fits (light lines) are good: r.m.s. deviations from the fitted curves are only about 1.5 times the estimated measurement error. Observations: ○△, relative to the mean assuming a 13.8-yr period; ● A, relative to the mean assuming an 11-yr period. Triangles indicate observations which are still preliminary. The shaded drawings illustrate the appearance of Titan at zero phase angle according to the two contrast models. The drawings exaggerate and simplify the contrast for clarity. b, Comparison of the solar cycle of smoothed sunspot number¹¹ with the ratio of observed to predicted relative variations in Titan's blue geometric albedo. Sunspot numbers are plotted along an inverted scale. The dashed portion of the sunspot curve indicates predicted behaviour based on previous cycles. The error bar indicates short term prediction uncertainty. Beyond 1983 the uncertainty is comparable with the predicted value.



assume the same albedo south of 60° S as between 52.5° S and 60° S, and ignore variations north of 85° N in finding a mean between 70° and 90° N. This normal albedo distribution is valid for 1980.9, the time of the Voyager 1 encounter. The fixed contrast model assumes the same distribution at all times. We describe possible seasonal variations in normal albedo in a simple model assuming (1) the normal albedo distribution for 1980.2 (the time of the last equinox) was the same as observed by Voyager for 1980.9, (2) the distribution for 1966.4 (time of the previous equinox) was as for 1980.2 except for a north-south reversal, and (3) at each latitude the normal albedo varied sinusoidally between the 1966.4 and 1980.2 extremes.

For fixed albedo contrast we find peak-to-peak modulation amplitudes of $\sim 10.5\%$ for violet and blue geometric albedos, and 8.2% for the green. For seasonally-varying contrast the corresponding values are 5.6% and 4.2% respectively. Figure 4a illustrates the time dependence of the geometric albedo based on the normal albedo models for the blue intensities. For fixed albedo contrast, p(t) varies in phase with the subsolar motion, reaching extreme values at the solstices. For the seasonal contrast model, p(t) attains its mean value at every equinox, when both hemispheres are equally illuminated and viewed, and at every solstice, when the interhemispheric normal albedo contrast is absent. In this case the extreme values occur midway between solstice and equinox. The shaded drawings (Fig. 4a) provide a simplified illustration of Titan's time dependent appearance according to these two contrast models.

Comparison with Earth-based photometry of Titan

The Lockwood measurements of Titan's b magnitude are plotted in Fig. 4a as relative brightnesses (brightness/time-averaged brightness). The most recent observations should be considered preliminary (G. W. Lockwood, personal communication).

The brightness variation calculated assuming a fixed normal albedo distribution is completely at odds with the Earth-based photometry. The predicted brightness is at a maximum in 1973 and beginning to decline, while the measurements are near the estimated mean value and rapidly increasing. This implies that the mean normal albedo of Titan's clouds and the relative albedo distribution with latitude cannot both be time-indepen-

dent. If the relative spatial distribution of normal albedo were fixed, then the disk-averaged mean value would have to vary dramatically to compensate as in Fig. 4b, which shows the ratio of Lockwood's blue observations to the predictions. As the ratio (for fixed contrast) changes most dramatically before 1976 when solar indicators (such as sunspot number, Fig. 4b) are changing relatively slowly, the possibility of fixed contrast and a large compensation by the solar UV mechanism seems highly unlikely.

If the relative latitudinal variation of normal albedo has the seasonal model time dependence, the predicted geometric albedo variation has roughly the same modulation period and phase as the Earth-based measurements. The assumed seasonal phase lag of 90° provides nearly optimal agreement with the Earth-based b brightness variation (Fig. 4a), although the optimal phase lag for fitting both b and y brightness curves is 82° ± 7°, where the uncertainty is based on the difference between best fit values for the two colours. The predicted peak-to-peak modulation amplitude based on the green contrast measurements is ~50-70% of the observed y brightness modulation, while the predicted blue variation is about 50-60% of the observed b brightness modulation, where the amplitude uncertainties are due to the uncertainties in Titan's mean brightness. For agreement with the Earth-based photometry the mean normal albedo can be modulated by only \sim 5% in blue and only 2.5% in the green (or y filter). This implied variation in disk-averaged mean normal albedo (Fig 4b) is roughly in phase with that expected from the solar cycle modulations of UV output (Fig. 4b), although the magnitude of the solar cycle effect in the present case would be only about half the amount suggested before Titan's brightness contrast had been discovered.

Implications of Neptune's brightness variation

The seasonal contribution to Titan's brightness variation could be significantly different from the above estimate if the boundary between high and low albedo also exhibited seasonal variation: it would be greater if the boundary moved opposite to the subsolar point motion (as suggested by Pioneer 11 measurements) and smaller if it moved in the same direction.

Thus dominance by either solar cycle or seasonal contributions remains possible. Earth-based photometry of Neptune is helpful in evaluating this possibility.

As Neptune's orbital period of 164.8 yr seems to preclude any significant seasonal changes during the 1972-80 period of the Earth-based photometry, we might expect its brightness curve to be dominated by the solar UV mechanism. The asymmetric form of Neptune's brightness variation supports this expectation: after relatively smooth increases between 1972 and 1976.5, at an average rate of 0.54% yr⁻¹ in the blue and $0.68\%\ yr^{-1}$ in the yellow, the brightness curves for both colours dropped by more than 2% in the yellow and almost 3% in the blue within the next year, with relatively little additional change from 1978 to 1980 (ref. 2 and G. Lockwood, personal communication). Thus Neptune's brightness curves are relatively well correlated with the asymmetric variation of the indicators of solar activity (Fig. 4b).

If the shape of Neptune's brightness curve represents the relative time dependence of the solar cycle contribution to Titan's brightness, then the solar cycle mechanism cannot be dominating Titan's variation. The very symmetric form of Titan's brightness curve is instead consistent with dominance by the seasonal mechanism. However, between 1972 and 1976 Titan's b magnitude increased ~70% faster than the y magnitude, while between 1976 and 1980 they both changed at about the same rate. We interpret this spectral difference as evidence for a non-seasonal component. From the amplitude of Neptune's brightness variation we infer that the solar cycle component in Titan's brightness curve would contribute ~30% of the total variation. Although one might expect a larger effect because Titan's haze layer is optically thick, while Neptune's is thought to be optically thin, a contribution much greater than 30% should produce a noticeable asymmetry in Titan's brightness curve. Thus a simple seasonal component probably accounts for more than half of Titan's secular variation, with the remainder being provided by the solar cycle mechanism.

Constraints on the contrast mechanism

As most photons reflected to space by Titan interact with the atmospheric aerosols, the hemispheric brightness asymmetry is almost certainly due to variations in their properties. The phase angle dependence of the brightness asymmetry gives an indication as to which property of the aerosols has a dominant role. Theoretical simulations with the aerosol parameters of Rages and Pollack9 indicate that the contrast declines considerably and even disappears as the phase angle decreases from 30° to 10° for a model involving variations in mean particle size, whereas little contrast alteration is expected over the same range of phase angles for models involving variations in aerosol absorption coefficient or optical depth. Thus the latter models seem to be in better agreement with the observed lack of significant contrast variation with phase angle.

The spectral dependence of the contrast also suggests that it is a reflection of differences in the sub-micrometre aerosol properties rather than differences in albedo of an underlying methane cloud layer. If the contrast was due to an albedo difference deep in the atmosphere it would be attenuated by aerosols in the upper atmosphere. However, to explain the spectral variation of Titan's geometric albedo the visible absorption coefficient of these particles must increase significantly with decreasing wavelength9. As their inferred optical depth is higher at shorter wavelengths their attenuation of the north-south contrast of an underlying cloud layer should result in significantly more contrast at long rather than short wavelengths. As this is opposite to the observed spectral variations, the contrast is more likely to be present in the properties of the sub-micrometre particles in the upper atmosphere.

The large phase shift of the contrast relative to the seasonal forcing functions shows that the contrast-producing mechanism cannot be a direct interaction between the solar irradiance and the atmosphere at the unit optical depth level. A significant depth of Titan's atmosphere must be involved to produce the time delayed response. As a relatively small north-south thermal contrast is expected at levels corresponding to large radiative time constants, a direct thermal effect on the composition or optical depth of the aerosol particles seems unlikely. The observed vertical stability of Titan's temperature profile 10 also argues against an interhemispheric difference in small-scale vertical convection as a modifying influence. This suggests that large-scale circulation asymmetries may be responsible. Possibly differences in meridional circulations, driven by an interhemispheric seasonal thermal contrast, are affecting the aerosol properties by changing the mix of trace gases available at the formation level, or by modifying their rates of removal from the formation region.

Summary and conclusions

Titan's normal albedo distribution is primarily axially symmetric with a substantial north-south contrast. As the contrast is essentially independent of phase angle it is likely to result from differences in the composition or optical depth of Titan's layer of sub-micrometre aerosols rather than differences in size distribution. The spectral variation of the contrast suggests that it is not caused by differences in the albedo of an underlying methane cloud. The contrast seems to vary with time in response to seasonal variations in solar irradiance and lags the seasonal forcing by a large phase angle, indicating that it originates probably well below the unit optical depth level. It seems to be produced indirectly, perhaps by circulation currents driven by thermal contrast at lower levels which affect the rate of removal or composition of the aerosol particles at the unit optical depth level. The contrast has a significant effect on Titan's diskintegrated brightness, and is probably responsible for most of the observed long term variation, with solar cycle effects accounting for the remainder.

There are still many uncertainties regarding the nature and origin of the contrast. A longer record of observations, both of the contrast and the disk-integrated brightness would yield a significant improvement in understanding. The nature of the contrast variation should be better defined by Voyager 2 observations in August 1981, and by the Space Telescope in the mid-1980s. If the simple seasonal model is correct we would expect the contrast to decrease to zero by the end of 1987.

Continued Earth-based photometry of both Titan and Neptune is also essential to resolve the remaining uncertainties. Between 1972 and 1978 the solar cycle component and the seasonal component have been approximately in phase, and neither has yet experienced a full cycle of variation, making their separate contributions difficult to distinguish from the brightness curves alone. The two components will become readily separated with another 5-10 years of observations as they grow increasingly out of phase, moving in opposite directions between 1981 and 1984.

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^{1.} Lockwood, G. W. Icarus 32, 413-430 (1977).

Lockwood, G. W. & Thompson, D. T. Nature 280, 43-48 (1979). Suess, S. T. & Lockwood, G. W. Solar Phys. 68, 393-409 (1980).

Vidal-Madjar, A. in The Solar Output and its Variation (ed. White, O. R.) 213-236 (Colorado Associated University Press, Boulder, 1977).

^{5.} Cook, J. W., Brueckner, G. E. & Van Hoosier, M. E. J. geophys. Res. 85, 2257-2266

Pollack, J. B., Rages, K. & Toon, O. B. Geophys. Res. Lett. 7, 829-832 (1980).

Smith, B. A. et al. Science, 212, 163-191 (1981). Toon, O. B., Turco, R. P. & Pollack, J. B. *Icarus* 43, 260-284 (1980).

Rages, K. & Pollack, J. B. *Icarus* 41, 119-130 (1980). Hanel, R. et al. Science 212, 191-200 (1981).

^{11.} Solar Geophysical Data, prompt reports, No. 425 (US Dept of Commerce, 1980).

Density waves in Saturn's rings

Jeffrey N. Cuzzi*, Jack J. Lissauer† & Frank H. Shu†

*Ames Research Center, NASA, Moffett Field, California 94035, USA †Department of Astronomy, University of California, Berkeley 94720, California, USA

Certain radial brightness variations in the outer Cassini division of Saturn's rings may be spiral density waves driven by Saturn's large moon Iapetus, in which case a value of ~ 16 g cm⁻² for the surface density is calculated in the region where the waves are seen. The kinematic viscosity in the same region is ~ 170 cm² s⁻¹ and the vertical scale height of the ring is estimated to be a maximum of ~ 40 m.

VOYAGER imaging observations of Saturn's rings¹ have revealed a wide spectrum of radial structure, indicating a variety of dynamical processes in the ring disk. Here we focus on one example of radial structure and explore its possible origin. A sequence of smoothly undulating brightness fluctuations is observed in the outer Cassini division within a band located between ~120,700 and 121,900 km from the centre of Saturn. The observed behaviour of these variations agrees with the expected behaviour of spiral density waves driven by a resonance between the apsidal motion of local ring particles and the mean motion of Saturn's outermost large satellite, Iapetus.

Basic theory

The existence of spiral density waves driven at Lindblad resonances with Saturn's major moons and propagating radially in Saturn's ring disk was suggested by Goldreich and Tremaine² as a means of transferring angular momentum over large radial distances within the disk and thereby clearing wide gaps. Similar density waves have been proposed to underlie the spiral structure of disk galaxies³. The earlier work was extended by Goldreich and Tremaine^{4,5} to the density waves that can be driven in thin disks by external and internal satellites.

Consider an axisymmetric disk of ring particles orbiting Saturn where centrifugal equilibrium at a radius r requires circular motion at angular speed $\Omega(r)$. If the particles possess small random motions in the plane of the disk (small eccentricities), Lindblad's theory of orbits⁶ allows us to think of the particles as gyrating at epicyclic frequency $\kappa(r)$ about the guiding centre of the reference circular orbit of the same specific angular momentum $r^2\Omega(r)$. Then

$$\kappa^2 = \frac{1}{r^3} \frac{\mathrm{d}}{\mathrm{d}r} (r^4 \Omega^2) \tag{1}$$

with κ^2 being equal to Ω^2 if the latter satisfies Kepler's third law: $\Omega^2 \propto r^{-3}$. For Saturn's rings, κ^2 is slightly less than Ω^2 because of the oblateness of Saturn. The small departure of κ^2 from Ω^2 is crucial to the nature of the resonant driving of the observable spiral density waves.

Suppose a moon of mass M orbits Saturn outside the rings at a mean angular speed $\Omega_{\rm M}$. Its perturbing force on the ring particles can be Fourier decomposed in time t and azimuthal angle θ (ref. 2). The mth Fourier component, having dependence proportional to $\exp[im(\Omega_{\rm M}t-\theta)]$ with m being a positive integer, will resonate with ring particle motions at a radius $r_{\rm L}$ where

$$\Omega(r_{\rm L}) - \kappa(r_{\rm L})/m = \Omega_{\rm M}. \tag{2}$$

Following the nomenclature used in galactic dynamics, we call the value r_L which satisfies equation (2) the *m*th inner Lindblad resonance of the moon M.

Ring particles slightly inside and outside $r_{\rm L}$ are induced to travel in eccentric orbits with longitude of periapse differing by π/m , leading to overlapping of their orbits. For m>1, collisions would then depopulate the overlapping orbits and clear natural gaps about the stronger resonances with fractional widths $\Delta r/r_{\rm L} \sim (M/M_{\rm S})^{1/2}$, where $M_{\rm S}$ is the mass of Saturn. Applied to Mimas's 2:1 resonance (the m=2 inner Lindblad resonance),

the above formula fails by more than two orders of magnitude to explain the observed width of the Cassini division.

To account for this discrepancy and to explain why Mimas's 2:1 resonance occurs near the inner edge of Cassini's division, Goldreich and Tremaine² invoked the self-gravity of the ring material. Consider free spiral density waves, of arbitrary wave frequency ω and azimuthal symmetry m, in a flat disk of particles which collectively behave as a fluid. Independent of their source, these waves satisfy the dispersion relation⁷:

$$(\omega - m\Omega)^2 = \kappa^2 - 2\pi G\sigma |k| + c^2 k^2 \tag{3}$$

where σ is the surface mass density, c is the one-dimensional velocity dispersion, and k is the radial wavenumber in a WKBJ analysis. Equation (3) states that the allowed square of the Doppler-shifted wave frequency equals the square of the natural frequency (the epicyclic frequency) apart from modifications required by self-gravity and acoustic effects.

From equation (3), it is easy to show that stability to axisymmetric (m = 0) disturbances of all wavenumbers requires the fluid analogue of Toomre's criterion⁸:

$$Q \equiv \frac{\kappa c}{\pi G \sigma} \ge 1 \tag{4}$$

When $Q \ge 1$, equation (3) predicts that the disk can support two types of free density waves with

$$|\mathbf{k}| = \frac{\pi G \sigma}{c^2} \left[1 \pm (1 - Q^2 D / \kappa^2)^{1/2} \right]$$
 (5)

where the upper sign corresponds to short waves and the lower to long waves, and where the departure of

$$D \equiv \kappa^2 - (\omega - m\Omega)^2 \tag{6}$$

from zero measures how far we are from exact Lindblad resonance. The value of r where

$$\omega - m\Omega(r) = -\kappa(r) \tag{7}$$

is called the inner Lindblad resonance of the wave of radian frequency ω and azimuthal mode number m. For a wave driven by an external moon of pattern speed $\omega/m = \Omega_{\rm M}$, the radial position corresponding to equation (7) is $r = r_{\rm L}$ (equation (2)).

The waves of interest here are the long waves. Near the inner Lindblad resonance of the wave, $Q^2D/\kappa^2 \ll 1$, and the dispersion relation for long waves becomes independent of c (see, for example, equation (3) for small |k|):

$$|k| = \frac{D}{2\pi G\sigma} \tag{8}$$

Equation (8) predicts that these waves formally become infinitely long at the waves' inner Lindblad resonance where D=0. Because the characteristic wavelength far from resonance, $4\pi^2G\sigma/\kappa^2(\simeq 4\pi H/Q)$ where $H\equiv c/\Omega$ is the scale height of the disk), amounts only to $\sim 10^2$ m in Saturn's rings, it is the reduction of the wavenumber |k| by the factor D/κ^2 that allows the waves at inner Lindblad resonance to be coupled to the slow spatial variation of the forcing by the external moon^{2,3}. The long waves—in particular the m=1 long waves—have the added observational advantage that they can be resolved by Voyager 1.

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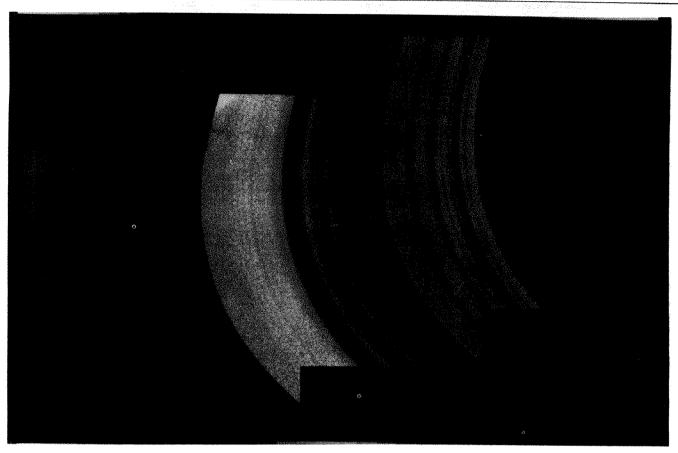


Fig. 1 A mosaic of the Cassini division of Saturn's rings, obtained from Voyager 1 observations with resolution of ~10 km per line pair (see Table 1). The region is seen from the unlit face. The prominent bright band in left centre contains a sequence of features of alternating brightness which are plotted in Fig. 2.

To understand why the m=1 inner Lindblad resonances (where the rate of apse precession of the ring particles is equal to the mean motion of the moon) are of special interest, we determine the radial range over which the waves remain significantly longer than the characteristic wavelength of 10^2 m. Because D=0 at $r=r_{\rm L}$ for driven waves, a Taylor series expansion of D about $r=r_{\rm L}$ for driven waves yields

$$D = \mathcal{D}x \tag{9}$$

where

$$\mathcal{D} = \left(r \frac{\mathrm{d}D}{\mathrm{d}r}\right)_{r_{L}} = \left(2\kappa r \frac{\mathrm{d}}{\mathrm{d}r} \left(\kappa - m\Omega\right)\right)_{r_{L}} \tag{10}$$

and $x \equiv (r - r_{\rm L})/r_{\rm L}$ is the fractional distance from Lindblad resonance. Equation (8) now implies that the radial spacing, or wavelength $\lambda = 2\pi/|k|$, between successive wave crests should be inversely proportional to the nondimensional distance x from the resonance:

$$\lambda = \frac{4\pi^2 G\sigma}{\mathcal{D}_X} \tag{11}$$

with the proportionality constant being larger for smaller values of the parameter \mathcal{D} . But in Saturn's rings, κ nearly equals Ω , so equation (10) now implies that the most readily resolved density waves correspond to m=1. Of the larger exterior moons, the only ones whose m=1 inner Lindblad resonance lies within the ring system are Iapetus, Hyperion, and Titan.

In the m=1 case, to calculate \mathcal{D} (but not to locate the resonance), it is sufficiently accurate to take

$$\kappa - m\Omega \simeq -J \left(\frac{GM_{\rm S}}{r^3}\right)^{1/2} \left(\frac{R_{\rm S}}{r}\right)^2 \tag{12}$$

where J = 0.0244 and $R_s = 60,330$ km are, respectively, Saturn's dynamical oblateness and equatorial radius. This yields for equation (10):

$$\mathcal{D} \simeq 7J\Omega_{\rm L}^2 (R_{\rm S}/r_{\rm L})^2 \tag{13}$$

Let us consider the amplitude distribution of the propagating waves whose source is m = 1 resonant driving at $r = r_L$ by an external moon. Free long waves that propagate conserving the

angular momentum luminosity F given them by a source can be shown^{2,9} to increase their fractional amplitude linearly with dimensionless distance x from the inner Lindblad resonance, $|\Delta\sigma/\sigma| = x/x_{\rm NL}$, where $x_{\rm NL}$ is the characteristic scale for waves to become nonlinear:

$$x_{\rm NL} = \frac{2\pi^2}{\mathfrak{D}} \left(\frac{r\sigma}{F}\right)^{1/2} (G\sigma)^{3/2} \tag{15}$$

For weak forcing, as occurs with Iapetus on Hyperion, $x_{\rm NL} \ge 1$, and nonlinear effects (for example, shocks¹⁰) are not important for realistic values of x. In the presence of particle collisions, however, small-amplitude density waves will suffer a viscous damping factor²

$$\exp\left[-\int_0^x k_1 r_L \, dx\right] \quad \text{where } k_1 = \frac{7}{3} \left[\frac{\kappa \mathcal{D}^2}{(2\pi G\sigma)^3}\right] \nu x^2$$

if the bulk viscosity is much less than the kinematic shear viscosity ν . Taking into account both amplitude variation factors, we obtain the wave amplitude profile near the position of the inner Lindblad resonance:

$$\frac{\Delta\sigma}{\sigma} = \left(\frac{x}{x_{\rm NI}}\right) \exp\left[-\left(\frac{x}{x_{\rm D}}\right)^3\right] \tag{16}$$

where

$$x_{\rm D} = \left(\frac{9}{7}\right)^{1/3} \frac{2\pi G\sigma}{(\kappa \nu \mathcal{D}^2 r)^{1/3}} \tag{17}$$

is the characteristic dimensionless damping length.

If spiral density waves are observed in Saturn's rings, a measurement of their wavelength yields, through equation (11), a determination of the surface mass density σ ; a measurement of the fractional amplitude variation then yields, through equations (16) and (17), a determination of the kinematic viscosity ν . Under certain assumptions, we can relate the kinematic viscosity ν to ring optical depth τ and velocity dispersion c. The vital parameters σ and ν are difficult to obtain empirically by other methods.

Table 1 Observational parame	eters of Cassini bright band
Image FDS counts used	34934.25, 34934.29
Resolution	10 km per line pair
Ring tilt angle to line of sight	74°
Normal optical depth	0.14 ± 0.02
Fit to wavelength: $\lambda(x) = A_1/(x-x_1)$	$A_1 = 0.45 \pm 0.07 \text{ km}$ $x_1 = -0.0001 \pm 0.0012$
Fit to amplitude $\frac{\Delta \sigma}{\sigma} = A_2 x e^{-(x/x_2)^3}$	$A_2 = 1.69 \pm 0.2$
-	$x_2 = 0.0115 \pm 0.001$
Theoretical amplitude $A_2 = (x_{NT})^{-1}$	1.3 (using $M/M_s = 4 \times 10^{-6}$)
Derived σ	$16 \pm 3 \mathrm{g cm^{-2}}$
Derived v	$170 \pm 80 \text{ cm}^2 \text{ s}^{-1}$

Observations and derived ring properties

During the Voyager 1 encounter with Saturn¹, several observations of the Cassini division region were obtained with spatial resolution of ~10 km per line pair (see Table 1). As viewed from the unlit face, an optically thin region of ~4,800 km width is observed between the classical A and B rings; we refer to this entire region as the Cassini division (see also ref. (11)). In the outer part of the Cassini division, at a distance from Saturn's centre of $\sim 120,700-121,900 \text{ km} \ (\pm 700 \text{ km})$ is a noticeably brighter band filled with regular, low contrast, radial brightness variations having spacings that decrease outward (see Fig. 1). Independent of the high resolution observations shown in Fig. 1, the band was observed against the bright limb of Saturn and the average normal optical depth of the band was determined as discussed by Smith et al. (see Table 1). The band appears brighter in diffuse transmission than nearby regions in the inner Cassini division because the band's optical depth is a factor of ~1.4 larger than the plateau structures in the inner Cassini division.

To obtain useful results from the very noisy data evident in Fig. 1, an azimuthal averaging technique was employed, in which data were averaged along loci of constant distance from the centre of Saturn. This method employs numerical fitting of ellipses to observed ring features at the boundaries of the region. To first order, geometrical distortion in the camera and due to the finite separation between the spacecraft and the planet is thereby removed. Typical azimuthally-averaged results, which incorporate most of the data contained in the frame, are shown in Fig. 2. The vertical brightness scale has been calibrated to reflectivity DN = I/F, in units of 10^{-4} , where I is the observed intensity and πF is the incident solar flux. The noise level of the result may be estimated from inspection of the darkest area, which is essentially empty. In particular, the mildly undulating variations located between 120,700 and 121,900 km from the centre of Saturn are real.

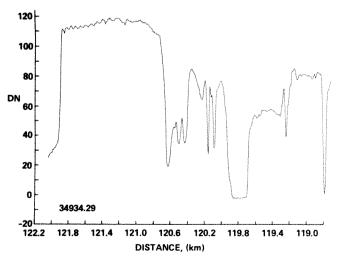


Fig. 2 Photometric scan across part of the data shown in Fig. 1. Averaging is done along lines of constant distance from Saturn, and the tracing includes most of the information contained in Fig. 1. The noise level is shown in the dark (empty) gap near 119,800 km. Radial distances shown are only accurate in an absolute sense to ±700 km or so, but relative distances are much more precisely known.

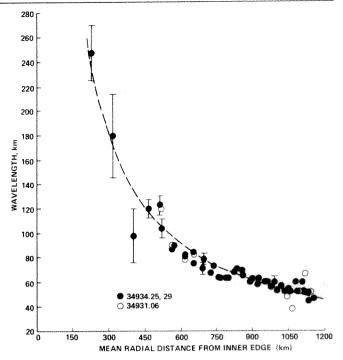


Fig. 3 Wavelength, or peak-peak/trough-trough separation, of the wave features of Figs 1 and 2 as a function of mean distance from the inner half-power point of the bright band. The best-fit curve of the form of equation (11) is shown by the dashed curve. The inverse variation of wavelength with distance is characteristic of long density waves near Lindblad resonance.

The horizontal distance scale in Fig. 2 is obtained from final spacecraft trajectory data and contains residual uncertainties of ±700 km for this observation. This uncertainty is large compared with the uncertainty <100 km to which the theoretical radial location of a given gravitational resonance may be calculated. However, relative distances are quite accurately represented in Fig. 2, and therefore, resonances can be located relative to one another once one of them has been identified.

We have calculated the predicted locations and strengths of the strongest resonances in this region of the rings following the approach of Goldreich and Tremaine⁵. The strongest resonance is the m=2 inner Lindblad resonance with Mimas. An m=1 Lindblad resonance with Iapetus lies at $2.008~R_{\rm S}$, but is several orders of magnitude weaker in torque. If we associate the Mimas 2:1 resonance with the inner edge of the Cassini division (which might entail a slight error 11), relative separations locate the Iapetus resonance at $100\pm150~{\rm km}$ outward of the inner halfpower point of the brightest band in Fig. 2. Because the undulations in brightness start near this location a more detailed investigation of the hypothesis that these undulations represent a radial slice through a tightly-wrapped spiral density-wave pattern whose source is the Iapetus resonance is necessary.

We have analysed the azimuthally averaged data obtained from two independent frames for the wavelength and amplitude behaviour of the wave pattern. For the low optical depths characterizing the region, we equate fractional brightness variations with fractional surface density variations. The fractional separations $x = (r - r_0)/r_0$ of the centroids of the peaks and troughs from an arbitrary radial location r_0 (the inner half-power point of the bright band) were measured. The peak-to-peak as well as trough-to-trough wavelengths were then plotted as functions of the distance from this arbitrary reference point. The results are shown in Fig. 3 with a best fit to the data of the form of equation (11):

 $\lambda = \frac{A_1}{r - r} \tag{18}$

where A_1 and x_1 were varied to minimize residuals. The resulting zero point offset $x_1 = -0.0001 \pm 0.0012$ implies that the source of the observed wave pattern is indistinguishable from the inner half-power point of the bright band. This is slightly inward of the theoretical location deduced on the basis of identifying the inner edge of the Cassini division with Mimas's

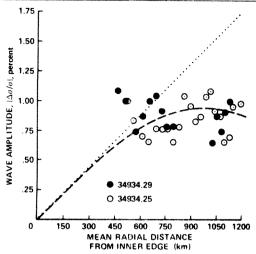


Fig. 4 Wave amplitude data (1/2 of peak-trough contrast) are plotted for the wave features of Figs 1 and 2 as a function of distance from the inner edge of the bright band. These data have been fit to a function of the form of equation (16), and the best-fit curve is shown by the dashed line. In the absence of damping, the amplitude would continue to increase linearly throughout the region instead of leveling off and then declining.

m = 2 inner Lindblad resonance, but the displacement is within the allowed uncertainties.

Comparing equations (11) and (18), we see that determination of A_1 allows us to calculate the local mass surface density σ . Our best-fit result yields $\sigma = 16 \pm 3$ g cm⁻². This is the most secure determination of a local ring property from our analysis and constitutes, to our knowledge, the only direct estimate for the ring mass density. The normal optical depth τ and surface mass density are related by

$$\tau = K\sigma \tag{19}$$

where K is the opacity, or mean cross-sectional area per unit mass of ring particles of density ρ :

$$K(r) = \frac{\int n(R, r) \pi R^2 dR}{\int n(R, r) \rho (4\pi R^3/3) dR}$$
 (20)

with n(R, r) representing the local distribution of particle radii R. Independent measurements of τ and σ yield a constraint on the ratio of integrals, K. Our analysis gives $K = \tau/\sigma \approx (9 \pm 2) \times 10^{-3} \text{ cm}^2 \text{ g}^{-1}$ for the region of interest.

For comparison, for identical particles of radius R_0 : $n(R, r) \propto$ $\delta(R - R_0)$, $K = 3/4\rho R_0$ whereas, for a power-law distribution: $n(R, r) \propto R^{-3}$ from R_1 to R_2 , as has been deduced from groundbased studies¹², $K = [3/(4\rho(R_2 - R_1))] \ln (R_2/R_1)$. Voyager radio science results¹³ for the region of our analysis indicate an effective particle radius $R_{\rm eff} \sim 8-10$ m. If this corresponded to identical particles of radius $R_0 \sim R_{\rm eff}$ and $\rho \sim 1~{\rm g~cm}^{-3}$, it would imply $K \sim 9 \times 10^{-4}$ cm² g⁻¹. In contrast, the power-law distribution, with $R_1 \sim 1$ cm (ref. 12) and $R_2 \sim R_{\text{eff}}$ (ref. 13) would predict $K \sim 6 \times 10^{-3}$ cm² g⁻¹, in much better agreement with our determination. Our analysis therefore supports a power-law distribution with an exponent of -3, although the precise value of the exponent depends on our choice for R_1 and R_2 . In particular, a steeper spectrum would allow a larger value of R_2 , possibly including many thousands of kilometre-sized bodies within the ring system¹

We now investigate whether the observed amplitude profile of the wave is consistent with the behaviour predicted by equation (16). There are two separate parameters, $x_{\rm NL}$ and $x_{\rm D}$, to be considered. First, the best-fit value for $x_{\rm NL}$ using equation (16) or the data of Fig. 2 may be compared with the expected value obtained from equation (15) using a theoretical calculation for the strength F of the Iapetus apsidal resonance and the observational determination of σ given above. Second, the best-fit for $x_{\rm D}$ using equation (16) may be used to obtain an estimate for the kinematic viscosity ν from equation (17).

The data for fractional wave amplitudes from our two pictures are shown in Fig. 4 with the minimum-residual fit for a

functional form given by equation (16). Although the data exhibit considerable scatter the waveform for density waves damped by viscosity does provide an acceptable fit to the observations. Moreover, the derived value of $x_{\rm NL}$ is within a factor of 1.3 of the theoretically expected value for wave excitation and propagation in a region of constant σ (see Table 1). This slight discrepancy may be accounted for by uncertainties in Iapetus' mass or by noting that the resonant source probably lies in a region of larger surface mass density than the region of wave propagation. Within these uncertainties, Iapetus apsidal resonance appears to contain sufficient torque to account for the observed wave amplitude.

We proceed to estimate the value of ν from our best fit for the damping length x_D . Using equation (16) and our value of σ , we obtain $\nu \sim 170$ cm² s⁻¹ (see Table 1). Although this value is less precise than our value for σ , the presence of appreciable viscosity is unquestionable given the clear departure seen in the data of Fig. 4 from an undamped linear increase of fractional wave amplitude with radial distance from the resonance.

As the waves suffer damping, the negative angular momentum they carry is absorbed by the ring particles, causing the latter to drift inward towards the inner Lindblad resonance. But this process itself does not seem to explain why the region containing the waves is a region of lower optical depth than its surroundings, as the total angular momentum transfer induced by Iapetus over the entire age of the Solar System fails by two orders of magnitude to produce the required mass removal. We believe the question of the low optical depth of the outer part of the Cassini division to be related to that of the origin and maintenance of the low optical depth of the entire Cassini division¹¹.

Under certain assumptions, the derived value of ν may be used to obtain the particle velocity dispersion c and thus the local vertical scale height $H = c/\Omega$. For instance, for particles of a single size, Goldreich and Tremaine¹⁵ have derived a form for ν which can be approximated by

$$\nu = \frac{c^2}{2\Omega} \left(\frac{\tau}{1 + \tau^2} \right) \tag{21}$$

This expression neglects gravitational focusing and scattering 16 , and is valid only if the distribution of ring particles contains few bodies with radii much in excess of 10 m. (The free fall speed onto a 10-m particle is comparable with the value of c we derive below using equation (21).) As equation (21) ignores contributions to the viscosity other than physical collisions, our determination of c based on ν yields only an upper limit. If we adopt equation (21) and set $\nu \sim 170~{\rm cm}^2~{\rm s}^{-1}$ and $\tau \sim 0.14$, we obtain $c \leq 0.6~{\rm cm~s}^{-1}$, corresponding to a ring scale height $H = c/\Omega \leq 40~{\rm m}$. From the values for σ and c, we obtain $Q \leq 30$ for the stability index, equation (4). The upper limit Q = 30 is still small enough to justify our use of the approximation $Q^2D/\kappa^2 \approx Q^2 \mathcal{D} x/\kappa^2 \ll 1$ to derive equation (8) for the region in question.

Discussion

The above results have several general implications. If ν has everywhere the same value, $170\,\mathrm{cm\,s^{-1}}$, as it does in the outer part of Cassini's division, the unconstrained spreading time $\Delta r^2/\nu$ of Saturn's rings as a whole would be comparable with the age of the Solar System. Smaller features would, of course, be much more difficult to maintain for the age of the Solar System. Indeed, unless the coefficient in the $\nu-\tau$ relation decreases rapidly with increasing optical depth (see equation (21)), ν is likely to be several times larger in the B and A rings than in the low optical depth region studied here. The persistence of even these gross features over the age of the Solar System would be called into question without another confinement mechanism.

In contrast to constant ν or c, a value for Q near its upper limit 30 everywhere would imply that the collective effects of the self-gravity of the rings are generally unimportant except very near a resonance. In the case of the m=1 waves, the waves are extremely close in frequency space to exact Lindblad resonance. In general, however, density waves become evanescent beyond

the distance from their source where equation (5) produces complex wavenumbers. This location, known in galactic dynamics as a 'Q barrier' 17, occurs in close spatial proximity to the source for all $m \neq 1$ in Saturn's rings unless Q is close to unity. For example, for density waves originating at the Mimas m=2 inner Lindblad resonance to propagate across even a continuously filled region as wide as Cassini's division, Q must be <3. If Cassini's division was cleared by such density waves Q and therefore c have to be so small that the primary contribution to the viscosity comes from gravitational scatterings by massive ring particles. In any case, our measured value of ν does require some means of maintaining the present Cassini division at a low surface density against radial diffusion by the B and A rings in a time $\Delta r^2/\nu \leq 4 \times 10^7$ yr.

The Titan apsidal resonance at a theoretical radial location of $1.288 R_{\rm S}$ is also orders of magnitude stronger in torque than the Iapetus apsidal resonance, but unlike the Mimas m=2 resonance, the Titan m=1 Lindblad resonance is free of difficulties with Q barriers, and the waves it excites should be resolvable. Yet we see no evidence, in high resolution studies, of density waves near 1.288 Rs, only a gap of a few hundred km width exists 18.

We believe that the seemingly paradoxical presence of observed density waves associated with the weak Iapetus resonance and their apparent absence from the much stronger Titan resonance has a simple explanation in the very strength of the latter resonance. It and the other strong resonances are so strong that their driven density waves become nonlinear in a short distance², $\Delta r = x_{NL}r_L$, and shock¹⁰, depositing most of their negative angular momentum in close proximity to the resonance, and thereby quite likely opening up a gap. The distance to nonlinearity for both the Mimas 2:1 resonance and the Titan apsidal resonance is 10² km, as compared with 10⁵ km for the weak Iapetus apsidal resonance. Goldreich and Tremaine² pointed out that the behaviour of the wave becomes highly uncertain after the material within $x_{NL}r_{L}$ of the resonance has been removed, and they also expressed concern in the case of the Mimas 2:1 resonance as to why the entire A ring had not been drawn into the Cassini division.

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- 1. Smith, B. A. et al. Science 212, 163 (1981)
- Goldreich, P. & Tremaine, S. Icarus 34, 240 (1978). Lin, C. C. & Shu, F. H. Astrophys. J. 140, 646 (1964). Goldreich, P. & Tremaine, S. Astrophys. J. 233, 857 (1979).
- Goldreich, P. & Tremaine, S. Astrophys. J. 241, 425 (1980).
- Chadrasekhar, S. Principles of Stellar Dynamics (University of Chicago Press, 1942).
- Chadrasckiai, F. H. in Astrophysics and General Relativity Vol. 2 (eds Chretian, M., Deser, S. & Goldstein, J.) 236–329 (Gordon and Breach, New York, 1968).
 Toomre, A. Astrophys. J. 139, 1217 (1964).

The presence of an empty region to the outside of a resonance diminishes the direct coupling of the satellite's resonant forcing to the disk on the outer side of the gap by an exponential factor if the waves must tunnel through a smoothly varying Q-barrier (edges not sharper than an inverse wavenumber). This factor in the amplitude may be roughly estimated from equation (53) of ref. 17, in the limit of small σ , as

$$\exp\left[-\frac{2}{3}\left(\frac{\mathcal{D}\Delta r}{\kappa^2 r}\right)^{1/2}\frac{\Delta r}{H}\right]$$

where Δr is the observed width of the gap. The magnitude of the exponent is >10 for the observed gap near Titan's apsidal resonance and even larger for the strong nonapsidal resonances in the rings. Thus, direct inward transport of material by strong resonances with outer moons may be self limiting. If density waves play a major part in transporting matter in Saturn's rings, it must be possible to excite waves at nonresonant positions via interactions with preexisting small-scale structure (Goldreich, personal communication).

Thus strong evidence exists in Saturn's rings for satellitedriven density waves, one in particular being driven by Iapetus at its apsidal resonance. Based on this identification, we obtain values for ring properties such as $\sigma = 16 \text{ g cm}^{-2}$; $K = \tau/\sigma =$ $9 \times 10^{-3} \text{ cm}^2 \text{ g}^{-1}$; $\nu = 170 \text{ cm}^2 \text{ s}^{-1}$; while $c \le 0.6 \text{ cm s}^{-1}$, $H \le$ 40 m, and $Q \le 30$. Firm lower bounds for c and H can be obtained by requiring axisymmetric gravitational stability when $Q \ge 1$, then our value of σ gives $c \ge 0.02$ cm s⁻¹, $H \ge 1.5$ m. Narrowing the ranges for c, H, and Q requires a careful evaluation of the relative contributions to ν by gravitational scatterings and physical collisions. This will depend critically on whether the power-law distribution of ring particles extends to bodies $>10 \, \text{m}$

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- Shu, F. H. Astrophys. J. 160, 99 (1970).
 Shu, F. H., Milione, V. & Roberts, W. W. Astrophys. J. 183, 819 (1973).
 Lissauer, J. J., Shu, F. H. & Cuzzi, J. N. Nature 292, 707-711 (1981).
 Cuzzi, J. N. & Pollack, J. B. Icarus 33, 233 (1978).

- 13. Tyler, G. L. et al. Science 212, 201 (1981).
- Henon, M. Nature (in the press).
 Goldreich, P. & Tremaine, S. Icarus 34, 227 (1978).
- Cuzzi, J. N., Durisen, R., Burns, J. A. & Hamill, P. Icarus 38, 54 (1979).
 Lin, C. C. & Lau, Y. Y. Stud. appl. Math. 60, 97 (1979).
 Collins, S. A. et al. Nature 288, 439 (1980).

Moonlets in Saturn's rings?

Jack J. Lissauer*, Frank H. Shu* & Jeffrey N. Cuzzi

*University of California, Berkeley, California 94720, USA †NASA/Ames Research Center, Moffett Field, California 94035, USA

The brightness structure within Cassini's division in Saturn's rings is explained in terms of perturbations produced by moonlets embedded within an optically thin disk of smaller ring particles. The moonlets exert gravitational torques on neighbouring ring particles and create gaps; diffusion acts to fill the gaps. A new explanation is offered for the inner edge of the Cassini division being located at the 2:1 resonance with Mimas.

VOYAGER 1 observations have revealed that Saturn's rings consist of a surprisingly large number of ringlets and narrow gaps'. Some of the features in the rings appear to be associated with resonant forcing by external moons². We have argued that some observed undulating variations of small amplitude in the outer Cassini division can be interpreted as propagating spiral density waves whose source is resonant driving by Iapetus³. However, there also exist ring structures which cannot be easily explained in terms of external gravitational perturbations.

Ring particles typically have sizes of 0.01-10 m (ref. 4), and Voyager 1 discovered several small new moons with diameters

of 30-200 km just outside the main ring system¹. Thus, we suspected the existence of bodies of intermediate size, and we investigated the idea that some of the prominent structures in Saturn's ring system might be caused by the gravitational influence of such relatively massive bodies orbiting within the ring system (see also ref. 5).

To have enough cohesive strength to survive inside the Roche limit of Saturn, the perturbing bodies must have diameters ≤100 km (ref. 6). To exert enough gravitational torque to clear gaps larger than their own sizes, the bodies must have diameters ≥2 km (see equation (14)). We refer to bodies embedded in the

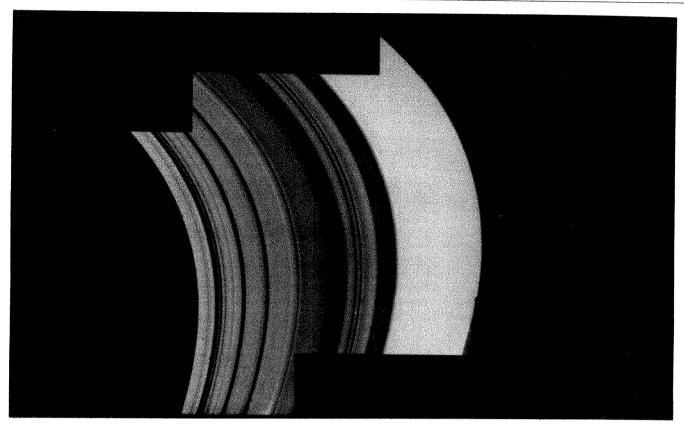


Fig. 1 Mosaic of the Cassini division in diffuse transmission. The B ring at the left and the A ring at the right appear dark because the relation, diffuse brightness proportional to optical depth, breaks down when the multiple scatterings occur. The inner Cassini division contains a series of plateaux separated by three dark narrow gaps and bounded by two dark wide rifts. The outer Cassini division (between the outer rift and the A ring) contains a faint wavelike pattern that is analysed in ref. 3.

rings which are massive enough to clear gaps larger than their own sizes as moonlets. We aim here to obtain mass estimates for unseen moonlets that may be responsible for the clearest gaps and to analyse whether such bodies could be directly detected by future spacecraft observations. We focus on the series of plateaus in the inner Cassini division which are separated by narrow gaps and set off from the B ring and the outer Cassini division by two wide gaps or 'rifts' (see Fig. 1).

Our analysis of this region follows that of ring dynamics given by Goldreich and Tremaine 7.8 and by Lin and Papaloizou 9. After the discovery of the rings of Uranus 10, Goldreich and Tremaine proposed an ingenious mechanism for creating and maintaining narrow planetary rings 11. Their mechanism invoked small unseen satellites between the rings that keep the ring particles from spreading under the influence of collisions. The discovery of two satellites 1, 1980 S26 and 1980 S27, which seem to 'shepherd' Saturn's narrow F ring supports the hypothesis.

A moonlet embedded within the ring system will exert a systematic torque on ring material located at the moonlet's outer and inner Lindblad resonances, where the ratio of the mean revolution rates of the ring Ω and of the moonlet Ω_{M} satisfy $\Omega/\Omega_{\rm M} = m/(m \pm 1)$, with m as a positive integer. The direction of this torque always acts to move ring material away from the moonlet. Collisions between ring particles broaden the resonances, and near the orbit of the moonlet, the Lindblad resonances of large m pile up and overlap. The torques can then be considered as spaced continuously over the ring material. Each moonlet thus sweeps ring material on both sides away from itself. If the moonlets are large or closely spaced, particles between the two moonlets may be pushed into quite a narrow ringlet, as is the case with the uranian rings and Saturn's Fring. Counteracting this effect, viscous diffusion tends to maintain the ringlet at a finite width. If the relative role of diffusion is increased—by an increase in the amount of material between adjacent moonlets or in the random velocities of the ring particles, by a decrease in the masses of the moonlets, or by an increase in their separation—the ringlets can spread and become plateaux wider than the gaps between them. We suggest this occurs for the prominent plateau structures in Fig. 1.

Basic equations

In a ring system where the normal optical depth τ is of the order unity, collisions between ring particles occur on time and length scales of the ring orbital period and thickness. For structures of longer duration and larger dimension, we may treat the ring material as a viscous fluid, confined to a thin sheet. Ignoring the effects of gravitational focusing and scattering 12, and assuming a single particle size, Goldreich and Tremaine 13,14 proposed a formula for the kinematic viscosity that we approximate with

$$\nu = \frac{c^2}{2\Omega} \left(\frac{\tau}{1 + \tau^2} \right) \tag{1}$$

where c is the one-dimensional velocity dispersion of ring particles. The visual optical depth τ is related to the surface mass density σ and mean cross-sectional area per unit mass of ring particles, 'opacity' K, by

$$\tau = K\sigma \tag{2}$$

In contrast, if the size distribution of embedded bodies extends from centimetres to kilometres (or larger) on a single-power law of sufficient flatness, a more important source of effective viscosity than physical collisions arises from the tendency of the larger bodies to exert torque on their neighbours in such a way as to repel the latter⁵. The same mechanism operates on all size scales, but a natural separation occurs at the size R_2 that separates bodies large enough to clear gaps around themselves (moonlets) from bodies too small to do so (ring particles). We treat the latter collection as a fluid with an effective kinematic viscosity ν given by dimensional arguments as

$$\nu = \alpha R_2 \frac{G\sigma}{\Omega} \tag{3}$$

where α is a dimensionless constant of the order of unity whose exact value depends on the exponent of the power-law that characterizes the ring-particle size-distribution.

Although equations (1) and (3) have different origins, they both give ν proportional to σ for small τ . This is the only important feature for our modelling of the inner Cassini divisions as the coefficient of proportionality between ν and τ or σ can be determined empirically. We therefore adopt equation (1) with the understanding that the quantity c should be regarded, not as the true velocity dispersion of ring particles, but merely as a parameter in the ν - τ relation. Further, we assume c to have a spatially constant value \sim 0.5 cm s⁻¹, given approximately by our determination of ν through the damping of spiral density waves in the outer Cassini division³. An equivalent procedure would have been to take $\alpha R_2 = 0.2$ km in equation (3); virtually identical results would then have been obtained in Fig. 2.

The tangential stress exerted by material outside radius r (distance from Saturn) on the material inside radius r, integrated over the thickness of the rings, is $\sigma vr \partial \Omega/\partial r$. This stress has a lever arm r and acts (in an axisymmetric system) over a circle of circumference $2\pi r$. The net torque exerted on a narrow annulus is the difference between these effects at r+dr and r; therefore, the viscous torque T_{ν} exerted per mass unit is given by 15

$$2\pi r \sigma T_{\nu} = \frac{\partial}{\partial r} \left(2\pi r r \sigma \nu r \frac{\partial \Omega}{\partial r} \right) \tag{4}$$

In the present problem, we can ignore the self-gravity of the rings and the oblateness of Saturn; we approximate $\Omega(r)$ by Kepler's third law; and equation (4) becomes

$$T_{\nu} = -\frac{3}{2r\sigma} \frac{\partial}{\partial r} (\sigma \nu r^2 \Omega) \tag{5}$$

Moonlets clear gaps around themselves by exerting torques on

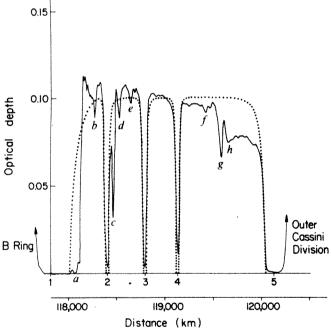


Fig. 2 Comparison of the theoretical optical depth profile with the measured diffuse transmission in the inner Cassini division. The solid curve gives the observed elliptical ring scans as a function of distance from Saturn; the dotted curve gives a theoretical fit to the data. The theoretical model adopts constant velocity dispersion, two stationary rifts moonlets, 1 and 5, and three gap moonlets, 2, 3 and 4, which have evolved to quasi-equilibrium positions. The moonlets have the properties listed in Table 1. Additional smaller moonlets may exist at positions a-h, but they were not modelled. In particular, moonlet a is required to confine the small bump (which is an optically thick narrow ring) between moonlets 1 and a, and to sharpen the inner edge of the first plateau. In these medium-resolution scans the optical depth does not reach zero at the positions 2-4, but this is an artefact as high-resolution scans show that clear gaps exist at these positions. Equilibrium solutions were also calculated for c increasing with τ . Resultant profiles have sharper gap edges, as expected from the slower diffusion rates in the lowest optical depth regions. If c decreases slowly with increasing τ , rounder edges will result. Rapid decrease of c with τ can result in diffusive instabilities20

Properties of the moonlets	
M_i/c (g cm ⁻¹ s)	$D_i (km)^*$
2.5×10^{19}	29
7.0×10^{17}	9
7.0×10^{17}	9
5.2×10 ¹⁷	8
6.9×10 ¹⁸	19
	$ 2.5 \times 10^{19} 7.0 \times 10^{17} 7.0 \times 10^{17} 5.2 \times 10^{17} $

^{*} The calculation for D_i from M_i/c assumes $\rho = 1$ g cm⁻³ and c = 0.5 cm s⁻¹.

ring particles in various kinds of resonances. If the eccentricity of a moonlet of mass M_i and radial position $r_i(t)$ is small, the torque $T_i(r, t)$ it exerts on a unit mass of fluid at radius r can be obtained from equation (18) of ref. 8, specialized to a keplerian velocity field:

$$T_{i} = 0.40 \text{ sgn} (r - r_{i}) \left[\frac{GM_{i}}{\Omega (r - r_{i})^{2}} \right]^{2}$$
 (6)

Once moonlets have emptied gaps larger than their own sizes, we may ignore accretion by and erosion of moonlets. Mass conservation of ring particles leads to the equation of continuity

$$\frac{\partial \sigma}{\partial t} + \frac{1}{r} \frac{\partial}{\partial r} (r\sigma u) = 0 \tag{7}$$

where u is the azimuthally averaged radial fluid velocity of the ring particles. Given the separate conservations of mass of ring particles and moonlets, we write the equations governing the transfer of angular momentum between the ring fluid and the J moonlets as

$$\frac{\mathbf{D}}{\mathbf{D}t}(r^2\Omega) = T_{\nu} + \sum_{i=1}^{J} T_i \tag{8}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[r_i^2\Omega(r_i)] = -\frac{1}{M_i} \int_0^\infty T_i(r,t) 2\pi r \sigma(r,t) \,\mathrm{d}r + T_i^{\mathrm{ext}}$$
 (9)

where $D/Dt \equiv \partial/\partial t + u\partial/\partial r$ is the substantial derivative and T_j^{ext} is the specific torque exerted on the jth moonlet because of possible resonant locking to a massive external moon¹⁶. In equation (8), we have assumed an absence of direct perturbations on the ring material by external moons; and in equation (9), we have ignored the possibility of resonant moonlet-moonlet interactions. The latter interactions are small for the moonlet masses and separations implied by the ring structure in Cassini's division.

In the keplerian approximation, $\partial (r^2\Omega)/\partial t = 0$ and $\partial (r^2\Omega)/\partial r = r\Omega/2$; thus, equation (8) becomes

$$\frac{1}{2}ur\Omega = T_{\nu} + \sum_{j=1}^{J} T_{j}$$
 (10)

Given c, Ω , K, M_i and T_i^{ext} , equations (1), (2), (5), (6), (7), (9) and (10) form a closed set to solve for ν , τ , T_{ν} , T_{ρ} , σ , r_i and u. We now show how quasi-equilibrium conditions simplify the solution to these equations.

Approximate equilibrium behaviour

From equations (10), (5) and (6), we readily estimate the fluid velocity u with which the jth moonlet will clear a ring gap Δr where the viscous torque has a magnitude comparable with that of the moonlet's gravitational torque:

$$ur\Omega \sim \frac{vr\Omega}{\Delta r} \sim \left[\frac{GM_i}{\Omega(\Delta r)^2}\right]^2$$
 (11)

so the characteristic time scale Δt for the gap clearing process is

$$\Delta t \sim \frac{\Delta r}{u} \sim \frac{(\Delta r)^2}{\nu} \sim \frac{(GM_i)^{4/3}}{\Omega^2 r^{2/3} \nu^{5/3}}$$
 (12)

which, with typical ring properties³, is of the order of several years for a moonlet of 2 km diameter, and scales as the moonlet diameter to the fourth power. Therefore, moonlets randomly placed among ring fluid will clear gaps around themselves in a time short in comparison with the age of the Solar System. The

ring particles must quickly move to accommodate a near balance between the torques on the right-hand side of equation (10). Approximating the spatial variation of c, Ω , r and K to be small in comparison with the resulting distribution of τ , we may set the right-hand side of equation (10) to zero and obtain

$$\frac{\tau}{1+\tau^2} + \arctan(\tau) = -\frac{0.18}{r\Omega^2} \sum_{j=1}^{J} \frac{(GM_j/c)^2}{|r-r_j|^3} + C_0$$
 (13)

where C_0 is a dimensionless integration constant. For ring material far from any moonlets, $C_0 = \tau_0/(1+\tau_0^2) + \arctan(\tau_0)$, and equation (13) predicts an optical depth profile which takes the form of a plateau $(\tau = \tau_0)$, with a steep decline as r approaches the position of the nearest moonlet on either side of the plateau. The edge of the gap, $\tau = 0$, is reached at a value of r where the right-hand side of equation (13) is zero. Equation (13) is not valid beyond this point.

For given local disk properties, there is a minimum size of a body which can lead to a clear gap. This minimum moonlet size is found by requiring that the equilibrium gap width exceed the size of the body embedded within the gap. With a spherical moonlet of internal density ρ , from equation (13) the criterion for the diameter D of a moonlet is

$$D \ge 1.4(C_0 r)^{1/3} \left(\frac{\Omega c}{G\rho}\right)^{2/3}$$
 (14)

where C_0 is a monotonic function of the plateau optical depth τ_0 . For small τ_0 , as in Cassini's division, C_0 is of the order of $2\tau_0$. With values which apply to Cassini's division today³, equation (14) implies that D must exceed roughly 2 km.

If an unconstrained moonlet $(T_i^{\text{ext}} = 0)$ were to find itself between two plateaus of different τ_0 (and therefore σ), the right-hand side of equation (9) will have a sign that forces this moonlet to move away from the high-density plateau towards the low-density plateau. The high-density plateau then spreads by viscous diffusion while the low-density plateau is compressed by the approaching moonlet. This reduces the density difference $\Delta \sigma$ between the two plateaus. When $\Delta \sigma$ has been reduced sufficiently and the evolving gap has developed enough width, the motion of the moonlet will be quite slow in comparison with the time required to produce the quasi-equilibrium ring profile given by equation (13) for fixed moonlet positions.

This suggests a simple procedure for computing the slow phase of the evolution of Saturn's ring system (most of the age of the Solar System). We calculate the moonlets' radial velocities by substituting the fluid distribution between adjacent moonlets implied by equation (13) into equation (9). New quasi-equilibrium fluid distributions can then be calculated from equation (13) after the moonlets are moved, keeping the amount of ring material constant between each pair of moonlets (Fig. 2).

Unless the boundaries of (part of) the disk are constrained by external effects (for example, moonlets locked to heavy exterior moons), no true equilibrium is possible for the ring system. (A monolayer with c = 0 and $\sigma \neq 0$ would not spread, but it would be unstable to collective gravitational disturbances.) If the inner and outer boundaries correspond to moonlets, there will be no rings to either side, and unbalanced torques in equation (9) will force these moonlets to move away from the middle. Similarly, if the inner and outer boundaries correspond to ring fluid, there will be no moonlet torques to keep the ring particles from spreading past the old boundaries. In either case, the radial spreading of the entire ring system would provide the ultimate release of orbital energy needed to sustain a non-zero level of random velocities¹⁸. In contrast, if embedded and/or boundary moonlets are constrained by resonant locking to outer moons, the maintenance of random velocities can come ultimately at the expense of a very small change in orbital characteristics of the heavy moon¹⁹. This may invalidate upper limit estimates of c based on lifetimes of Saturn's rings against viscous diffusion¹

These general comments may bear on the question of the maintenance of Cassini's division. Currently, Cassini's division and B ring material give a net outward push on the proposed moonlet ('guardian') embedded within the inner rift with a torque per unit mass which can be obtained by substituting $T_i \approx -T_{\nu}$, with T_{ν} given by equation (5), into the integral on the right-hand side of equation (9):

$$\frac{\mathrm{d}}{\mathrm{d}t} \{r_i^2 \Omega(r_i)\} = -\frac{3\pi}{M_i} [\sigma \nu r^2 \Omega]_{\mathrm{B ring}}^{\mathrm{Cassini \ division}} + T_i^{\mathrm{ext}}$$
 (15)

where the quantity in the brackets is to be evaluated well inside the Cassini division and the B ring. If the guardian is unconstrained, it could not prevent the closing of the Cassini division until the ring material reached surface densities comparable with the B ring and could exert an equal but opposite torque to balance that of the B ring. We believe that this compression is prevented by locking of the guardian in the classical 2:1 resonance with Mimas, which is itself locked to Tethys¹⁹. Using estimates of the guardian moonlet's mass, the viscosity and surface density of the B ring, we find that the Mimas 2:1 lock may barely have enough strength T_i^{ext} to keep the guardian in place. (Relevant formulae can be found in ref. (17).) Unfortunately, the proposed lock feeds eccentricity into Mimas' orbit, and unless there is a means to damp the eccentricity growth, the small current value of Mimas' eccentricity could pose a severe difficulty for this proposed mechanism.

The outer rift moonlet lies between two regions of roughly equal surface density; therefore it may remain stationary even if it is not locked to a heavy outer moon. (Note that the outer rift is located within current uncertainties at the 5:4 resonance with 1980 S27.) The outer edge of Cassini's division is a mystery. It is unlikely that a moonlet maintains the sharp density discontinuity between the division and the A ring. First, no gap is observed here down to the resolution limit of 10 km, so any moonlet present would have to be quite small. Second, the strongest resonance in this region, the 5:4 resonance with 1980 S26, has neither the right sign (for stability) nor sufficient magnitude to hold a small moonlet against the inward push of the A ring.

Independently of the mechanisms operating beyond the rift moonlets, we may use the observed locations of the gaps to fix the moonlet positions r_i at the present epoch. We can then adjust the moonlet masses and the amount of ring matter between pairs of moonlets (M_i/c) and C_0 in equation (13)) to give optical depth profiles that best resemble the major structures of the inner Cassini division. An internal check is available as the resulting structure yields, as it should, no appreciable moonlet motions if the rift guardians are held fixed. The best fit using five moonlets is given in Table 1. The resulting theoretical optical depth profile with equal heights of the plateaux (Fig. 2) is a natural consequence of the adopted physics and not of any adjustable parameter of the model. The observed approximately equal spacings of the moonlets may imply nearly uniform conditions in which the moonlets were initially formed.

Discussion

Embedded moonlets may explain why the inner rift of Cassini's division is located at the Mimas 2:1 resonance, and how large optical depth variations can be maintained between the division and the B ring. They also explain Encke's gap, which has sharp edges and no correspondence with resonances of known moons, as well as many of the gaps in the C and D rings. We conjecture that the many ringlets and grooved patterns in the B ring result from bodies massive enough to exert appreciable gravitational torques, but too small to clear resolvable empty gaps. Finally, Voyager 2 observations may detect rift moonlets if they have the sizes listed in Table 1. Gap moonlets could be harder to detect.

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- Smith, B. A. et al. Science 212, 163-191 (1981). Collins, S. A. et al. Nature 288, 439-442 (1980). Cuzzi, J. N., Lissauer, J. J. & Shu, F. H. Nature 292, 703-707 (1981). Tyler, G. L. et al. Science 212, 201-206 (1981).

- Henon, M. Nature (in the press).
 Jeffreys, H. Mon. Not. R. astr. Soc. 107, 260-262 (1947).

- Goldreich, P. & Trememe, S. Astrophys J 223, 857-871 (1979)
- Goldreich, P. & Tremanne, S. Astrophys. J. 241, 425-441 (1980)
 Lin, D. N. C. & Papaloncou, J. Mon. Not. R. autr. Soc. 186, 799-812 (1979)
- Elliott, J. L., Dunham, E. & Mink, D. Nature 267, 328-330 (1977)
- 11. Goldreich, P. & Tremame, S. Nemer 277, 97-99 (1979).
- Cozzi, J. N., Durisen, R. H., Burrat, J. A. & Hamill, P. Icarus 36, 54-68 (1979)
 Goldreich, P. & Tremune, S. Icarus 34, 227-239 (1978).

- Goldruch, P. & Tremime, S. Lesrer 34, 240-253 (1978)
 Lynden-Bell, D. & Pringle, J. E. Mon. Not. R. ant. Soc. 168, 603-637 (1974)
 Goldreich, P. Mon. Not. R. ant. Soc. 136, 159h181 (1965)
 Sinclair, A. T. Mon. Not. R. ant. Soc. 160, 169-187 (1972)
 Braine, A. Antrophys. 54, 895-907 (1977).

- Posle, S. J. A. Ren. Astr. Astrophys. 14, 215-246 (1976).
 Lm, D. N. C. & Bodenbeuner, P. Astrophys. J. Lett. 248 (in the press)

Distribution of neutral gas and dust near Saturn

E. C. Sittler Jr', J. D. Scudder & H. S. Bridge

* NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Greenbelt, Maryland 20771, USA † Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

The distribution of neutral gas and dust within the magnetosphere of Saturn has been inferred from the electron velocity distribution functions measured by the Voyager 1 plasma science experiment. Substantial enhancements of neutral material near Titan and in the vicinity of Enceladus are found. The E ring is also shown to be larger than previously thought.

DURING the Voyager 1 encounter with Saturn, the electron plasma in the energy range 10-5,950 eV was sampled by the plasma science experiment (PLS). The electron distribution function in the vicinity of Saturn is comprised of smoothly joined thermal and suprathermal components; it is not well represented by a single gaussian distribution. We define here the location where the electron distribution departs from this typical behaviour of smooth dependence of phase space density with energy and use this to indicate the presence of neutral material in Saturn's vicinity.

We have reported that in the wake of the satellite Titan anomalous electron distribution functions were sampled by the PLS experiment. Figure 1 shows a representation of the distribution function, f(v), sampled as a function of time as Voyager passed through Titan's wake. The principal anomaly in these spectra is the 'bite-out' region above 700 eV where f(v) is abruptly reduced to values below instrumental threshold. This energy-dependent reduction should be contrasted with the smooth and otherwise regular spectra of the thermal/suprathermal type seen on either side of the bite-out feature. This 'quenching' of the suprathermal part of the spectrum has been interpreted as a result of inelastic collisions of electrons with Titan's extended neutral atmosphere. The extinction becomes strong when the electron gyroradius becomes comparable to, or greater than, the local scale height of the neutral atmosphere1. A corresponding enhancement of the low-energy population is observed, which is theoretically expected when the primary electrons become degraded and produce secondaries, and/or when the neutral gas is photoionized.

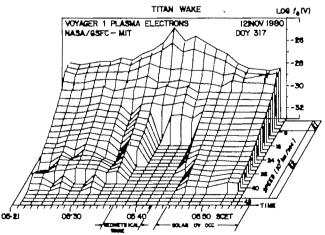


Fig. 1 The observed electron distribution functions in the Titan wake region. Energy increases towards the viewer An individual distribution is plotted at a time corresponding to measurement of its lower energy portion E1 The low and high electron energy modes of the instrument E1 and E2are indicated, respectively, where energy mode E2 is sampled 48 s after E1.

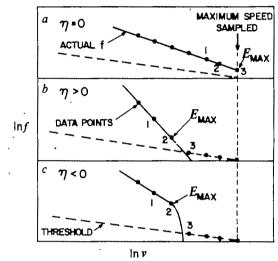


Fig. 2 Log of the distribution function plotted against the logarithm of the speed for three possible values of the curvature parameter (a, b, c). The minimum flux level or threshold and maximum speed sampled by the instrument are indicated. The data points indicated by dots represent the observed distribution function while the actual distribution function being measured by the instrument is displayed by the solid curves. The speed channel for computing $E_{\rm max}$ for each case is denoted. In computing η points 1, 2, and 3 are used. To remove any artificial scatter in η when flux levels are low, the data points 1 and 2 must be five times or more above instrument noise. If not, E_{\max} is reduced until this condition is satisfied.

We have systematically located analogous bite-out features in the distribution function samples acquired during encounter. The easiest way to quantify these features is to evaluate the curvature of the distribution function at the maximum energy (E_{max}) that the distribution is observed above the PLS instrument's flux threshold. Here we evaluate a dimensionless curvature indicator, η , given by

$$\eta = \frac{\mathrm{d}^2 \ln f(v)}{\mathrm{d} (\ln v)^2} \bigg|_{H_{-}}$$

the second derivative of the logarithm of the observed distribution with respect to the logarithm of the speed, centred at the last energy channel E_{max} which has detectable fluxes above the instrument noise and telemetry thresholds; η vanishes when the local spectrum near E_{max} is a power law in particle speed. This is usual in the magnetosphere in our energy range. Further, the curvature index has been constructed to differentiate between an intrinsic spectral change near threshold ($\eta < 0$) and a spectrum-preserving reduction in the particle flux $(n \ge 0)$.

Figure 2 shows three possible transitions of the observed speed distribution from above to below instrument threshold. A power law is assumed for the distribution function f(v) where different spectral slopes in Fig. 2a, b, c are used only for illustration. In Fig. 2a the spectrum remains above threshold for the

full energy range of the instrument: here $\eta = 0$. Figure 2b illustrates the situation where there is a spectral preserving reduction in the actual distribution function; here, because of the instrument threshold level, $\eta > 0$. Figure 2c illustrates the negative curvature situation, similar to that shown in Fig. 1 for Titan's wake. Here the spectral trend below $E_{\rm max}$ is shallower than the trend suggested by the fluxes above $E_{\rm max}$. Because the threshold level is greater than or equal to the incident particle flux above E_{\max} , an upper limit for η is determined using the threshold value as the third point in the numerical evaluation of the second derivative. The curvature parameter computed near threshold gives a qualitative measure of the curvature in f(v) at E_{max} ; and is used to identify regions where bite-out features are present and to give general trends about the energy dependence of the reduction experienced by the suprathermal electrons. Hence $\eta \ge 0$ indicates no bite-out signature, whereas $\eta < 0$ signifies a bite-out. The more negative the curvature parameter the greater the reduction in f(v).

Observations

We have processed all available electron data acquired close to and inside Saturn's magnetosphere. The results are shown in Fig. 3 with abscissa the equatorial radius, ρ , of the observer in a cylindrical coordinate system with its z axis aligned with Saturn's rotation axis. Within the plot of the L shell parameter the annotated arrows show the equatorial distance, ρ , of field lines which simultaneously thread the spacecraft and various inner satellites according to a model of the magnetic field which incorporates both an intrinsic potential model of Saturn's magnetic field and the first-order effects of a distributed ring current outside $8.7~R_{\rm S}$ (Saturn radii)³. Positions of the bow shock and magnetopause crossings¹ are also indicated, so changes in electron distribution function can be discussed in relation to the boundaries of the magnetospheric system. Note that the density scale is different for inbound and outbound passes.

In the magnetosphere the curvature parameter is usually near zero whenever E_{max} is at the upper speed channel of the instrument; this supports our interpretation that the parent distribution for the suprathermal electrons is a power law within Saturn's magnetosphere. However, in many places the η -curvature parameter is noticeably and significantly negative. The bite-out signature displayed in the three-dimensional plot of the Titan feature is retrieved as indicated by the sharp reduction in $E_{\rm max}$ and the accompanying increase in the magnitude of the negative curvature. On either side of the principal Titan feature, at least two and possibly more subsidiary density enhancements previously called 'plumes' comprised of electrons cooler than the surrounding magnetospheric electrons can be seen (19.1 and 21 R_s). These density enhancements are associated with changes in the curvature index to more negative values, but at larger values of $E_{\rm max}$ than that at Titan's wake. According to the 'plume' model¹, the density enhancement at $21 R_s$ should be of most recent origin, because it has a higher density and colder temperature, presumably due to recent contact with the Titan atmosphere. Note that E_{max} for this plume is smaller than that for the other plume seen at $19 R_s$, which should be older. In addition to these relatively clear instances of remnants of neutral plasma interactions, there is evidence for bite-out signatures in the suprathermal electrons outside of Titan's orbit all the way to the magnetopause boundary and possibly even into the magnetosheath. Centrifugal forces resulting from co-rotation would tend to move plasma which has interacted with Titan towards the magnetopause. Bite-out signatures observed in the magnetosheath could therefore result from an open magnetic topology, where the suprathermal electrons, which have encountered Titan in the past, have leaked out of the magnetosphere into the magnetosheath. Note that the intrinsic magnetosheath electron spectrum may not be a true power law, but rather maxwellian, in which case the normal curvature of the distribution function crossing the instrument threshold would be

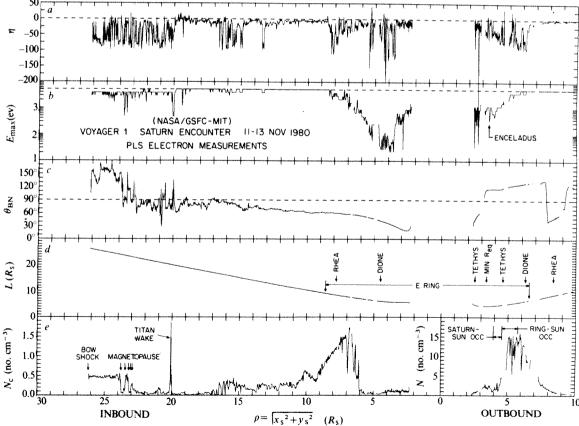


Fig. 3 Plots of spacecraft equatorial radius, ρ , against: a, η : b, maximum energy E_{\max} for which electron fluxes are observed above instrument threshold; c, angle $\theta_{\rm BN}$ between sensor look direction and current magnetic field direction 2 ; d, the dipole L shell parameter of the spacecraft; e, the electron density for the thermal electrons (low energy component). The Saturn–Sun occultation and ring–sun occultation periods are indicated. The density estimates for these periods show the trend of the data. Complications in estimating the spacecraft floating potential when solar occultation occurs means the analysis is incomplete. Any variations in the density during this period could be artificially produced by unaccounted for variations in the spacecraft floating potential and should be ignored. The spacecraft positions are computed using the Connerney $et\ al.$ model³ and not from the dipole L shell parameter.

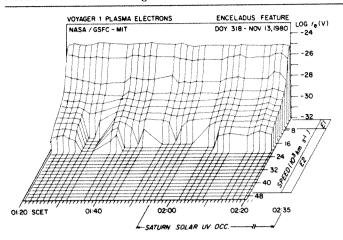


Fig. 4 Plot of the observed electron distribution function for the bite-out event observed near closest approach with Enceladus's L shell (see Fig. 1). When the spacecraft is in shadow, it may reach negative potentials of -10 V significantly below the E_{max} energies reported here. Therefore, only minor trajectory perturbations can be induced by these size potentials for electrons with energies near E_{max} . Hence little effect on the intrinsic bite-out signature is expected when the spacecraft goes into shadow in this region. Data gaps that are \sim 5 min are centred at 01:36, 01:48, 02:02, and 02:11.

negative. If true, negative curvature signatures for η in the magnetosheath may not be signatures of a bite-out.

Inside Titan's orbit on the inbound traversal between 15 and 17 R_s and briefly at 13.4 R_s there are additional intervals of large negative curvature signatures. In these intervals there is no known satellite or suggested ring or dusty material. We have considered the possibility that when Titan is near local midnight, magnetic field lines which have had close encounters with Titan could map into these smaller radial distances at local noontime. The solar wind compresses Saturn's magnetosphere at noon local time moving field lines in closer to Saturn, and distends them at midnight local time moving the field lines out to greater radial distances. The bite-out signatures inside Titan's orbit at noon local time may then be vestiges of plasma that have experienced a close encounter with Titan near midnight local time. The magnetopause of Saturn has been observed^{4.3} as 17.3 Rs to Saturn by Pioneer 11 which supports this interpretation. This interpretation also requires a persistence (lifetime) of the curvature signature over a period that is comparable to one-half the orbital period of Titan (8 days or 18 Saturn rotation periods). Losses from these field lines would be smaller than those loaded with plasma by Titan at local noon, as the latter field lines should come much closer to the magnetopause surface, and be extended further down the tail at local midnight where the field lines can open up and lose plasma^{6,7}.

E ring signature

The curvature parameter indicates another region of suprathermal extinction between 8.4 R_s inbound (317:16:56) and about 6.7 R_s outbound (318:04:45). At the extremities of this interval the curvature parameter is suddenly sharply increased in magnitude, and while remaining negative then begins a recovery towards smaller magnitude but still negative values. The value of E_{max} also exhibits a systematic variation within this interval, decreasing as we proceed towards closest approach and then recovering on the outbound traverse. The amplitude of this variation is remarkable with a range of 6,000-30 eV. Note that the onset of the sharp inbound bite-out signature occurs while the density is still rising so the decrease in the $E_{\rm max}$ parameter cannot result from decreased fluxes without a corresponding spectral change. When the sharp bite-out commences at $8.4 R_{\rm S}$ inbound the maximum energy at which particle fluxes are observed above threshold is higher than it is in the weakest curvature parts of this interval. At the outbound termination of the bite-out period at $6.5 R_s$, a similar phenomenon is observed: the erosion of f(v) is the most severe as E_{max} rises back towards the maximum PLS energy.

We suggest these signatures are produced by the interaction of particulate matter with the local thermal plasma. We associate our observations with the optically defined E ring first imaged by Feibleman8 and whose impact on the trapped particles was theoretically predicted by Thomsen and Van Allen. Thomsen and Van Allen showed that the extinction of low energy electrons (E < 6 keV) becomes more pronounced as the particle energy is raised. This is essentially the effect we have observed in the relation between the curvature index and E_{max} . The strong energy dependence arises either because the degradation of electrons is proportional to the bounce frequency which is in turn proportional to the particle speed for particles with magnetic mirror points above or below the E ring or, when this is not the case, the collision frequency is proportional to the particle speed (geometrical cross-section).

Thomsen and Van Allen make a prediction about the angular distributions expected for particles interacting with a thin ring of dust, which has been essentially confirmed by Pioneer observations^{10,11}. Particles with equatorial pitch angles near 90° are preferentially removed as their path lengths through the ring material are larger and bounce periods shorter than those whose pitch angles are near 0°. Throughout most of the interval given by the Ering in Fig. 3, the PLS electron detector had a mean field of view including particles having 90° pitch angles. For the present energy range and the pitch angles sampled, using known ranges of optical opacities of the E ring¹² and measured diffusion coefficients within Saturn's inner magnetosphere 13, the attenuation of the speed distribution is probably exponentially dependent on the electron speed. This explains the sensitivity of the curvature index as E_{max} rises, and why a sharp definition of the outer edge of the E ring results from this analysis. The PLS definition of the E ring corresponds closely to the definition of the inner boundary of the ring current by the MAG team^{3,14} The energies of electrons used here to define the E ring are substantially below that used by the Pioneer investigations; so they are more sensitive to smaller column densities than previous studies since the stopping distances or ranges for charged particles through neutral material increase with particle energy. The extension of the E ring to these radial distances also explains why the distributed currents of the outer magnetosphere seem to stop in this vicinity. Energetic ions which have ranges or stopping distances less than that of a 6 keV electron are probably the principal current carriers of the azimuthal ring current. These current-carrying ions are then removed by the E ring which determines the inner edge of the ring current.

The radial extent of the E ring is not symmetrical about Saturn; the ring extends to nearly $L = 9.0 R_s$ in the noon hemisphere and extends to $L = 6.5 R_{\rm S}$ in the outbound traverse. A similar asymmetry has been observed^{10,15} by Pioneer 11, suggesting this is a permanent feature of the E ring plasma interaction—but no explanation is available.

Within the broad E ring region another feature occurs which is not consistent with the overall trend of the bite-out signatures of the E ring itself. Between 3.7 R_s (day 317 at 21:28 LT) inbound and nearly 2.8 R_s (day 318 at 00:34 LT) outbound there is an anomalous enhancement of the E_{max} parameter. During this interval the magnetic field vector becomes increasingly aligned with the sensor look direction. This change in the relative viewing angle could be reconciled with the observation of the field aligned portion of the theoretically expected dumbbell pitch angle distribution⁷, which is outside the field of view of the instrument during the earlier portion of the E ring measurements. Alternatively, this region could be a new particle regime, with a different set of forces and history for the observed particles. This view relies on the results of Acuña et al. 4 who report that during this anomalous period a 'non-potential' feature in the magnetic field indicates a distinct spatially localized disruption of the otherwise dipolar magnetic topology. During this period the last channel above threshold increases from 50 eV to nearly 1 keV in near coincidence with the non-potential field perturbation. This shift may represent an energization of the particles or simply an enhancement of their number density. As the magnetic anomaly requires a field-aligned current and as the high mobility of the electrons argues that they would be the principal carriers, the enhanced electron fluxes in this plasma anomaly may represent the signatures of field aligned currents. The plasma data by themselves cannot, however, determine if there is a net current flow.

Enceladus signature

For the brief interval between 01:20 and 02:10 SCET on 13 November, 1980, within the general E ring bite-out, a strong reduction in the suprathermal electrons occurred similar to that shown in Fig. 1 for the Titan wake, and signified by the sharp depression in E_{max} and the curvature parameter between 3.4 and $4 R_{\rm S}$ spacecraft equatorial distance. Figure 4 shows the distribution function for this feature. The L shell distance of the strongest depression in E_{max} centred on 01:30 SCET is 4.35 R_{S} , very near the minimum L shell of 4.34 R_s predicted by Connerney et al.³ As the dipole L parameter plot indicates, L is approximately constant in this interval and it is therefore difficult to determine the equatorial distance to be ascribed to the strongest bite-out. This distance is close to, but outside, the radial distance of Enceladus, which has been suggested by Terrile and Tokunoga¹⁶ to be the source of the E ring. Terrile and Tokunoga observed a brightening of the E ring in the vicinity of Enceladus. If this interpretation is correct, we have observed bite-out signatures on flux tubes that have traversed this enhancement of dust about Enceladus. At the energies considered here, the previous explanation of the bite-out at Titan is inappropriate because the flux tubes do not come within a gyroradius (<2 km) of Enceladus or any other satellite and the scale lengths for these features are of the order of a Saturn radius. The possibility of an interaction with a localized dense cloud of gas with neutral column densities $\sim 10^{12}$ cm⁻² cannot be ruled out by these measurements, but the lack of evidence for such a dense cloud of gas from UV observations¹⁷, makes this explanation unlikely.

Conclusions

Based on our suggestion that neutral absorbing material in the vicinity of Saturn can be sensed by studying the spectral modifications to the speed distribution of low energy electrons (E < 6 keV) we have remotely identified the neutral gas around Received 13 May; accepted 15 July 1981.

- Bridge, H. S. et al. Science 212, 217-224 (1981). Ness, N. F. et al. Science 212, 211-216 (1981). Connerney, J. E. P., Acuña, M. H. & Ness, N. F. Nature 292, 724-726 (1981). Wolfe, J. H. et al. Science 207, 403-407 (1980).
- Smith, E. J. et al. Science 207, 407-410 (1980).
- Hill, T. W., Dessler, A. J. & Michel, F. C. Geophys. Res. Lett. 1, 3 (1974)
- Carbary, J. F., Hill, T. W. & Dessler, A. J., J. geophys. Res. 81, 5189 (1976). Feibelman, W. A. Nature 214, 793-794 (1967).

Titan which is a strong quencher of the (otherwise commonplace) suprathermal electrons. A similar feature has been used to delineate the E ring, which is considerably larger than previously thought. This results from the use of lower energy electrons as the probes of dust in the ring, as they are stopped by smaller dimension particles. In our energy range the E ring is also asymmetric in local time. The present definition of the extremities of the E ring correspond closely to the inner edge of the ring current as modelled by the MAG team, which argues indirectly that ions with ranges through matter (dust) less than that of a 6 keV electron are the prominent ring current carriers.

The progressive reduction of the suprathermal electrons with decreasing radial distance within the E ring strongly suggests that the suprathermal electrons are diffusing inwards (source must be outside of E ring), whereas outside Titan's orbit we found evidence for outwards diffusion.

The removal of the suprathermal electrons within the E ring explains the apparent collapse of the plasma sheet1. Due to extinction of the suprathermal electrons, which dominate the electron pressure outside the E ring, the mean energy of the electrons decreases by a factor of ~5. Consequently, the ability of electrons to leave the equatorial plane and pull off the ions by the polarization electric field, is reduced. The scale height of the density variation should then be smaller within the Ering regime than outside. Estimates of the scale heights within and outside the E ring assuming a composition of singly ionized nitrogen are in good agreement with those inferred by Bridge et al.

We interpret a prominent extinction feature in the vicinity of Enceladus to be the result of enhanced dust around Enceladus: this may support earlier speculations that Enceladus¹⁶ is the primary source of the E ring material.

Finally, the electron fluxes are strongly enhanced relative to the ambient conditions within the non-potential magnetic field disturbance within the E ring traversal. These combined observations suggest a localized field aligned current system with the electrons being the principal current carriers.

We acknowledge the advance availability of the magnetic field data from the Voyager magnetometer team and N. F. Ness, and magnetic coordinates provided by the magnetic modelling effort of the MAG group. We thank K. W. Ogilvie and N. F. Ness for helpful comments and M. C. Harrison and L. J. Moriarty for technical assistance.

- 9. Thomsen, M. F. & Van Allen, J. A. Geophys. Res. Lett. 6, 893-896 (1979).
- Simpson, J. A. et al. J. geophys. Res. 85, 5731-5762 (1980).
 Bastian, T. S., Chenette, D. L. & Simpson, J. A. J. geophys. Res. 85, 5731-5762 (1980).
- Smith, B. A. The Saum System 105-111 (NASA Conference Publication 2068, 1978). Van Allen, J. A. Thomsen, M. F. & Randall, B. A. J. geophys. Res. 85, 5709-5718 (1980).
- Acuña, M. H., Connerney, J. E. P. & Ness, N. F. Nature 292, 721-724 (1981).
 McDonald, F. B., Schardt, A. W. & Trainor, J. H. J. geophys. Res. 85, 5813-5830 (1980).
 Terrile, R. J. & Tokunaga, A. Bull. Am astr. Soc. 12, 701 (1980).
 Broadfoot, A. L. et al. Science 212, 206-211 (1981).

Low-frequency plasma waves near Saturn

B. M. Pedersen*, M. G. Aubier*, & J. K. Alexander*

Observatoire de Paris, Section d'Astrophysique de Meudon, 92190 Meudon, France † Laboratory for Extraterrestrial Physics, Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

Voyager planetary radio astronomy observations of low frequency emissions detected around the time of closest approach to Saturn and near the outbound ring plane crossing are presented. Near the ring plane an electron density of between 5 and 20electrons cm⁻³ at distances of $\sim 6R_S$ is estimated.

THE low-frequency plasma wave emissions presented here are reminiscent of emissions observed within the Earth's magnetosphere1 and more recently within the jovian magnetosphere^{2,3}. Their most striking feature consists of intense spiky bursts, interpreted as local electrostatic upper hybrid resonances accompanied by odd-half-harmonic cyclotron, (n + $1/2)f_{\rm g}$ waves where $f_{\rm g}$ is the electron gyrofrequency. These emissions are preceded by two distinct components at frequencies lower than the normal Saturn kilometric radiation (SKR)⁴: the first one is a polarized drifting emission between 3 and

40 kHz; the second, appearing at still lower frequencies, is unpolarized and narrow banded (1-3 kHz). By analogy with observations in the terrestrial magnetosphere, these two emissions are interpreted respectively as non-thermal continuum radiations⁵ and VLF hiss.

The characteristics of the Voyager 1 trajectory close to Saturn are shown in Fig. 1. During the inbound segment, the spacecraft crossed the saturnian equatorial plane downwards at the orbit of Titan. The planetary radio astronomy (PRA)⁶ observations during this Titan flyby will be discussed elsewhere⁷. Up to the

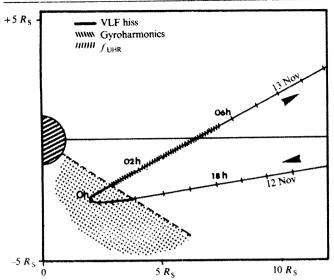


Fig. 1 A meridian plane projection of the Voyager 1 trajectory around closest approach is coded to indicate the locations where three different classes of emission are detected by the PRA experiment. From the $f_{\rm UHR}$ resonance we deduce electron densities between 5 and 20 electrons cm⁻³ (see Fig. 3). The dashed line corresponds to magnetic latitude $ML = -28^{\circ}$.

time of the closest approach, the spacecraft continued towards higher negative saturnian latitudes (also magnetic latitudes), while moving around the planet. During the outbound pass through the saturnian magnetosphere, Voyager 1 returned towards the ring plane, which it crossed at the orbit of the moon Dione $(6R_s)$ and then continued towards high positive latitudes.

The different low-frequency emissions observed near periapsis are shown as a function of time on the fixed frequency plots in Fig. 2. Each channel has a 1-kHz bandwidth, and each point represents a 6-s measurement interval. Two traces in a particular frequency channel correspond to signal levels in each of the opposite senses of circular polarization and indicate that the emission is polarized.

The emission appearing at frequencies above $\sim 60~\rm kHz$ in Fig. 2 is freely propagating SKR⁸, whereas the broad-banded emission present in all channels around the time of the outbound ring plane crossing does not show normal SKR characteristics. In particular, it does not appear at the typical saturnian longitudes for the low-frequency SKR component. We cannot explain this emission which is confined to a region around the equatorial plane. We now consider the activity which occurs only in the frequency range below $60~\rm kHz$ and which can provide information about plasma conditions in the vicinity of the spacecraft.

Impulsive emissions appear between ~1 h and 6 h in the three lowest-frequency channels. The very intense emission in the 1.2-kHz channel which saturates our receiver most of the time (intensity level $> 10^{-11} \, \text{V}^2 \, \text{m}^{-2} \, \text{Hz}^{-1}$) appears below half the local electron gyrofrequency and can be thus interpreted as VLF noise⁹. Another type of emission in this time interval consists of brief spiky bursts (Fig. 2). This impulsive emission first develops at low frequencies, moves towards high frequencies and then back to low frequencies. We interpret this pattern as a variation of the local upper hybrid resonance frequency along the spacecraft trajectory. This interpretation is supported by the resemblance to similar narrow-banded emissions observed in the magnetospheres of Earth¹ and Jupiter^{2,3}: The electrostatic noise at $f_{\text{UHR}} = (f_p^2 + f_g^2)^{1/2}$, where f_p is the local plasma frequency, allows a direct estimate of the electron density at the position of the spacecraft. The resulting density curve around closest approach is shown in Fig. 3 together with density curves estimated by Voyager 1 plasma wave (PWS)9 and plasma science experiments (PLS)¹⁰. A time discrepancy is noted with respect to PWS results, whereas our maximum density (~20 electrons cm⁻³) is only slightly higher than the PLS value.

Preceding this developing f_{UHR} line we observe smooth emission humps of longer duration on the 20.4 and 39.6 kHz

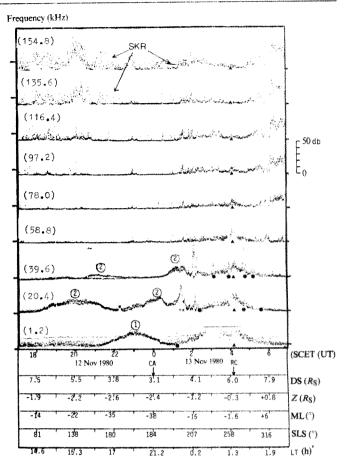


Fig. 2 Fixed frequency plots (intensity in dB μ V⁻¹ kHz⁻¹) of the lowest PRA frequencies as a function of the distance (D_s) from Saturn. The distance (Z) from the equatorial plane, the magnetic latitude (ML), the Subspacecraft Saturn Longitude (SLS) and the local time (LT) are shown. CA denotes the point of closest approach, and RC denotes the ring plane crossing. The broad-banded emission around RC (Δ), the $In + 1/2if_g$ waves (\times), the $In + 1/2if_g$ waves (\times), the VLF hiss (Ω) and escaping (Ω) continuum radiations are indicated. The intense spiky emission from 2 h to 5 h on the 1.2-kHz channel is interpreted as VLF noise. The emissions observed throughout the period at frequencies ≥ 60 kHz are due to freely propagating Saturn kilometric radiation.

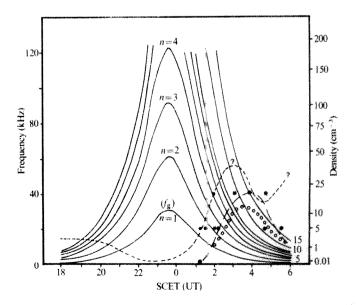


Fig. 3 Appearance of electrostatic emissions $(\times, \text{ smooth } (n+1/2)f_k$ humps; \bullet , f_{UHR} spiky resonances) with respect to the evolution of the gyroharmonic pattern as calculated from the measured magnetic field (left ordinate). The density profile deduced from the PRA observations (---) is compared with the Voyager PLS¹⁰ (200000) and PWS⁹ (----) results (right ordinate).

channels (Fig. 2). We interpret these emissions as $(n+1/2)f_n$ harmonic waves. Such waves are also observed in the vicinity of the Earth1 and within the Io plasma torus2.3 and generally accompany f_{UHR} waves. Figure 3 shows the position of these waves with respect to the gyroharmonic pattern calculated from Voyager 1 magnetic field measurements¹¹. The location of the gyroharmonic waves in relation to the f_{UHR} waves can be interpreted in terms of the relative temperatures of a twocomponent electron distribution consisting of a 'cold' background plasma and a 'hot' component that provides a source of free energy for the waves³.

In the Earth's and Jupiter's magnetosphere¹² the whole pattern of (n+1/2) f_g and f_{UHR} electrostatic waves in some cases seems to be closely associated with two components of non-thermal electromagnetic radiation: an escaping and a trapped continuum component. The emission of type 2 in Fig. 2 resembles such a non-thermal continuum: this polarized emission drifts between frequencies ≤ 20 kHz and ~40 kHz and is typical of an apparently distinct narrow-banded component of SKR that is seen intermittently in the PRA data throughout the encounter period4. Its characteristics are similar to the narrowband jovian kilometric radiation (nKOM)¹³, and both may

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- Christiansen, P. J. et al. Space Sci. Rev. 22, 383-400 (1978). Warwick, J. W. et al. Science 204, 995-998 (1979).
- Birmingham, T. J., Alexander, J. K., Desch, M. D. Hubbard, R. F. & Pedersen, B. M. J. geophys. Res. (in the press).
- Warwick, J. W. et al. Science 212, 239-243 (1981). Gurnett, D. A. J. geophys. Res. 80, 2751-2763 (1975)

originate in the same physical process as the escaping non-thermal continuum radiation¹². At lower frequencies around 1 kHz a smooth, intense and unpolarized emission 1 (on Fig. 2) is observed during a short period at latitudes lower than -28° (Fig. 1). Gurnett et al⁹ interpret this low frequency component, below the plasma frequency and far below the gyrofrequency, as VLF noise.

We have shown that the different emission components of the low-frequency spectrum observed during the Saturn encounter constitute a similar pattern to the spectra observed elsewhere (see Fig. 9 of ref. 12). The combination of strong, impulsive bursts at f_{UHR} and smooth emissions at $(n+1/2)f_g$ is common in plasma populations consisting of a mixture of relatively cold and hot energetic magnetospheric plasmas. This mixture, as pointed out for the terrestrial and jovian magnetospheres by Kurth et al.12, agrees with the emission mechanism proposed by Melrose 14, according to which the non-thermal radio continua result from conversion of upper hybrid waves into electromagnetic waves by coalescence with low-frequency waves. Finally our estimate of the electon densities near the time of the outbound ring plane crossing $(4 \le R \le 7R_s)$ agrees reasonably well with the direct measurements by the PLS team 10.

- 6. Warwick, J. W., Pearce, J. B., Peltzer, R. G. & Riddle, A. C. Space Sci. Rev. 21, 309-327
- Daigne, G., Pedersen, B. M., Desch, M. D. & Kaiser, M. L. J. geophys. Res. (submitted).
- Daigne, G., Pedersen, B. M., Desch, M. D. & Kaiser, M. L. J. geophys. Res.
 Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731–733 (1981).
 Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 235–239 (1981).
 Bridge, H. S. et al. Science 212, 217–224 (1981).
 Ness, N. F. et al. Science 212, 211–217 (1981).

- Nets., N. F. et al., Sterice 212, 21 (221) (1991). Kurth, W. S., Gurrett, D. A. & Anderson, R. R. Preprint 80-42 (University of Iowa 1980). Kaiser, M. L. & Desch, M. D. Geophys. Res. Lett. 7, 389-392 (1980).
- Melrose, D. B. J. geophys. Res. 86, 30-36 (1981)

Impulsive radio discharges near Saturn

David R. Evans*, James W. Warwick*, Jeffrey B. Pearce*, Thomas D. Carr* & John J. Schauble

> *Radiophysics, Inc, 1885 33rd Street, Boulder, Colorado 80301, USA †Department of Astronomy, University of Florida, Gainesville, Florida 32611, USA

An unexpected type of emission was observed during the Voyager 1 Saturn encounter. This consisted of periodic episodes of many impulsive discharges throughout the frequency range (20.4 kHz to 40.2 MHz) of the planetary radio astronomy experiment.

NON-THERMAL radio emissions from the Saturn system were first detected by the Voyager planetary radio astronomy (PRA) experiment on board Voyager 1 in January 1980. Since then emission between 100 kHz and ~1 MHz from the planet, termed Saturn kilometric radiation (SKR), has been detected almost continuously. Observations of SKR are detailed elsewhere1. For only a few days around encounter a broadband (100 kHz to at least 40 MHz) emission was observed. Initial observations of this form of emission are given elsewhere²; here we detail the further analysis of these data.

In its normal mode, the PRA receiver scans downward in frequency through 198 channels between 40 MHz and 1.2 kHz and samples each channel for 30 ms. Each scan includes experiment status information, and requires 6 s (ref. 3). Figure 1 shows part of a typical dynamic spectrum obtained by the receiver in this mode near encounter, in which several vertical streaks are apparent. We believe² that these streaks have a physical cause outside, and independent of, the spacecraft. The detailed structure of an individual streak (or 'event') can be determined only on the rare occasions that the PRA receiver is operating in its 'high rate' mode, when two preselected pairs of adjacent frequency channels are sampled simultaneously once every 140 µs. Although we only have 48 s of such data which exhibit clear events, obtained ~4 h after encounter, this is enough to examine the detailed structure of such events, which in many ways resembles the radio signature of a terrestrial lightning discharge. This, and properties previously listed², leads

us to conclude that we are observing the effects of short-lived electrostatic discharges originating in the vicinity of Saturn (hence Saturn electrostatic discharges (SED)).

To understand better the properties of SED, an algorithm was

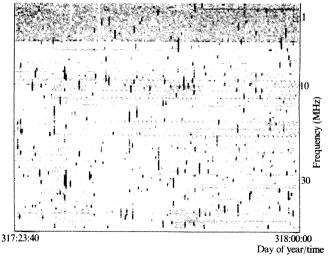


Fig. 1 Dynamic spectral scans from the PRA experiment near closest approach. The SED appear as short vertical streaks whose duration is proportional to the height of the streaks. The broadband nature of the phenomenon is clear.

written to identify and log each individual discharge event occurring in the regular low rate data. This proved to be much more sensitive than the plotting of dynamic spectra as in Fig. 1. The rate of discharge occurrence varied markedly throughout the encounter period with the SED being generally confined to apparently periodic episodes. Given the relatively limited data base, and the rapidly changing geometry of the Saturn system as seen from the spacecraft during encounter, the inherent periodicity of these episodes cannot be estimated precisely. We calculate, however, that the observed events are consistent with a source revolving about the planet with a period of ~10 h 10 min (±5 min), radiating away from the planet. The possibility of a corotating phenomenon (of period near 10 h 40 min), previously thought to be a plausible explanation of the observations, is now clearly excluded. Generally, SED episodes are observed over slightly more than 50% of this orbit, which is located about 1.81R_s (Saturn radii) from the centre of the planet. Figure 2 shows all the clearly defined episodes we observed; each frame represents one revolution of a reference frame rotating with period 10 h 10 min about the centre of Saturn (0° is defined as that longitude facing the vernal equinox of Saturn at 1.0 January 1970), with spacecraft motion taken into account. The episodic nature of the phenomenon is clear. For convenience, the episodes have been assigned identifying numbers as in Fig. 2.

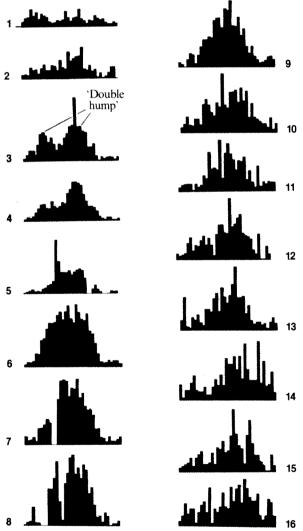


Fig. 2 The episodic gross structure of SED which shows the 16 relatively well-defined episodes (closest approach takes place during episode 5). Each episode consists of 36 bins each 10° longitude wide, representing the sub-spacecraft longitude of a system rotating about Saturn's centre once every 10 h 10 min. The length of each bar is a function of the numbers of observed SED events and the spacecraft distance from $r = 1.81R_s$. This convolution increases the relative noise background with increasing distance from $r = 1.81R_s$.

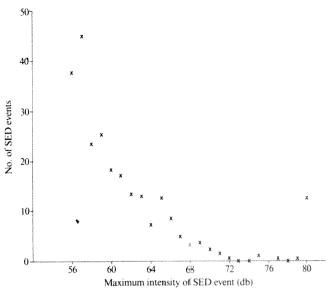


Fig. 3 Maximum intensity of SED bursts for the available high-rate data. Instrument cutoff occurs at +80 dB. The number of points at or above cutoff is highly anomalous and indicates indirectly the possibility of two distinct populations of SED.

Dynamic spectra such as Fig. 1 exhibit clearly the broadband nature of SED. Generally whilst within an episode, we observed SED in all channels which were not swamped by SKR emission. This normally limited us to frequencies ≥800 kHz. For part of the encounter episodes, however, SKR was absent at the lowest frequencies, and SED was clear even below 100 kHz. To test the broadbandedness of SED, spectra consisting of the number of SED events observed per channel on an episode-by-episode basis were calculated. At encounter (episode 5) the spectrum appears remarkably flat; what features are present are due to the sensitivity curve of the receiver, and the changing shape of the spectrum as the spacecraft moves in the days around closest approach indicates that at encounter relatively few discharges go undetected.

Note also that the onset of an SED episode is not uniformly simultaneous at all frequencies. In particular, the encounter episode was observed at 40 MHz some 4.5 h before it was seen at 10 MHz. The start of other episodes and the end of all episodes seem to be independent of frequency.

A marked feature of Fig. 2 is the asymmetry about closest approach; the earliest recognizable SED were observed only ~36 h before closest approach, whereas post-encounter episodes continued for some days. Two of the pre-encounter episodes exhibit a clearly double-humped envelope, a feature

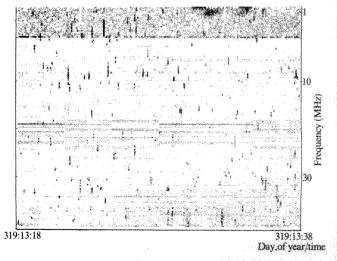


Fig. 4 Dynamic spectrum similar to Fig. 1 for episode 6. The alternating light and dark squares during many SED events indicate the presence of circular polarization, in this case overwhelmingly in the left-hand sense.

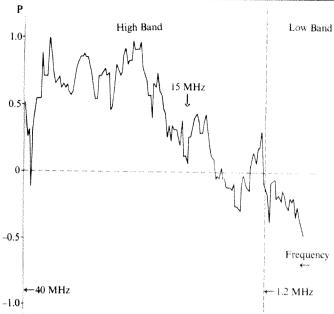


Fig. 5 Polarization as a function of frequency for post-encounter episode. Frequency runs between 40 MHz at left to ~800 kHz at right. A three-point running mean has been used to smooth the curve. Very few points were available at the top of the high band, but at other frequencies above about 15 MHz (marked with arrow) most bursts are clearly left-circularly polarized. Below 15 MHz there is little evidence for any systematic sense of polarization.

which does not appear to be present after the encounter. There is some evidence that this could be due to a smaller source of SED revolving about the planet with a longer period ($\sim 10\,h$ 30 min). Pre-encounter, the two sources are just resolved. During the period displayed in Fig. 2 the faster source catches up with, and may overtake, the slower source, and so for the strongest episodes (numbers 3–9), the two are unresolved. In support of this, the early episodes seem to have a slightly greater angular width than the later ones. Support for a two-source model comes from an investigation of the intensity characteristics of the bursts detected in the high rate mode, which exhibit results typical of the superposition of two or more populations (Fig. 3). This hypothesis cannot be rigorously checked until the Voyager 2 encounter.

In the normal mode, the PRA receiver is sensitive to circular polarization. The sense of polarization sampled alternates from channel to channel as a 6-s scan progresses. (Alternating 6-s scans start with alternating polarization sampling of the first

channel.) Consequently, should a given SED event show circular polarization, it would appear as a streak of alternating light and dark squares on a dynamic spectrum plot. A search for this phenomenon yielded many examples, such as shown in Fig. 4. We defined an index of polarization for the *i*th channel, P(i), by

$$P(i) = (L(i) - R(i))/(R(i) + L(i))$$

where R(i), L(i) are the number of 30 ms samples of right-hand and left-hand polarization of the ith channel in which an SED signal was detected. An examination of the SED on the basis of polarization showed that episodes 1–5 contained very few clearly polarized events out of many thousands. Post-encounter episodes, however, were generally strongly left-hand circularly polarized. Polarization/frequency plots were produced for each episode. Figure 5 shows the results for the first post-encounter episode. The SED are clearly strongly left-circularly polarized above ~ 15 MHz. Below this frequency, the degree of circular polarization is much less, and the sense is frequency-dependent.

We have previously citch nn on polarization as being evidence of a discharge². Despite the present finding, we still believe that we are observing electrostatic discharges, which are polarized by virtue of either magnetic or curved electric fields, and consequently we have retained the term 'SED'.

In conclusion, some eight characteristics have now been fairly well defined by the Voyager 1 encounter:

- (1) a very flat, broadband frequency spectrum;
- (2) a period of $\sim 10 \text{ h } 10 \text{ min}$;
- (3) a change in the envelope shape of episodes between preand post-encounter;
- (4) an intensity population structure typical of plural populations;
- (5) episodic structure of width ~180°;
- (6) post-encounter episodes continue for about three times as long as pre-encounter ones;
- (7) post-encounter bursts are left-circularly polarized at high frequencies;
- (8) at least one episode shows the onset of high frequency events some time before that of lower frequency ones.

Any theory for the generation of SED should account for all these characteristics.

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- 1. Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731-733 (1981).
- Warwick, J. W. et al. Science 212, 239-243 (1981).
 Lang, G. J. & Peltzer, R. G. IEEE Trans. AES-13, 466-471 (1977).

X-ray and energetic neutral particle emission from Saturn's magnetosphere

E. Kirsch*, S. M. Krimigis†, W. H. Ip* & G. Gloeckler‡

*Max-Planck Institute for Aeronomy, D-3411 Katlenburg-Lindau 3, FRG † Applied Physics Laboratory, The Johns Hopkins University, Laurel, Maryland 20707, USA ‡ Department of Physics and Astronomy, University of Maryland, College Park, Maryland 20742, USA

Although Voyager 1 was not equipped for the detection of X-rays and neutral particles, its low energy charged particle detector (LECP) records suggest a significant flux of these radiations. X-rays could be due to substantial precipitating electron fluxes in the auroral region or the rings whereas energetic neutrals could be due to charge-exchange between trapped ions and Saturn's neutral hydrogen disk.

THE production of auroral X rays and energetic neutral particles is well known in the Earth's magnetosphere (X rays¹; charge exchange energetic neutral particles²⁻⁵) and can be expected at Jupiter and Saturn. In the case of the outer planets, besides

charge exchange with the planetary exospheric neutrals, the magnetospheric particles are also subject to similar loss processes due to interaction with the neutral clouds emitted from satellites⁶.

We present heré observations of the Voyager 1 spacecraft obtained a few days before encounter of the Saturn magnetosphere in November 1980. As Voyager 1 has no special experiment onboard for X-ray and neutral particle detection, we use the sensitivity and directional measurements of the LECP (low energy charged particle) analyser to identify the neutral radiation above the charged particle background.

Method of detection

The method has been outlined earlier and will be summarized only briefly here. The LECP telescope uses a silicon detector of 96.5 μ m thickness and 0.08 cm² area to accumulate counts in eight separate sectors near the ecliptic plane. Electrons 400 keV are magnetically deflected and cannot reach the detector. A stepwise integration of the X-ray sensitivity of the silicon detector for channel PL01 which has an electronic threshold of 26–31 keV, yields an average sensitivity $\bar{\epsilon}_1 \approx 2.38\%/\text{keV}$, and for channel PL02 $\bar{\epsilon}_2 \approx 0.8\%/\text{keV}$ (E=31-60 keV). The detection process for energetic neutral particles is the same as for charged particles: the neutrals become ionized when they hit the detector surface and deposit their energy through ionization energy loss in the detector.

The energy thresholds for neutral or charged particles are:

PL01: 40-53 keV ($\varepsilon \approx 0.4$) PL02: 53-85 keV ($\varepsilon \approx 1$) PL03: 85-139 keV ($\varepsilon \approx 1$)

Figure 1 shows the trajectory of Voyager 1 for days 307-330, 1980 and the viewing directions of the eight sectors of the instrument. It can be seen that X rays and energetic neutrals escaping from the magnetosphere should appear in sector 5. Encounter of the bowshock occurred at ~23:26 UT on day 316 (11 November 1980), thus the days before 316 can be used for our study. Criteria for the identification of neutral radiation during the pre-encounter phase (Fig. 1 and Kirsch et al. unpublished data) can be summarized as follows:

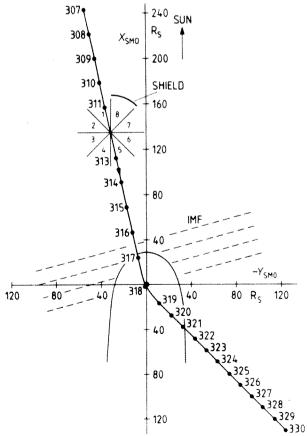


Fig. 1 Projection of the Voyager 1 trajectory on the ecliptic plane (days 307-330, 1980). Indicated are the eight viewing directions of the experiment. Sector 8 is permanently blocked by an aluminum shield.

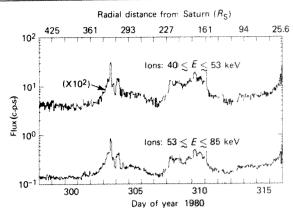


Fig. 2 Scan-averaged measurements of the ion channels PL01 and PL02 for days 298-316, 1980. Interplanetary particle acceleration by Corotating Interaction Regions (CIR) are evident beginning on days 302 and 308.

- (1) The interplanetary magnetic field must be nearly perpendicular to the Saturn-Sun line (in the nominal direction) to exclude charged particles escaping from the planet.
- (2) The pre-encounter measurements must not be disturbed by solar particles or particles accelerated in corotating structures (CIR) in the interplanetary medium.
- (3) Statistically significant enhancements above normal background must appear in the sector which is pointing towards the planet.
- (4) The energy spectrum measured within the sector pointing towards the planet should exhibit differences from spectra measured in the other sectors.
- (5) The additional flux (after subtracting background) must show a $1/R^2$ dependence (R distance from the planet).

The magnetometer data (ref. 10 and N. F. Ness, personal communication) show the interplanetary magnetic field direction to be almost perpendicular to the Saturn-Sun line, which satisfies criterion (1). To investigate the remaining criteria we show in Fig. 2 the scan-averaged particle measurements for days 298-314. Days 298-300 show the lowest count rates, which are probably due to penetrating galactic cosmic rays. Days 301-305 and 308-311 must be excluded due to the presence of ions associated with typical CIRs (see criterion (2)). On days 313-314 a slight increase was observed which is probably due to solar particles. Thus, we shall examine days 298-300, 306-307, 312-316 (~07:00 UT) in the context of criteria (3)-(5) for the presence of neutral radiation.

Observations

Figure 3a shows the sector count rates of channels PL01, 2, 3 for the selected days and the net flux (ΔP) for these channels after the galactic cosmic-ray background was subtracted. Figure 3b shows the count rate ratios before, and Fig. 3c after, subtraction of the galactic background flux (average of days 298, 299 and 300 has been used as galactic background). From day 312 onward sector 5 shows an increase in comparison with the neighbouring sectors which is ~4 times the statistical error. However, similar peaks exist in sector 1 throughout this period, and in sector 7 from day 313 onwards. Because both of these sectors are generally viewing the solar direction (Fig. 1), we attribute their response to low level fluxes of energetic solarinterplanetary particles. The solar background complicates our detection method. As pointed out in Fig. 1, sector 8 is an experiment calibration sector and cannot be used for further diagnostics of the solar particle contribution. Enhanced fluxes in sector 1 for channels PL02, 3 are most likely produced by the $\mathbf{E} \times \mathbf{B}$ drift. The enhanced rate in PL01, sector 1, is due to residual sensitivity to solar X rays, as the Sun was viewed by this sector at this time.

A clue to the identity of the observed fluxes can be obtained by examining the directional energy spectra through the channel ratios. It is evident from the channel ratios presented in Fig. 3b (background included) and especially Fig. 3c (background subtracted) that the additional flux counted in sector 5 has a

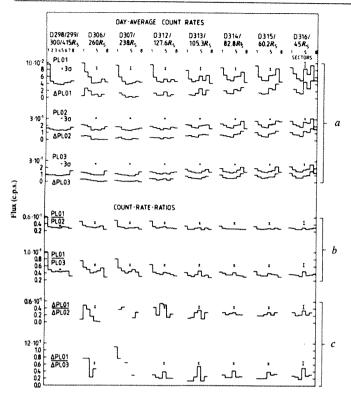


Fig. 3 a, Sector-averaged measurements for the days indicated for channels $PLO_{1,2,3}$. $\Delta PLO_{1,2,3}$ indicates that the galactic background is subtracted. The bars on sector 5 represent 3 times the statistical error. b, The count rate ratios PLO1/PLO2, PLO1/PLO3. c, $\Delta PLO1/\Delta PLO2$, $\Delta PLO1/\Delta PLO3$ (after background subtraction) show that sector 5 measured an additional flux which has a softer spectrum than the flux in neighbouring sectors.

softer spectrum than the flux of the neighboring sectors. As sectors 6, 7, 1, 2 and probably 3 are most likely to contain interplanetary particles, we compare sector 5 to only sector 4, in an attempt to estimate the additional flux in 5, as follows:

$$\Delta P_{1,2,3} = [(S_5 - S_4) - (Sq_5 - Sq_4)]_{1,2,3} \tag{1}$$

where $S_{5,4}$ are sector count rates and Sq_5 , Sq_4 the quiet time sector rates averaged over days 298, 299 and 300. The excess count rates obtained by use of equation (1) are then normalized by multiplication with $(R/45\ R_{\rm S})^2$ and listed in Table 1. The statistical errors have been calculated as

$$(\overline{\sigma}_{1,2,3})^2 = \{ (\overline{\delta S_4})^2 + (\overline{\delta S_5})^2 + (\overline{\delta Sq_4})^2 + (\overline{\delta Sq_5})^2 \}^{1/2}$$
 (2)

where

$$(\overline{\delta S_{4,5}})^2 = \frac{1}{5} \sum_{n=1}^{5} (\delta S_{4,5})_n^2 \left(\frac{R}{45R_S}\right)_n^2$$
 (3)

with n=1 to 5 representing days 312 to 316, 1980, and δSq_4 , δSq_5 are the statistical errors of the quiet days average 298, 299 and 300. The errors in the count rate ratios in Fig. 3b,c shown for each day were calculated using an equation similar to equation (2). Table 1 contains the normalized count rates $\Delta P_{1,2,3}$, the corresponding fluxes and the statistical errors calculated with equation (2). Note that the r.m.s. scatter in the rates for ΔP_1 is larger than the statistical error, and is likely to be due to time variations in this softest part of the spectrum.

The averaged excess count rates in sector 5 shown in Table 1 are statistically significant. The additional counts could be due to planetary or ring X rays, energetic neutrals and/or interplanetary charged particles reflected off the bow shock, so that only upper limits for the neutral radiation can be derived. The averaged X-ray flux j_X can be calculated from the count rate by

$$j_{X} = \frac{\Delta P_{1,2,3}}{A \varepsilon_{X} \Delta E_{X}} \tag{4}$$

where A is detector area, ε_X is X-ray efficiency and ΔE_X is the electronic channel width. Similarly, the neutral particle flux j_n

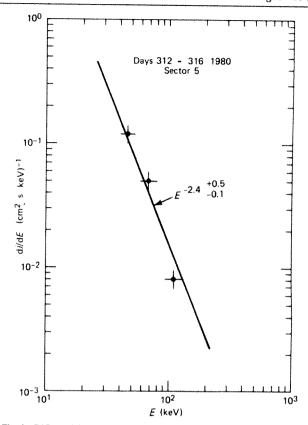


Fig. 4 Differential energy spectrum of the excess radiation, on the assumption that the detector response is due to energetic neutral hydrogen (Table 1).

can be calculated from equation (4), but using the appropriate values for ε and ΔE . The results, normalized to a distance of 45 $R_{\rm S}$ from the planet, are included in Table 1.

Discussion

The significance of the fluxes listed in Table 1 must be examined in the context of processes known or expected to take place in the vicinity of Saturn. Production of X rays can occur in auroral processes recently observed to take place at Saturn¹¹ over a relatively narrow range in latitude, $78^{\circ}-81.5^{\circ}$. If the electron precipitation takes place over the area of the observed UV signature $(A \sim 10^{19} \text{ cm}^2)$, that is, 2% of the surface), then the emitted photon flux at the source is given by

$$j_{\rm X} < \frac{j_{\rm X} 4\pi R^2 \Delta E}{A} = 3.4 \times 10^6 \text{ photons cm}^{-2} \text{ s}^{-1}$$
 (5)

with $\Delta E \sim 7$ keV, $R = 45 R_s$. The corresponding electron flux

Table 1 Excess	rates, fluxes in sect	tor 5 (normalized	to 45 R _s)
Day (normalization)*	$\Delta P_1(\text{c.p.s.})$	$\Delta P_2(\text{c.p.s.})$	$\Delta P_3(\text{c.p.s.})$
312 (8.1) 313 (5.44)	7.89×10^{-2} 7.38×10^{-2}	1.66×10 ⁻¹ 1.69×10 ⁻¹	7.5×10^{-2}
314 (3.4) 315 (1.78)	4.16×10^{-2} 2.80×10^{-2}	1.38×10^{-1} 0.79×10^{-1}	2.4×10^{-2} 2.1×10^{-2}
316 (1.0)	3.43×10^{-2}	0.76×10^{-1}	2.5×10^{-2}
Ave† $(c.p.s.) \pm 1\sigma$	0.051 ± 0.008	0.126 ± 0.014	0.36 ± 0.012
Flux (counts cm ⁻² s ⁻¹)	0.64 ± 0.1	1.57 ± 0.18	0.46 ± 0.15
$j_{\mathbf{x}}(\text{photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1})$	5.4 ± 0.9	6.1 ± 0.7	
j _n (cm ² s keV) ⁻¹ Energy range (keV)	0.12 ± 0.02	0.05 ± 0.01	0.008 ± 0.003
X rays Neutrals/charged	26-31 40-53	31–63 53–85	63–121 85–139

^{*} Factors in parentheses are obtained from $(R/45 R_{\rm S})^2$ and are used to normalize the excess rate to 45 $R_{\rm S}$.

† Averages normalized to 45 Rs.

precipitating into the atmosphere can be estimated by using a conversion efficiency of $\sim 2 \times 10^{-5}$ photons per electron¹²; we find $J \sim 1.7 \times 10^{11}$ electrons cm⁻² s⁻¹ which is some 10³ larger than the maximum fluxes measured by the LECP experiment within the magnetosphere of the planet¹³. Hence, we conclude that the observed upper limit, if interpreted in terms of X rays, cannot be reasonably related to precipitating magnetospheric electrons. The same conclusion is reached even if precipitation is assumed to occur over the entire visible hemisphere.

Another possible X-ray source is the interaction of radiation belt electrons with the rings of Saturn, which extend out to $\sim 2.5 R_s$. The surface area of the rings on the sunlit side is

$$S = (2.5 R_s)^2 \pi - (1 R_s)^2 \pi = 6 \times 10^{20} \text{ cm}^2$$
 (6)

If we assume -1% of the area to be filled with matter, the estimated X-ray production in the rings in the energy range 31-63 keV (PL02), is

$$J_{\rm X} = \frac{6.1 \times 4\pi (43 R_{\rm S})^2 \times 32}{6 \times 10^{18}} = 2.7 \times 10^7 \,\text{photons cm}^{-2} \,\text{s}^{-1}$$
 (7)

The corresponding electron precipitation is $\sim 1.4 \times 10^{12} \, \text{cm}^{-2}$ s^{-1} , again $\sim 10^4$ larger than the maximum flux measured by the same instrument in the magnetosphere with this channel¹³. Note that there is a sharp cutoff in particle fluxes at the edge of ring A, as shown by Pioneer 1114

The Voyager 1 UV experiment¹¹ found that Saturn is surrounded by a torus of neutral hydrogen with a concentration $n_{\rm H} \approx 10 {\rm cm}^{-3}$. The torus extends from $\sim 25 R_{\rm S}$ to $\sim 8 R_{\rm S}$ and its thickness could be $\sim 6 R_s$. Thus charge exchange processes between radiation belt protons and the neutral hydrogen concentration can be expected. For a flux estimation we use the cross-sections given by Tinsley⁵. The product σv (crosssection × velocity of the particles) is as follows for the lowest three channels

PL01:
$$(40-53 \text{ keV})$$
: $\sigma v_{H}^{+} \approx 3 \times 10^{-8} \text{ cm}^{3} \text{ s}^{-1}$

PL02:
$$(53-85 \text{ keV})$$
: $\sigma v_{H}^{+} \approx 1.4 \times 10^{-8} \text{ cm}^{3} \text{ s}^{-1}$

PL03:
$$(85-139 \text{ keV})$$
: $\sigma v_{H}^{+} \approx 3 \times 10^{-9} \text{ cm}^{3} \text{ s}^{-1}$

The lifetime against charge exchange is then

PL01:
$$\tau_1 = (n_H \sigma v)^{-1} = (10 \times 3 \times 10^{-8})^{-1} = 3.33 \times 10^6 \text{ s}$$

PL02:
$$\tau_2 = (n_H \sigma v)^{-1} = (10 \times 1.4 \times 10^{-8})^{-1} = 7.14 \times 10^6 \text{ s}$$

PL03:
$$\tau_3 = (n_H \sigma v)^{-1} = (10 \times 3 \times 10^{-9})^{-1} = 3.33 \times 10^7 \text{ s}$$

The LECP instrument measured an average density outside the orbit of Rhea at $E \ge 40 \text{ keV}$ of $\sim 5 \times 10^{-3} \text{ cm}^{-3}$ (ref. 13). Taking the neutral hydrogen cloud volume of $\sim 2 \times 10^{33}$ cm³ (ref. 11) we can estimate the total energetic ion content as

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- Kremser, G. Scientific Ballooning (ed. Riedler, W.) 161 (Pergamon, Oxford, 1979). Moritz, J., J. geophys. Res. 38, 701 (1972). Hovestadt, D., Haeusler, B. & Scholer, M. Phys. Rev. Lett. 28, 1340 (1972).

- Blake, J. B. J. geophys. Res. 81, 6189 (1976). Tinsley, B. A. J. geophys. Res. 81, 6193 (1976).

- Cheng, A. F. Astrophys. J. 242, 812 (1980).
 Kirsch, E., Krimigis, S. M., Kohl, J. W. & Keath, E. P. Geophys. Res. Lett. 8, 169 (1981)

$$N = nV = 5 \times 10^{-3} \text{ cm}^{-3} \times 2 \times 10^{33} \text{ cm}^{3} = 10^{31}$$

The total energetic neutral particle production is given by

$$\dot{N} = \frac{nV}{\tau} = \frac{10^{31}}{10^7} = 10^{24} \text{ neutrals s}^{-1}$$

The loss rate may be obtained by estimating the total surface area of the neutral hydrogen disk,

$$A \simeq 2\pi [(25 R_S)^2 - (8 R_S)^2] \simeq 1.3 \times 10^{23} \text{ cm}^2$$

Thus energetic $(E \ge 40 \text{ keV})$ neutrals are lost at a rate

$$\frac{\dot{N}}{A} = \frac{10^{24}}{1.3 \times 10^{23}} \sim 8 \text{ cm}^{-2} \text{ s}^{-1}$$

The flux at 45 R_s would be decreased by a factor $\sim (20/45)^2 =$ 0.2, that is, N/A would be ~ 1.6 cm⁻² s

We now need to compare the expected flux at $45 R_s$ with the observations as summarized in Table 1. The differential flux from Table 1 has been plotted in Fig. 4; the form of the spectrum can be described by a power law of the form $E^{-\gamma}$ with $\gamma \sim 2.4$. To obtain the total flux we evaluate the integral

$$J = \int_{40 \,\mathrm{keV}}^{\infty} KE^{-2.4} \,\mathrm{d}E$$

and find $J \sim 4.3 \text{ cm}^{-2} \text{ s}^{-1}$. This value compares well with the expected value of $\sim 1.6 \text{ cm}^{-2} \text{ s}^{-1}$ derived above.

We have demonstrated the existence of radiation above the detector background emanating from the vicinity of Saturn, upstream from the bow shock. The excess counts are most plausibly attributed to neutral radiation, that is, X rays and/or neutral energetic particles. Estimates of the number of energetic electrons necessary to produce the observed counts by precipitation in either the saturnian auroral regions or the rings are too high by factors $\sim 10^3 - 10^4$ over electron intensities observed within the magnetosphere of Saturn. Thus the observed excess counts are probably not due to X rays even if satellite surfaces are considered as possible X ray sources. We conclude that charge exchange of energetic ions with satellite tori is an important loss mechanism at Saturn as well as at Jupiter15

The efforts of many at APL/JHU, the Universities of Maryland and Kansas and Bell Laboratories contributed to this investigation. We thank our colleagues on the LECP team: T. P. Armstrong, W. I. Axford, C. O. Bostrom, E. P. Keath and L. J. Lanzerotti for various contributions, J. F. Carbary for assistance with graphics, and especially L. J. Lanzerotti for useful comments. This research was supported in part by NASA under Contract N00024-78-C-5384 between The Johns Hopkins University and the Department of the Navy, and under subcontract to the University of Maryland.

- Krimigis, S. M. et al. Space Sci. Rev. 21, 329 (1977)
 Bridge, H. S. et al. Science 212, 217 (1981).
- 10. Ness, N. F. et al. Science 212, 211 (1981)
- Ness, N. F. et al. Science 212, 211 (1981).
 Broadfoot, A. L. et al. Science 212, 206 (1981).
 Evans, R. D. The Atomic Nucleus 619 (McGraw-Hill, New York, 1955).
- Krimigis, S. M. et al. Science 212, 225 (1981).
 Van Allen, J. A., Thomsen, M. F., Randali, B. A., Rairden, R. L. & Grosskreutz, C. L. Science 207, 415 (1980).
- Cornwall, J. M. J. geophys. Res. 77, 1756 (1972).

Topology of Saturn's main magnetic field

M. H. Acuña, J. E. P. Connerney & N. F. Ness

NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Greenbelt, Maryland 20771, USA

The Voyager 1 magnetic field observations at Saturn confirm the principally dipolar topology of the planetary magnetic field and suggest the need for more general models which incorporate non-potential field sources external to the planet and within the planetary magnetosphere.

THE close flyby of Saturn at 3.07 R_s (1 $R_s = 60.330$ km) by Voyager 1 in November 1980 provided a second opportunity to study in situ the intrinsic planetary magnetic field. The first detection of the Saturnian field was accomplished by experiments aboard Pioneer 11 during its close flyby of Saturn at $1.35 R_s$ in September 1979^{1-4} . The most surprising characteristic derived from these magnetic field measurements was the near-alignment of the magnetic axis with the rotational axis of the planet. A small but non-zero axial displacement with respect to the planet's centre was also obtained. These features and the purely dipolar nature of the planetary field have significant implications for the general problem of the generation of magnetic fields by massive rotating objects.

The detection of Saturn at kilometric and hectometric wavelengths by the Voyager planetary radio astronomy experiment and identification of periodicities at 10:66 indicate that emissions originate from a restricted range of planetcentred longitudes⁵. This constitutes a paradox in terms of what would be intuitively expected from an axisymmetrical magnetic field, assuming that the emissions originate near the feet of the field lines. A more detailed analysis of the data obtained by Voyager 1 during its encounter with Saturn is presented here, with the emphasis on the uncertainties associated with the determination of model parameters describing the main magnetic field. The results of a preliminary analysis use data available immediately after the time of the encounter. Since then determinations of spacecraft attitude, position and alignment of the magnetometer sensors with respect to the main body of the spacecraft have been made, which can have a direct impact on the models derived from the observations. The independent results obtained from Voyager 1 allow a more general interpretation of Pioneer 11 observations and provide an insight into the phenomena taking place in the saturnian magnetosphere.

The flyby trajectories for Pioneer 11 and Voyager 1 were such that different ranges of planet-centred latitudes, longitudes and corresponding radial distances were sampled during each encounter. These characteristics are illustrated in Fig. 1 where the sub-spacecraft latitude and longitude for each trajectory during the closest approach phase are shown. The planetocentric radial distance to each spacecraft is indicated as well as the spacecraft event time (SCET) for Voyager 1. Figure 1 shows that the Pioneer 11 trajectory is largely equatorial, remaining within $\pm 6^{\circ}$ of the planetographic equator for $r < 8 R_s$.

In contrast, Voyager 1 approached Saturn from the south, reaching a maximum latitude of -41° at a radial distance of $3.14 R_s$ just before periapsis at $3.07 R_s$. Voyager 1 crossed Saturn's equatorial plane into the northern hemisphere at $6.3 R_s$ while outbound from periapsis. The larger latitude coverage of Voyager 1 is important to the modelling process, primarily because it allows a more accurate determination of hemispherical asymmetries, such as the polar offset derived from the Pioneer measurements^{2,4}. Note also in Fig. 1 the narrow range of planet centred longitudes sampled by Pioneer 11 inside $6 R_s$ ($\sim 120^{\circ}$). Voyager 1, in addition to the greater

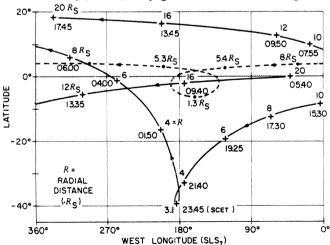


Fig. 1 The close flyby trajectories of Voyager 1 (solid line) and Pioneer 11 (dashed line) in Kronographic coordinates. The locus of sub-spacecraft points in SLS-longitude and latitude is shown. Voyager 1 crossed the equatorial plane from north to south at $20~R_{\rm S}$, reaching a maximum latitude of -40° . In contrast the Pioneer 11 trajectory remained within $\pm 6^{\circ}$ of the equator. SCET, spacecraft event time.

latitude coverage, extends this range to $\sim 135^{\circ}$, although it remains entirely in the southern hemisphere within the same radial distance range.

The analysis of magnetic field observations along a spacecraft flyby trajectory has been described elsewhere and presents several problems related to the uniqueness and uncertainties of parameters in the mathematical models derived from the measurements⁷⁻⁹. The usual assumption made is that the observations are performed in a region that is current-free, that is, external (or internal) to the sources of the measured magnetic field $(\nabla \times \vec{B} = \mu_0 \vec{J} = 0)$. In these conditions, the field can be represented as the gradient of a scalar potential function V, as $\vec{B} = -\nabla V$. The scalar potential has traditionally been represented by means of spherical harmonic functions with suitably normalized coefficients¹⁰. Two potential functions are normally used, one representing sources which reside in the interior of the planet and a second one which accounts for sources outside the region where the measurements are obtained. This approach was used in the preliminary report on the Voyager 1 magnetic field observations at Saturn⁶. The condition of $\nabla \times \vec{B} = \mu_0 \vec{J} = 0$ is reasonably well satisfied within the radial range $R < 6R_s$, 48-s averages of magnetic field data were used to derive a leastsquares model fit to the observations using an interior, centred, tilted dipole and an exterior uniform field (I1E1 spherical harmonic model with internal and external components both first order). These results yielded the Schmidt normalized coefficients reported by Ness et al.⁶, that is: $g_1^0 = 0.209 \,\text{G}$, $g_1^1 = 0.00220 \text{ G}, h_1^1 = 0.00124 \text{ G}; \text{ external } G_1^0 = -8.8 \times 10^{-5} \text{ G},$ $G_1^1 = -4.2 \times 10^{-5}$ G and $H_1^1 = 3.3 \times 10^{-5}$ G. These results agree well with those reported by the Pioneer 11 investigators 1-4. With respect to the dipole displacement from the centre of the planet, the preliminary Voyager 1 studies placed an upper limit of $0.02 R_{\rm S}$ to any possible offset. The most significant new results were that the 0.04 R_s northward offset derived from the Pioneer 11 measurements is not reproduced in the Voyager 1 models, and that a definite dipole tilt of $\sim 0.7^{\circ}$ at a SLS¹¹ longitude of 331° was obtained. Note, however, the assumptions made in the model: current-free observations as well as a uniform external field.

Additional studies of the magnetic field data obtained during the traverse of the middle magnetosphere of Saturn (6 $R_s \le R \le 16 R_s$) have led Connerney et al.¹² to conclude that Saturn is surrounded by an eastward-flowing ring current which extends from 8.5 to $15.5 R_S$ in the equatorial plane and with a total thickness of $\sim 5R_s$. The current density in this sheet decreases proportionally to distance from the planet and is an order of magnitude smaller than that found at Jupiter. A more realistic magnetic field model for the analysis of Saturn's main field in which the distributed ring currents are explicitly modelled is constructed as follows: an I1E1 model is first least-squares fitted to the observations obtained within $\sim 6 R_S$ of close approach. These internal field parameters constitute a first approximation to Saturn's main field: the external terms (uniform field) approximate the field due to magnetospheric currents. The internal field is then subtracted from observations obtained within $\sim 30 R_s$ and the resulting external field is modelled in terms of an azimuthal ring current. The (solenoidal) field of the ring currents is then adopted as a more accurate external field representation, and assumed in a least-squares fit to determine new internal field coefficients (I1E0).

This procedure does not necessarily converge to a unique set of model parameters and each iteration step must be guided by appropriate physical constraints as well as results from correlative investigations. The results of such a study are given in Table 1. As done previously, 48 s-averaged field values obtained for $R < 8R_s$ have been used. Two weighting schemes have been used, one in which uniform weights are attached to the measurements $(\omega_i = 1)$ and a second one in which the weights have been chosen inversely proportional to the magnitude of the measured field $(\omega_i \propto [0.01 \times |\vec{B}_i|]^{-1})$. The first is appropriate for observations assuming a uniform (random) noise level and the second for observations assuming a noise component which is

	Table 1 S	aturn's magnetic	field models from Voy	ager 1 observations		
Parameter	Case	1 I1E1*	2 I1E1	3 I1+Ring curr.†	4 I1E1	5 I1+Ring curr.†
. g°		20,900	21,121	20,926	20,940	20,966
\mathbf{g}_{1}^{1} (nT)		220	324	401	230	276
A		124	43	152	170	191
G_1^0		-8.8	-11	 .	-9	
$G_1^{!}$ (nT)		-4.2	-1		-3	
H_1^1		3 3	-1	_	1	
r.m.s. residual (nT)		2.7	2 0	1.9	2.7	2.8
Dipole tilt, 80		0.7°	0.89°	1.17*	0.78*	0.92*
Longitude, Ap [SLS]		331°	352°	339°	324°	` 325°
Radial distance range of data	•	<6R.	<8R _s	<8R _s	<8R	<8 <i>R</i> _a
Weights		1 -	$[.01 \times R]^{-1}$	$1.01 \times B^{-1}$	1	1

^{*} Ref. 6.

proportional to the local field magnitude. The derived tilt angles and longitude of the tilt are also indicated in Table 1. The r.m.s. of the residuals of the model fits show that all these models fit the observations very well. As discussed by Ness et al.⁶, second-order (quadrupole) models do not provide a significant reduction in the residuals. The implication of this is that the derived offset of the dipole from the centre of the planet is small and consistent with zero to the level that can be ascertained from the magnetic field measurements alone. Note from Table 1 that models incorporating the field due to the ring current explicitly, rather than the I1E1 models which represent this field as uniform, do not provide a significant reduction in the r.m.s. of the residuals. This is due to the model residuals being dominated by a localized and presumably non-potential feature unrelated to the ring current or internal field.

We now illustrate the residuals in the three orthogonal field components, ΔB_R , ΔB_{\bullet} , ΔB_{\bullet} after the internal field model plus that due to the modelled ring current are subtracted from the observations. This is shown in Fig. 2 for case 5 in Table 1. This particular model was chosen primarily for convenience. In general, the magnitude of the residuals is small, <10 nT. Some still unresolved engineering and attitude determination problems are evident in Fig. 2. The 'disturbance' at 00:43 UT and extending until ~01:00 is associated with a spacecraft roll manoeuvre and the lack of sufficiently accurate knowledge of spacecraft and therefore magnetometer sensor orientation during the manoeuvre. The sensor alignment with respect to the spacecraft reference axis was determined by means of an onboard calibration coil¹³ and the analysis of spacecraft manocuvres in steady-state fields during both cruise and planetary encounters at Jupiter and Saturn. This orientation is known to better than ±0.2°. Also evident in Fig. 2 are small discontinuities associated with instrument range changes, the updating and interpolation of spacecraft attitude matrices and analogue-todigital converter digitization uncertainties. In addition, the spacecraft limit cycle (±0.06°), which is unmodelled, contributes to the observed noise where the ambient field is large (1 nT in 103 nT).

The largest contribution to the residuals occurs between 21:00 on day 317 and 05:00 on day 318, particularly in the radial component, ΔB_R . An additional feature can be seen superimposed on ΔB_R and ΔB_{θ} curves occurring between 22:30 on day 317 and 01:00 on day 318. This quasi-abrupt enhancement of the residuals seems to be associated with a magnetospheric current system quite distinct from the azimuthal current system already modelled. This feature was observed as Voyager 1 passed through the dusk sector of Saturn's magnetosphere, nearly coincident with closest approach. In planetocentric coordinates, the spacecraft was moving primarily in latitude at this time (Fig. 1), actually reversing momentarily its westward motion, as viewed from Saturn.

The residuals of internal magnetic field models are dominated by a highly correlated, very localized and presumably nonpotential field contribution near periapsis. Thus the current representations of the field are not sufficient to describe the observed field to better than 10 nT. This includes models explicitly incorporating distributed azimuthal currents as well as potential field models. Note that the residuals illustrated in Fig. 2 are evidence of local currents but the plotted field components are not the magnetic field signature of such currents. The plotted residuals are only that part of the observed field that cannot be expressed with the limited models available. Certainly some part of the field contributed by local currents has been absorbed by the internal field coefficients and therefore does not appear in the residual plot. The problem of separating the different contributions to the observed field due to the planetary field, the ring current, and the other local currents is the primary challenge to be met in obtaining better estimates of Saturn's internal field. To do so requires a model of the inner magnetosphere in which the very localized currents are explicitly taken into account.

Several model current systems that might be expected on physical grounds to be active in this region of Saturn's magnetosphere are being considered. For example: (1) fieldaligned currents associated with the interaction of Saturn's corotating magnetosphere with a satellite, or particle ring, reminiscent of the currents linking Jupiter and its satellite Io, as observed by Voyager 114. The most promising candidates here seem to be the E-ring and the satellite Dione. Voyager passed \sim 20,000 km north of the field line linking Dione at \sim 00:10, day 318, as it traversed the dusk sector. (2) A dawn-dusk system of Birkeland currents driven by the solar wind-induced asymmetry. (3) A system of Birkeland currents driven by a nonazimuthally symmetric torus or an as yet unidentified surface magnetic anomaly similar to that proposed for Jupiter12 enhancement of high energy (1-2 keV) electron fluxes observed by the Voyager 1 plasma experiment in the dusk sector,

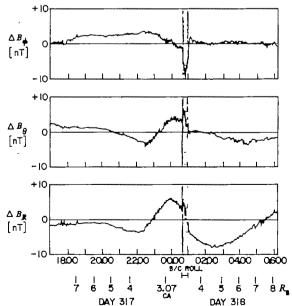


Fig. 2 Plot of the component residuals, ΔB_{π} , ΔB_{ϕ} and ΔB_{ϕ} obtained after subtracting a dipole model plus the field from the ring current as determined from the Voyager 1 observations. The abrupt enhancement observed between 22.30 and 01.00 h of day 318 is interpreted as evidence for a localized current system flowing in the inner saturnian magnetosphere.

[†] Ring current model of Connerney et al. 12

concomitant with the magnetic field signature, has been described by Sittler et al16. As additional correlative observations become available, we hope to offer a more detailed model.

Of the models in Table 1, cases 2 and 3, derived using weights inversely proportional to the local field magnitude, minimize the effect of the localized current system encountered near periapsis. This unmodelled 10-nT perturbation is less likely to affect significantly the internal field coefficients in the weighted leastsquares fits compared with the unweighted cases, as larger errors are tolerated in higher fields. Case 3 in Table 1 is regarded as best representing the global characteristics of the saturnian field, as deduced from Voyager 1 observations alone.

To estimate model parameter uncertainties we need to assess the impact on the internal field coefficients of the unmodelled current system. Lacking a detailed model, we can estimate the parameter uncertainties by comparing models relatively unaffected by the currents (weighted fits) with those that are more strongly dependent on the residuals near closest approach (unweighted fits). Such a comparison suggests a dipole moment of 0.21 ± 0.005 G $-R_s^3$, tilted $1.0 \pm 0.3^\circ$ towards an SLS longitude of $340^{\circ} \pm 20^{\circ}$. The northward displacement of the dipole by 0.04 R_s deduced from the Pioneer 11 observations is not

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- Acuña, M. H. et al. Science 207, 444-446 (1980).
- Smith, E. J. et al. Science 207, 407-410 (1980).
- Acuña, M. H. et al. J. geophys. Res. 85, 5675-5678 (1980). Smith, E. J. et al. J. geophys. Res. 85, 5655-5674 (1980).
- Kaiser, M. L. et al. Science 209, 1238-1240 (1980). Ness, N. F. et al. Science 212, 211-217 (1981).
- Acuña, M. H. & Ness, N. F. in Magnetospheric Particles and Fields (ed. McCormack, B. M.) 311-323 (Reidel, Dordrecht, 1976).
- Davis, L. Jr & Smith, E. J. in Magnetospheric Particles and Flelds (ed. McCormack, B. M.) 301-310 (Reidel, Dordrecht, 1976)

substantiated by the Voyager 1 observations, although we must take into account that the near-equatorial trajectory of Pioneer 11 was poorly suited for detecting hemispherical asymmetries. Voyager 2 will fly by Saturn in August 1981; its close encounter trajectory will provide a latitude coverage of $\sim \pm 30^{\circ}$ within 8 R_s and facilitate a more accurate estimate of a possible dipole polar

Certain aspects of Saturn's main magnetic field relevant to the planet's interior have been discussed by Stevenson¹⁷. In particular, the unexpectedly small dipole moment seems to be consistent with the gravitational settling of helium, which leads to a much smaller electrically conducting and convecting region than would be expected of a homogeneous distribution of hydrogen and helium. The IR studies by Voyager 1 have demonstrated such an atmospheric depletion of helium, obtaining an 11% helium mass fraction at Saturn as compared with the 19% observed at Jupiter and expected on a cosmochemical basis¹⁸. The near alignment of Saturn's magnetic and rotational axes is likewise consistent with the differential rotation of the conducting core in Stevenson's model of Saturn's interior¹

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- 9. Connerney, J. E. P. J. geophys. Res. (in the press).
- Chapman, S. J. & Bartels, J. Geomagnetism (Oxford University Press, 1940).

 Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 253–256 (1981).

 Connerney, J. E. P. et al. Nature 292, 724–726 (1981).

 Behannon, K. W. et al. Space Sci. Rev. 21, 235–257 (1977).

- Acuña, M. H. et al. J. geophys. Res. (in the press).

 Dessler, A. J. & Vasyliunas, V. M. Geophys. Res. Lett. 6, 37-40 (1979)
- Sittler, J. C., Scudder, J. C. & Bridge, H. S. Nature 292, 711-714 (1981). Stevenson, D. J. Science 208, 746-747 (1980).
- 18. Hanel, R. et al. Science 212, 192-200 (1981)

Saturn's ring current and inner magnetosphere

J. E. P. Connerney, M. H. Acuña & N. F. Ness

NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Greenbelt, Maryland 20771, USA

The Voyager 1 magnetic field observations at Saturn reveal an equatorial system of (eastward) azimuthal currents, very similar in certain respects to that responsible for the jovian magnetodisk.

IN November 1980, Voyager 1 became the second spacecraft to obtain in situ observations of the magnetosphere of Saturn: little more than a year earlier, the Pioneer 11 spacecraft returned the first evidence of Saturn's magnetosphere. Analyses of the Pioneer 11 magnetic field observations^{1,2} revealed a dipolar planetary magnetic field of moment $0.21 \,\mathrm{G} - R_{\mathrm{S}}^{3}$, rather less than that expected on the basis of scaling laws^{3,4}. [The units $G-R_s^3$ are dimensionally equivalent to standard units of magnetic dipole moment, the numerical value having been divided by the cube of the planetary radius.] More surprising was the near alignment of Saturn's rotational and magnetic dipole axes, a unique characteristic among the known planetary dynamos and therefore of central interest in dynamo theory5. A preliminary estimate of 0.7° angular separation of Saturn's rotational and magnetic dipole axes has recently been derived from the Voyager 1 magnetic field observations⁶

Saturn's lack of an appreciable dipole tilt results in an observational limitation insofar as studies of magnetospheric phenomena in situ by spacecraft are involved. Figure 1 shows the trajectories of each spacecraft to encounter Saturn (Pioneer 11 (P11) in September 1979; Voyager 1 (V1) in November 1980; and Voyager 2 (V2) in August 1981) in a cylindrical (ρ, z) planet centred coordinate system, which in this case is practically identical to the magnetic equatorial coordinate system. Each spacecraft remains essentially at constant magnetic latitude for a large percentage of the encounter, with only one (V2) or two

(V1, P11) crossings of the magnetic equator. Particularly evident is the rather singular nature of Pioneer 11's trajectory, which traced a nearly identical path outbound from periapsis as inbound. In contrast to the periodic variations in the magnetic

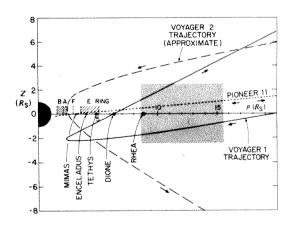
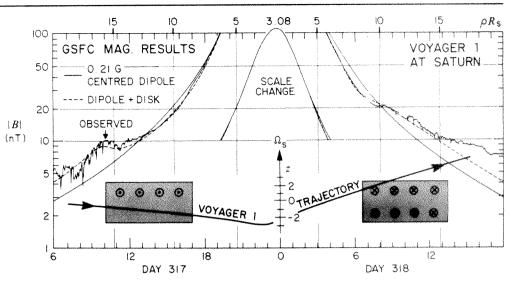


Fig. 1 Trajectories of Pioneer 11, Voyager 1 and Voyager 2 at Saturn in a cylindrical planetocentric equatorial coordinate system. Positions of the major satellites and rings are indicated; satellite diameters are exaggerated by a factor of 10. Stippled region is the region of (model) distributed currents in Saturn's magnetosphere.

Fig. 2 Magnitude of observed magnetic field within $\sim 20~R_{\rm S}$ of Saturn and comparison with an internal dipole model (solid line) and a model containing a dipole and distributed ring currents (dashed line) discussed in test. The Voyager 1 trajectory is illustrated beneath these observations, showing distance from the equator as a function of time. Note the different scale used for vertical dimension of trajectory insert.



field and charged particle environment observed in Jupiter's magnetosphere concomitant with the regular variation of magnetic latitude due to that planet's rotation and dipole tilt (9.6°) , at Saturn, the observations reflect more the systematic co-variation of ρ and z.

The Voyager 1 magnetic field observations at Saturn are shown in Fig. 2, in which the magnitude of the observed magnetic field is compared with that of a 0.21 G - R_s^3 centred dipole. The departure from a dipole is noticeable during the approach to periapsis at radial distances exceeding $\sim 8 R_{\rm s}$. whereas outbound the differences are noticeable at radial distances in excess of $\sim 5R_S$. The observed field magnitude is appreciably less than that of the model dipole at small radial distances ($\rho < \sim 10R_s$) and greater than the model dipole in the more distant magnetosphere. These characteristics can be understood by introducing a model current system similar to that originally applied to observations of the jovian magnetodisk⁷. In this model, large-scale azimuthal currents flow eastward in an annular disk extending from $8.5 R_s$ to $15.5 R_s$ in Saturn's equatorial plane. The current is distributed uniformly in \hat{z} over the total disk thickness of $5R_s$ and decreases with radial distance from Saturn as $1/\rho$. The physical dimensions of this annulus roughly correspond to those of the 'extended plasma sheet' observed by the Voyager 1 plasma experiment⁸. Because the radial extent of the disk, $7 R_s$, is almost equal to the thickness, and considering the radial decrease in model current density, we refer to this phenomenon as a ring current rather than a magnetodisk to distinguish it from the more two-dimensional geometry of the jovian azimuthal currents. The model illustrated in Fig. 2 has a current density of $\sim 0.3 \times 10^6 \text{ A}/R_S^2$ $(\sim 0.1 \text{ mA km}^{-2})$ at the inner edge of the annulus, decreasing to $\sim 0.15 \times 10^6 \text{ A/R}_s^2$ at its outer edge. These current densities are about an order of magnitude less than those observed at Jupiter.

The derived model is a result of a vector fit to the Voyager observations and self-consistently satisfies both of the observed radial and vertical field components. The magnetic field of this equatorial system of currents is largely solenoidal. The near axis field is essentially vertical and oppositely directed to Saturn's (equatorial) main field; at larger distances the two add. Above the plane of symmetry of the distributed current system, the radial field is positive outward and below the equator, positive inward, increasing approximately linearly with distance, Z, from the equatorial plane for Z < D. Thus the field lines are radially stretched in a manner reminiscent of the stretching or drawing out of field lines in the near equatorial regions of the jovian magnetodisk.

The total azimuthal current, and the distribution of the current in $\hat{\rho}$, is inferred from the radial dependence of the external vertical field component. The external field is estimated by subtracting from the observations a best-fitting dipolar

 $(0.21~{\rm G-R_s^3})$ internal field. The distribution of that current in \hat{z} is inferred from the variation of the radial component of the external field. While the Voyager trajectory at Saturn did not permit numerous traversals of the current-carrying region as at Jupiter, it did provide observations within the distributed currents sufficient to allow the localization of source currents in Saturn's magnetosphere. An earlier model⁶, characterized by currents confined to an annular disk of radial extent 8-16 R_s , with a total thickness of $4R_s$, fits the observations about as well if the current density is increased such that the linear current density in Saturn's equatorial plane remains unchanged.

The near-axis field of the azimuthal currents is ~ 10 nT, which agrees well with the external field inferred from spherical harmonic analysis 6.9 of the Voyager 1 encounter observations. The field of this model current system reaches a maximum (12.5 nT) along the trajectory as Voyager 1 approaches the inner edge of the annulus at hour 6, day 318 while outbound from periapsis. The field enhancement observed as Voyager 1 approached the outer edge of the annulus at 15.5 $R_{\rm s}$ while inbound to periapsis is understood as an edge effect. The lack of such a localized enhancement of the modelled field during the outbound traversal is a simple consequence of Voyager 1's higher latitude at this time. Beyond 15 $R_{\rm s}$ outbound the discrepancy between the model field and the observations grows as the contribution of unmodelled cross-tail currents 10 to the total field grows.

A model of Saturn's magnetosphere, constructed by superposition of the dipolar $0.21 G - R_s^3$ internal field and that of the model ring currents, is compared in Fig. 3 with a dipolar magnetic field. The radial stretching of field lines in the equatorial plane and the non-dipolar nature of the model field is evident at radial distances exceeding $5 R_s$. The global character of the field due to the azimuthal currents ensures a departure from a dipolar magnetic field configuration at high latitudes as well. This axisymmetric model magnetosphere neglects the

Table 1 Times at which Voyager 1 encountered field lines which pass through the equator at a radial distance occupied by a known absorber

	Radial	(Nearest minute)					
Feature	distance	Model MS			Dipole		
Inbound		Day	h	min	h	min	
Rhea	8.75	317	17	26	17	7	
Dione	6.28	317	20	32	20	17	
E Ring Outer Edge	5.0	318	0	7	0	2	
Tethys	4.92	318	0	11	0	7	
Outbound							
Tethys	4.92	318	2	45	2	46	
E Ring Outer Edge	5.0	318	2	52	2	53	
Dione	6.28	318	4	18	4	18	
Rhea	8.75	318	6	26	6	35	

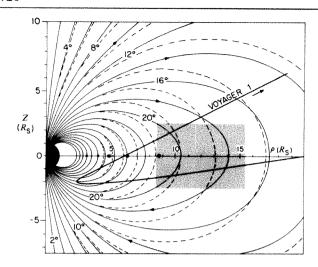


Fig. 3 Meridian plane projection of magnetosphere field lines for a dipolar field model (dashed) and a model containing a dipole and distributed ring currents (solid) discussed in the text. Field lines are drawn for 2° increments in co-latitude. Positions of the major satellites Mimas, Enceladus, Tethys, Dione, and Rhea are as in Fig. 1.

contribution of magnetopause and tail current systems driven by the solar wind interaction which become non-negligible in the more distant magnetosphere ($R > 15 R_s$).

Saturn's ring current has important implications for chargedparticle motion in Saturn's magnetosphere, particularly the absorption of trapped radiation by its many satellites and rings. Satellite absorption is conceptually simple: all charged particles that, in the course of their mirroring or latitudinal bounce motion, intersect an absorber are removed from the local particle population. In practice, charged particle absorption signatures associated with satellite sweeping are energy and species dependent, reflecting particle drift motion and finite gyroradius effects as well as particle diffusion. Thus, a complete description of charged particle motion relevant to satellite absorption requires a knowledge of the magnetic field line geometry and field gradient and curvature drifts over the particle's bounce path.

The absorption signature observed by the Voyager 1 cosmic ray experiment¹¹ near the orbital position of Rhea illustrates well the effects of Saturn's ring current on charged particle trajectories. Indeed, the premature occurrence of the absorption signature observed by Voyager 1 outbound in the vicinity of Rhea was cited as evidence of non-dipolar deformations of Saturn's post-midnight magnetosphere¹¹. Given the model magnetosphere deduced from the magnetic field observations, we have traced field lines from the Voyager trajectory to the equatorial plane to arrive at the results presented in Table 1. times at which Voyager 1 encountered field lines which pass through the equator at a radial distance occupied by a known absorber. Also listed are the corresponding times assuming a dipolar model magnetosphere. The actual time at which the Rhea absorption signature was observed was $\sim 06:25$, day 318, which agrees very well with the model results presented here and ·10 min before that expected in a dipolar field.

The Rhea absorption signature was observed while Voyager 1 passed ~4° east of Rhea's orbital position. Magnetospheric plasma corotates from Rhea to Voyager 1 in ~7 min; in the

corotating reference frame protons drift eastward, in the corotational direction, and electrons drift westward. Observation of an absorption signature in the 0.6-MeV electron data11 was interpreted as evidence that the detector was responding to lower energy electrons, as electrons of energy >0.57 MeV (60° pitch angles) have drifted sufficiently in a dipole field that they trail Rhea in its orbit about Saturn. While we have not performed the required bounce-averaged gradient and curvature drift computation using our model field, we note that in the vicinity of Rhea's orbit Saturn's magnetosphere is sufficiently non-dipolar so as to cast doubt on longitudinal drifts computed with a dipolar field. The equatorial field gradient at Rhea's orbital distance is only ~0.6 that of a dipole field, and the local field magnitude is also ~ 0.66 that of a $0.21 \,\mathrm{G} - R_5^3$ dipole. Particles of a particular energy with 90° pitch angles will thus drift with ~50% greater velocity than identical particles in a dipole field. In addition, the variation of the longitudinal drift rate with pitch angle is greater in the ring current model field than in a purely dipole field. Finally, we note that pitch angles derived assuming a dipole field geometry can be substantially in error (~20°) in the region of the magnetosphere where the Rhea absorption feature was observed. We conclude that in the cosmic ray experiments the 0.6 MeV detector may well have been responding to electrons with energies >0.6 MeV, as designed, and not to electrons below the nominal detection threshold of that instrument.

The total integrated ring current in Saturn's magnetosphere is $\sim 3 \times 10^6$ A, only a few per cent of the integrated azimuthal current flowing in the jovian magnetodisk. The near axis field of Saturn's ring current is ~ 10 nT; the ratio of this quantity, $B_c(0)$. to Saturn's equatorial dipole field, B_e , is $\approx 4.8 \times 10^{-4}$. The same quantity computed from a model of Jupiter's azimuthal disk currents⁷ using $B_r(0) \sim 200 \text{ nT}$ and $B_e = 4.2 \text{ G}$, is also $\sim 4.8 \times$ 10⁻⁴. This implies^{12,13} that the total kinetic energy of trapped particles in the magnetosphere (E) scales with the available dipole magnetic energy outside the planet's surface (U). We note that while the ratio E/U is the same for both of the giant planets, Saturn's total E is little more than 0.15% of Jupiter's. The same ratio, applied to the Earth's magnetosphere (assuming $B_e \sim 0.3$ G), leads to a near-axis ring current field of ~ 14.5 nT. This is well within the 10-23 nT range estimated for the nearaxis field of the Earth's quiet ring current on the basis of models fitted to Imp magnetic field observations¹⁴. That three so vastly different magnetospheres apparently have almost the same E/Uratio is indeed surprising and suggests a limiting process whereby such a ratio is maintained.

Saturn's magnetosphere is well approximated by an axisymmetric, but non-dipolar field within $\sim 15 R_s$ of the planet. The non-dipolar nature of Saturn's magnetosphere is evident at radial distances exceeding $\sim 5 R_s$ and can be expected to have a significant role in charged-particle motion in Saturn's magnetosphere. Beyond 15 R_s , the asymmetries introduced by the solar wind interaction become non-negligible, particularly the formation of a magnetotail in the night-side magnetosphere. We suspect that Saturn's ring current is a permanent feature of Saturn's magnetosphere, much like the jovian magnetodisk. A better understanding of possible temporal characteristics of Saturn's magnetosphere must await analysis of Pioneer 11 observations already obtained and those to be obtained by Voyager 2 in August 1981.

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Smith, E. J. et al. J. geophys. Res. 85, 5655-5674 (1980)

Acuña, M. H., Ness, N. F. & Connerney, J. E. P. J. geophys. Res. 85, 5675-5678 (1980).

Busse, F. Solar System Plasma Physics Vol. 2 (ed. Kennel, C. F., Lanzerotti, L. J. & Parker, E. N.) 293-317 (North-Holland, Amsterdam, 1979)

Dolginov, Sh. Sh. Rep. Izmiran, Moscow (1978). Todoeschuck, J. P. & Rochester, M. G. Mature 284, 250-251 (1980).

Ness, N. F. et al. Science 212, 211-217 (1981).

Connerney, J. E. P., Acuña, M. H. & Ness, N. F. J. geophys Res. 86, (in the press). Bridge, H. S. et al. Science 212, 217-224 (1981).

Acuña, M. H., Connerney, J. E. P. & Ness, N. F. Nature 292, 721-724 (1981). Behannon, K. W., Connerney, J. E. P. & Ness, N. F. Nature 292, 753-756 (1981). Vogt, R. E. et al. Science 212, 231-234 (1981).

Dessler, A. J. & Parker, E. N. J. geophys. Res. 64, 2239-2252 (1959). Carovillano, R. L. & Siscoe, G. L. Rev. Geophys. Space Phys. 11, 289-353 (1973).

^{14.} Mead, G. D. & Fairfield, D. H. J. geophys. Res. 80, 523-534 (1975).

Arc structure in Saturn's radio dynamic spectra

A. Boischot', Y. Leblanc', A. Lecacheux', B. M. Pedersen' & M. L. Kaiser'

*Observatoire de Paris, F-92190 Meudon, France †Goddard Space Flight Center, Code 695, Greenbelt, Maryland 20771, USA

The dynamic spectra of Saturn's kilometric radiation show arc structures whose main characteristics are described and compared with those observed in the decametre emission of Jupiter. The origin of the arcs is probably similar for the two planets.

ONE of the most spectacular findings of the Voyager 1 planetary radio astronomy experiment (PRA) was the discovery of nested arc-like structures in the dynamic spectra of Jupiter's decametric emission. These arcs have curvature in either a direction towards increasing time (vertex early arcs or VEA) or decreasing time (vertex late arcs or VLA)^{1,2}. Several explanations for their structure have been proposed³⁻⁷. Similar arc structures are also evident in Saturn kilometric radiation (SKR) (see Fig. 1). These structures appear superimposed on the strong intensity modulation which is controlled by the rotation of the planet (sub-Voyager longitude in SLS) and on fast and narrow band fluctuations which give a very large variability to the PRA observations on a 6-s, 20-kHz scale. This very fine structure seems to be always present.

Characteristics of the arcs

The arc structure for Saturn, although easy to recognize, is not as regular as it is for Jupiter. This is due to the superposition of fine structure, and also because the amplitude of modulation of the emission by the arcs is small, only a few decibels on average, compared with 10 or 20 db for Jupiter. Thus for each rotation only a few arcs are clearly distinguished. The arc structure extends over the whole frequency range of the emission, but is often more clearly seen near the high frequency limit. Some phenomenon (possibly absorption or refraction) probably fixes the low frequency limit of the observed emission, and this prevents the lower legs of the arcs being seen to their full extent.

During the month around encounter the shapes of the arcs do not change much with time or SLS (Fig. 2). We can draw an 'average arc' with its vertex around 400 kHz and symmetrical curvature (on a linear frequency scale) around this vertex. The total extent is about 20 min and the shape is the same for VEA and VLA. In several cases, successive arcs are separated by 10-30 min, so that they appear to be nested like jovian arcs, but this nesting is not as regular as for Jupiter.

The arc structure could be seen on PRA records even when the spacecraft was far away from Saturn: arcs were very clearly observed as early as July 1981, when Voyager 1 was still at 1 AU. From that distance, the arcs appear more pronounced than when observed closer to the planet (probably because the amplitude of modulation is larger) and their shape is slightly different, the vertex being at lower frequencies (Fig. 2c).

The few arcs which are clearly recognized during each rotation are generally at quite different SLS positions. We can study their occurrence in SLS by adding up several rotations. Figure 3 presents two histograms of the position of the arcs for the preand post-encounter periods. For each period, a particular sense of arc dominates: VEA before encounter and VLA after encounter, irrespective of longitude.

Arcs of opposite curvature are also observed superposed on the dominant variety, but only in the range of longitudes where the total emission is most intense. Hence, the sub-spacecraft longitudes of these arcs change after encounter. Saturn emission is, at least partly, circularly polarized, but the arc structure does not seem to affect this polarization. We conclude that the sense of polarization remains constant before and after encounter and that the two families of arcs that we recognize during one rotation have the same sense of polarization. A more detailed analysis is required to see whether the arc structure corresponds to a modulation of the degree of polarization.

The difference in the dominant sense of curvature of the arcs between pre- and post-encounter periods can be interpreted in two ways:

- (1) The difference could be due to a latitudinal effect because the average latitudes of the spacecraft were different during the inbound (~8°) and outbound (~26°) paths; but whatever the origin of the arc mechanism, it would be more natural to expect a change in the shape of the arcs rather than in their sense of curvature.
- (2) The change in the sense of the arcs is a local time effect, a source at fixed position in local time (as invoked to explain the pre- and post-encounter change of the occurrence probability histograms of the emission with sub-Voyager SLS) being seen from two nearly opposite directions before and after encounter. As a local time explanation is more natural for the occurrence histograms, it is also more likely for the change of arc direction.

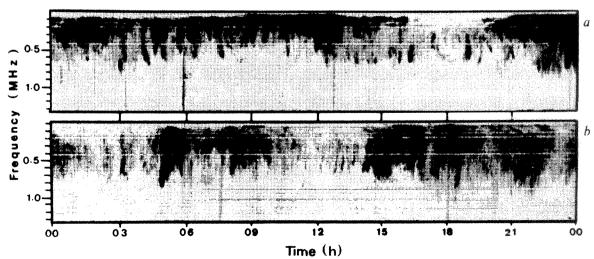
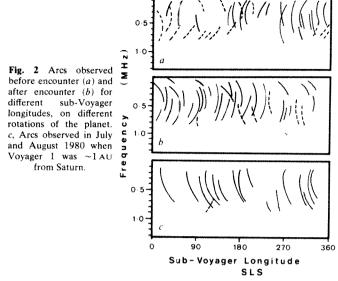


Fig. 1 Dynamic spectra of Saturnian kilometric radiation (SKR) for a day before encounter (10 November 1980, a) and a day after encounter (20 November 1980, b). In each plot the darkness is proportional to signal intensity as a function of wave frequency and hour of the day. More than two rotations of the planet are covered.

The arc structure and the reversal of the direction of the dominant family are clear.

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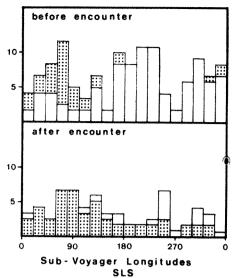


Fig. 3 Histogram of the arc-direction as a function of sub-Voyager longitude in SLS. White corresponds to vertex early arcs (VEA) and dark to vertex late arcs (VLA). At every dominate before counter and VLA after encounter, while arcs of the opposite direction are more localized in longitude. Because longer period studied before encounter more arcs were seen during this period.

Comparison with Jupiter

The arc structure in Jupiter appears mainly at frequencies larger than 1 MHz. At lower frequencies, the arcs are more difficult to follow, although many drifting features can be seen at the end of the low-frequency leg of the arcs, with their vertex between 5 and 10 MHz. On the other hand, Saturn's emission is observed only at frequencies lower than 1.2 MHz. This large difference in the frequency range of the emission must be considered when we compare the arc structure of the two planets.

The most important result is that arcs cover the whole frequency range of the emission from both Jupiter and Saturn. As the high frequency limit of Saturn's emission is fairly constant the arc shape is stable. In contrast the jovian arc shapes vary considerably with LCM. In particular the higher the frequency limit, the higher the frequency of the vertex. The inclination of the magnetic axis to the rotation axis is much more important for Jupiter than Saturn and explains the difference. As the jovian arc pattern varies consistently with the LCM, we consider that the variation of shape with LCM is principally due to the wobbling of the observer's position in magnetic coordinates due to this inclination. Such a variation does not exist on Saturn because the magnetic and rotation axis nearly coincide^{9,1}

Received 13 May; accepted 15 July 1981.

- Warwick, J. W. et al. Science 204, 995-998 (1979).
- Leblanc, Y. J. geophys. Res. 86 (in the press). Staelin, D. H. J. geophys. Res. 86 (in the press)
- 4. Goldstein, M. L. & Thieman, J. R. J. geophys. Res. 86 (in the press).

Another comparison concerns the sense of the arcs and its change at encounter. If we compare the arcs of Saturn with the lesser arcs of Jupiter¹⁻², whose emission is not controlled by Io, the variations are similar. The lesser arcs are predominantly VLA before encounter and VEA after encounter, but both types of arc are always present. Hence, although the character of the dominant family is opposite, the reversal at encounter is observed for both planets. Thus the main characteristics of the arcs in Jupiter's and Saturn's emission are similar, even if their frequency ranges are different.

At Jupiter, several types of arcs have been identified²: at least two types of lesser arcs, and several types of Io-controlled and non-lo-controlled high frequency arcs, while only two are observed for Saturn. This probably requires several distinct sources of jovian emission while only one or two are necessary to explain the Saturn's arcs. The larger variability of the arc with longitude observed for Jupiter can be explained simply by the change in the magnetic latitude of the observer during one rotation due to the inclination of the magnetic axis to the rotation axis. Therefore the cause of the arcs is probably related to the magnetic field and the similarities between Saturn's and Jupiter's arc structures mean that they are probably due to the same physical mechanism.

- Boischot, A. & Aubier, M. J. geophys. Res. 86 (in the press).
- Warwick, J. W. J. geophys. Res. 86 (in the press).
 Lecacheux, A., Meyer-Vernet, N. & Daigne, G. Astr. Astrophys. (in the press).
- Warwick, J. W. et al. Science 212, 239-243 (1981). Smith, E. J. et al. Science 207, 407 (1980).
- 10. Acuña, M. H. & Ness, N. F. Science 207, 444-446 (1980).

Arcs in Saturn's radio spectra

J. R. Thieman* & M. L. Goldstein

*Advanced Systems Planning Division, ORI Inc, 1400 Spring St Silver Spring, Maryland 20910, USA †Code 692, Laboratory for Extraterrestrial Physics, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

Arcs appearing in the dynamic spectra of Saturn radio emission have been modelled using the same techniques as applied to jovian arcs. Their properties suggest a source region several tenths of a Saturn radius above the cloud tops in the late morning local time sector.

ARC-LIKE structures appear sporadically in the dynamic spectra of Saturn radio emission measured by the Voyager 1 planetary radio astronomy (PRA) experiment. Arcs were pervasive in the dynamic spectra of Jupiter emission 1-3 but they are not nearly as prevalent in Saturn data. There are some differences in the properties of the arcs from the two planets, but a model used to fit jovian arcs has also been successfully applied to Saturn arcs. The curvature and frequency range of the Saturn arcs are best fitted by assuming the source is along magnetic field lines with small L values $(L \le 5)$.

Figure 1 shows examples of Saturn dynamic spectra and the types of arcs which occur: both vertex-early and vertex-late arcs (appearing as opening and closing parentheses, respectively) are indicated. A search of dynamic spectra between June and December 1980 resulted in a set of 82 arcs but data gaps during this period preclude determination of an exact occurrence rate. Table 1 summarizes the similarities and differences between the catalogued arcs and the arcs seen in jovian dynamic spectra. As Boischot et al.4 have considered some of these characteristics. only some of the entries will now be discussed.

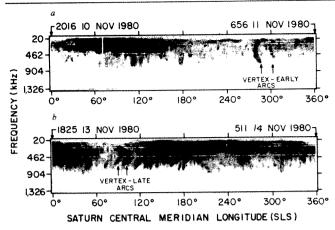


Fig. 1 Examples of arcs in the dynamic spectral plots of Saturn emission detected by the Voyager 1 spacecraft. Data from one rotation of Saturn before (a) and after (b) closest approach. The beginning and ending spacecraft data acquisition times are marked. Darkness is proportional to the signal strength computed for 1° longitude averages in each of the 70 frequency channels from 1.2 kHz to 1.32 MHz. The abscissa is the longitude of the meridian plane containing the spacecraft. Two vertex-early and two vertex-late arcs are indicated.

Although the right-hand polarization sense (as conventionally used by radio astronomers) is dominant for Saturn arcs, left-hand polarized arcs occur occasionally, mostly during closest approach to Saturn when the spacecraft trajectory was southernmost in latitude⁵. For the 82 arcs analysed, the duration ranged from 10 min to >1 h, with an average of \sim 45 min.

Figure 2 displays histograms of the minimum, maximum and vertex frequencies for the catalogued Saturn arcs. The average vertex frequency is slightly lower than the value of 400 kHz cited by Boischot et al.⁴, but their value was based on arcs identified near encounter only. The low frequency extent of the arc peaks sharply around 100 kHz, probably as a consequence of the plasma frequency cutoff due to the plasma density between the source and the spacecraft.

The most interesting difference between Jupiter and Saturn is that for the inbound passage of Voyager 1 to Saturn nearly all of the observed arcs had vertex-early curvature sense while the reverse was true outbound. The sense of curvature of some jovian arcs also shows some pre- and post-encounter dependence, but the dominant influence is longitude. Both vertex-early and vertex-late curvature are often superimposed, and the amount of curvature, the vertex frequency and the frequency extent for each type of curvature are directly dependent on jovian longitude. The inbound and outbound difference in curvature sense at Saturn is interpreted as a local time effect as there is some evidence that the source of emission is confined in local time.

Although differing means of arc generation are possible, the many similarities in the planets' arcs suggest similar mechanisms. Beaming of radio emission into a hollow conical sheet was a common hypothesis long before arcs were discovered. Previous modelling work demonstrated that a hollow conical beam which is centred on and rotating with a jovian magnetic field line can reproduce the observed jovian arcs if the cone opening angle is allowed to vary with distance along the field line. To apply this model to Saturn the direction and

Table 1 Saturn-Jupiter arc comparison Jupiter Saturn Nearly all right-hand Dominantly right-hand Polarization Total arc duration 20 min 45 min ~100 kHz to 40 MHz ~50 kHz to ~1.0 MHz Frequency range Nearly all high band radiation Occurrence rate in every rotation made of arcs At most a few per rotation Large number per group 1-3 per group Nesting Longitude dependence Curvature and frequency None Curvature

magnitude of the magnetic field at any point must be known. The model of Saturn's magnetic field which is used is based on Voyager magnetometer experiment results⁹. It can be modified to include the effects of a current sheet¹⁰, but the corrections were not of sufficient magnitude to affect the arc modelling results. The discrete nature of the arcs implies that activity takes place along individual magnetic field lines. For Jupiter the active field lines were usually assumed to have magnetic L values¹¹ of \sim 6, as those field lines pass through the orbit of the moon Io which is known to influence the occurrence of radiation. There is no a priori reason to limit L values of the field lines for Saturn.

The frequency of the emission is assumed to be just above the right-hand cutoff frequency ω_R so that right-hand polarized emission escapes the planet. The cutoff frequency is defined as:

$$\omega_{\rm R} = \Omega_{\rm e} [1 + (1 + 4\omega_{\rm pe}^2/\Omega_{\rm e}^2)^{1/2}]/2$$

where $\omega_{\rm pe} \equiv \sqrt{(4\pi Ne^2/m)}$ is the electron plasma frequency, $\Omega_{\rm e} \equiv |eB/mc|$ is the electron gyrofrequency, e, m and N are the electron charge, mass and number density, and B is the magnetic field strength.

The apparent emission sources for the Earth and Jupiter are in underdense plasma regions $(\Omega_e \gg \omega_{pe})$ close to the planets so that ω_R and the emission frequency are very close to the electron gyrofrequency. If we assume that the maximum emission

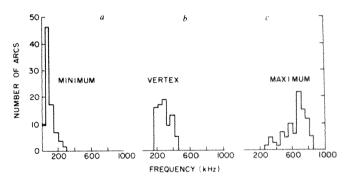


Fig. 2 Histograms of the distribution of the: a, minimum; c, maximum, and b, vertex frequencies in a catalogue of 82 arcs. The catalogue spans 19 June 1980 to 12 December 1980.

frequency in Saturn radiation corresponds to the maximum gyrofrequency above the planetary cloud tops, then the highest emission frequency would be ~1.5 MHz. Observed emission never exceeds 1.2 MHz and is rarely higher than 900 kHz, so there seems to be a fundamental difference from Jupiter. Low L-value field lines have maximum gyrofrequencies in the 900 kHz range, but the lowest gyrofrequencies on these lines are higher than the lowest radiation frequencies so the source region cannot lie on these field lines. Radio occultation measurements of the Saturn ionosphere show that the plasma frequency close to the planet is comparable with the gyrofrequency 12,13. The region where the gyrofrequency is ~900 kHz is several tenths of a Saturn radius above the cloud tops (for $L \sim 5$). In this region the plasma frequency is much less than the gyrofrequency (as it was at Jupiter), suggesting that an underdense plasma environment may again be necessary in the source region. Consequently, the maximum frequency in the models of the Saturn arcs has been determined by assuming the emission occurs only beyond a fixed distance from the planetary cloud tops. This is similar to the Earth's auroral kilometric radiation (AKR) which occurs well above the surface at a distance of ~2 Earth radii¹⁴

The vertex frequency is defined from observations to be a set fraction of the maximum frequency. The minimum arc frequency is difficult to determine since surrounding radiation often mixes with and obscures the low frequency end. The model assumes a fixed lowest frequency at a value determined from observations. The cone angles are allowed to vary with position along the field line from a minimum at the lowest frequency to a maximum at the nose frequency and then back to the minimum

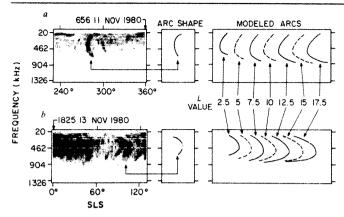


Fig. 3 A comparison of the forms of model arcs to the actual arc shapes derived from the data in Fig. 1. Seven magnetic L values were used to derive the location of seven possible source regions, yielding the arcs shown.

value at the maximum frequency. The formula below is used to vary the cone angle with frequency and is derived from jovian arc studies⁸.

$$\psi(f) = \psi_{\text{max}} \sin\left(\frac{\pi}{2}\right) \left(\frac{f_{\text{max}} - (1 - a)f - af_{\text{v}}}{f_{\text{max}} - f_{\text{v}}}\right) \qquad f \ge f_{\text{v}}$$

$$\psi(f) = \psi_{\text{max}} \sin\left(\frac{\pi}{2}\right) \left[\frac{(1 - a)f + af_{\text{v}} - f_{\text{min}}}{f_{\text{v}} - f_{\text{min}}}\right] \qquad f \le f_{\text{v}}$$

 ψ_{\max} and $\psi(f)$ are the maximum cone angle and the cone angle at frequency f. The minimum, maximum and vertex frequencies are represented by f_{\min} , f_{\max} and f_{ν} and the coefficient a is defined so that $\psi(f) = \psi_{\min}$ at both $f = f_{\max}$ and $f = f_{\min}$.

Arcs from Fig. 1 are compared in Fig. 3 with curves predicted by the arc model. For the vertex-early arcs the model assumes: a cone angle varying from a minimum of 75° to a maximum of 85°; a minimum frequency of 100 kHz; a nose frequency which is 0.6 of the maximum frequency; a minimum distance for the emission region of $0.2 R_s$ above the cloud tops; and the L values indicated. The same assumptions are used for the vertex-late arcs except for the cone angle which varies from 70° to 85° and the minimum frequency which is increased to 150 kHz. These values should not be considered to be unique as the parameters can vary within a small range without significantly altering the fits. Other arcs differ in shape from examples in Fig. 3 but the parameters can be changed to fit those arcs. The curvature of the modelled arcs is sensitive to the difference between the maximum and minimum cone angles; slight changes in this difference have a noticeable effect. The values of the maximum and minimum cone angles can vary by tens of degrees without noticeably affecting the curvature, provided the difference is held constant. Only values of the cone angle of the order of 60° or larger allow the cone of emission to intersect the line of sight of the Voyager spacecraft. This is consistent with the Jupiter emission model in which large cone angles are necessary to explain the 150° separation in longitude between the two major radio sources, which are believed to originate from opposite sides of the same cone.

Constraining the magnetic L value is an important goal. In general, higher L values cause an increased curvature at high frequencies because of the cone beaming geometry. No severe curvature is evident in the observations, which implies that the emission is confined to $L \leq 5$. For Jupiter, the ability to produce good fits assuming a source along the L=6 field line was consistent with the influence of Io on the emission. Four major satellites as well as Saturn's rings are threaded by field lines with L values ≤ 5 . The resolution of the arc fitting process is not sufficient to distinguish whether any of these objects may be affecting the emission.

The source location implied from the above limits agrees reasonably well with the location deduced by Kaiser et al.⁶. That location, however, is confined to a local time region near the noon meridian. For arcs to be formed from hollow conical

beams, the beams must rotate with the planet and so intersect the observer's line of sight. If the source is stationary in local time this is not possible. There may, however, be a limited activity region near the noon meridian in which charged particle precipitation and subsequent radio emission is stimulated along a field line as it rotates into the region, continues as the field line rotates, and then dissipates.

The confinement to a specific local time domain explains the dominance of vertex-early arcs in inbound observations and vertex-late arcs after encounter. Figure 4 displays the proposed emission geometry. Inbound, Voyager 1 approached along a local time line of ~ 13 h. Consequently, the leading edge of the conical beams would usually be the only emission detected. According to the model vertex-early arcs result from the leading edge of a cone, so this is consistent. The outbound trajectory of Voyager 1 was limited to local times around 3 h; thus the trailing edge of the cone was observed and, therefore, vertex-late arcs.

The discrete nature of the arcs for both Jupiter and Saturn remains a problem. For Jupiter, the arcs are discrete but quite regular and usually nested. Saturn arcs appear intermittently

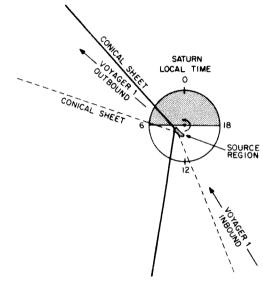


Fig. 4 Hollow conical beam emission from Saturn projected onto the Saturn orbital plane. The source region is confined to the late morning local time sector. Conical emission begins for a particular field line rotating into this region, and ends when the field line rotates out of the region. The directions of the inbound and outbound trajectories are indicated by the arrows. The leading (trailing) edge of the cone rotates through the inbound (outbound) Voyager 1 line of sight.

and are only occasionally nested. Gurnett and Goertz¹⁵ suggested a mechanism for jovian nested arcs which would produce sources along regularly spaced field lines threading Io's plasma torus. Lecacheux *et al.*¹⁶ proposed the production of discrete arcs by a Fresnel screen interference pattern resulting from the observation of the source region through the Io plasma torus. Because there is no similar plasma torus in the magnetosphere of Saturn these theories require modification to explain the discrete Saturn arcs. It may be necessary to look to the solar wind, moons, rings or other influences as sources of discrete excitation.

Our study suggests that the hollow conical emission model of Jupiter arcs can be adapted to fit the Saturn arcs and indicates (1) source regions located along magnetic field lines with $L \lesssim 5$, and (2) a source location several tenths of a Saturn radius above the cloud tops along high latitude field lines. The pre- and postencounter vertex sense of curvature is consistent with conical emission rotating through the late morning local time region.

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Received 13 May; accepted 23 July 1981.

- 1. Warwick, J. W. et al. Science 204, 995-998 (1979).
- 2. Warwick, J. W. et al. Science 206, 991-995 (1979).
- 3. Alexander, J. K., Carr, T. D., Thieman, J. R., Schauble, J. J. & Riddle, A. C. J. geophys. Res. (in the press).
- Boischot, A., Leblanc, Y., Lecacheux, A., Pedersen, B. M. & Kaiser, M. L. Nature 292, 727-728 (1981).

- Warwick, J. W. et al. Science 212, 239-243 (1981).
- Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731-733 (1981). Dulk, G. A. Learus 7, 173-182 (1967).
- Goldstein, M. L. & Thieman, J. R. J. geophys. Res. 86 (in the press). Ness, N. F. et al. Science 212, 211-217 (1981).
- 10. Connerney, J. E. P., Acuña, M. H. & Ness, N. F. Nature 292, 724-726 (1981).
- McIlwain, C. E. J. geophys. Res. 66, 3681-3691 (1961).
 Kliore, A. J. et al. Science 207, 446-449 (1980).
- 13. Tyler, G. L. et al. Science 212, 201-206 (1981).
- Alexander, J. K., Kaiser, M. L. & Rodriguez, P. J. geophys. Res. 84, 2619–2629 (1979).
 Gurnett, D. A. & Goertz, C. K. J. geophys. Res. (ia the press).
 Lecacheux, A., Meyer-Vernet, N. & Daigne, G. Astr. Astrophys. (in the press).

Saturnian kilometric radiation: statistical properties and beam geometry

M. L. Kaiser*, M. D. Desch* & A. Lecacheux*

* NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Greenbelt, Maryland 20771, USA † Observatoire de Paris, Section d'Astrophysique, F-92190 Meudon, France

Analysis of the average properties of Saturn's kilometre wavelength radio emission suggests that the source region is near the noon meridian in the northern auroral zone and/or the polar cusp.

THE detection of kilometre-wavelength radio emissions from Saturn (SKR) increases the possibility that useful comparative radio 'planetology' can be done, to provide insight into the similarities and differences in particle acceleration and precipitation processes on the three known non-thermal radio planets-Earth, Jupiter and Saturn. However, before Saturn's radio emissions can be studied, an adequate description of their average statistical properties is needed. We now present these properties and discuss the implications for source location, beaming, and magnetic surface anomalies. Such statistical properties are already well known for the Earth's auroral kilometric radiation (AKR). Jupiter's decametric wavelength radio emissions have been studied with ground-based instruments for 25 yrs, and space-borne radio telescopes have extended studies of the jovian spectrum to 10 kHz.

The statistical properties of AKR are a strong function of the observer's viewing geometry. Gurnett2 first showed that the observer's sub-spacecraft magnetic latitude and local time were the important coordinates in which to organize studies of the Earth's emission beam properties. The Voyager observations of Jupiter have shown that these same two coordinates are of prime importance over much of that planet's radio spectrum³⁻⁶. Figure 1 shows the magnetic latitude-local time coverage of the three radio planets by the Voyager spacecraft and ground-based observations. For the Earth, >90% of the possible viewing locations have been surveyed frequently enough to provide good statistics; only a small region above the southern polar cap has not been surveyed. For Jupiter, the ground-based observations and the two Voyager fly-bys have covered only $\sim 4\%$ of the possible observing plane, and for Saturn the total coverage will be <0.5% even after both Voyager encounters. This is

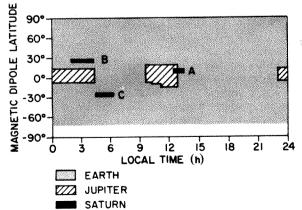


Fig. 1 Positions in the local time-magnetic latitude plane from which radio observations have been made of the Earth, Jupiter and Saturn. Local time is defined as the equatorial plane projection of the observer-planet-sun angle with $12 h = local noon = 0^{\circ}$. A, The inbound trajectories of both voyagers; B, the outbound Voyager 1 trajectory: C, outbound Voyager 2. Thin lines connect A and B, and A and C but they correspond to such brief durations that statistical properties cannot be determined for those viewing locations.

Table 1	Total power o	f SKR	
Occurrence Probability	50%	10%	1%
Inbound power (W) Outbound power (W)	$ \begin{array}{c} 2 \times 10^8 \\ 6 \times 10^7 \end{array} $	$\begin{array}{c} 3 \times 10^9 \\ 1 \times 10^9 \end{array}$	3×10 ¹⁰ 8×10 ⁹

largely due to the near perfect alignment of the Saturnian magnetic moment with the rotational axis⁷⁻⁹ which almost eliminates the 'rocking' in magnetic latitude experienced by both the Earth and Jupiter. We describe the average properties of SKR as observed from locations A and B in Fig. 1 by the Voyager 1 planetary radio astronomy (PRA) instrument 10,111 for a 2-month period centred on the 12 November 1980 encounter. Location A specifies the inbound (pre-encounter) geometry (at ~12.5 LT and +8° lat) and location B, the outbound (postencounter) geometry (at ~3.5 LT and +26°). Location C will be the Voyager 2 outbound trajectory in late 1981. The Saturn hemisphere from 13 to 01 h is only briefly entered by the Voyagers, specifically during their fast swing-bys at closest approach. Additional radio noise from a source in this region would not be detected by the Voyagers.

Occurrence probability

An analysis of the occurrence pattern of SKR as a function of Saturn's rotation phase has shown 12 that SKR occurrence is not continuous, but variable and roughly periodic. From preencounter observations, a Saturn longitude system12 (SLS) can be defined. SKR was found to be well organized in longitude with an occurrence rate maximum near 90° and a minimum at ~270° in sub-Voyager SLS. The PRA observations near closest approach 13 showed that the SKR occurrence pattern was fixed relative to the Sun with maximum occurrence rate near 100° in sub-solar SLS.

Figure 2 shows the absolute occurrence rate of SKR bursts stronger than 10^6 Jy (1 Jy = 10^{-26} W M $^{-2}$ Hz $^{-1}$) as a function not only of sub-solar SLS, but also of observing frequency. (We have adopted 1 AU as a 'standard' distance for normalizing flux density measurements.) The reported13 similarity in the occurrence patterns for both the inbound and outbound trajectories is clear. However, the absolute occurrence rate for the outbound observations is roughly half that of the inbound observations. Furthermore, the maximization of occurrence in the 100-200 kHz frequency range which is so evident in the inbound panel is replaced in the outbound panel with a broad maximum from 100 to 500 kHz. At the 5% occurrence level, SKR extends from ~ 40 to 600 kHz. SKR events extending to 1,200 kHz have been reported¹³, but the occurrence rate of these events (>10⁶ Jy) is «1%, with no discernable difference between inbound and outbound observations. SKR events as low as 3 kHz have also been reported14

Figure 3 shows, for an observing frequency of 174 kHz, the probability of detecting emission above a given flux level for both inbound and outbound trajectories. The probability of

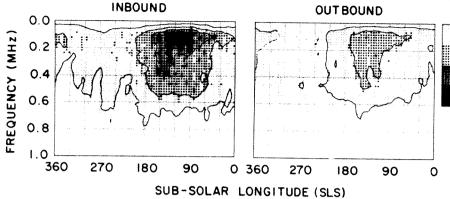


Fig. 2 The absolute occurrence rate of SKR above 10⁶ Jy is shown as a function of sub-polar SLS and observing frequency for the Voyager 1 inbound and outbound observing positions (corresponding to A and B in Fig. 1).

detecting a given flux density event is generally higher on the inbound leg than outbound. For example, the probability of detecting SKR stronger than 10^5 Jy is $\sim 58\%$ inbound, but only 32% outbound. For events stronger than 10^6 Jy, the probabilities are 18% inbound, and only 8% outbound.

Several authors^{12,13} have reported that SKR is largely right-hand (RH) polarized with left-hand (LH) SKR detected only during the approximately 2-month period immediately preceding encounter, and not at all after encounter. Figure 3 shows the occurrence probability of LH SKR. Its rate of occurrence is only a small fraction of the total SKR occurrence rate. The distribution of LH SKR in frequency and SLS is not well determined due to the sparsity of data, but there is evidence that the LH emission maximizes near 350 kHz at about 300 ° SLS. This is ~200 kHz higher in frequency and 180 ° displaced in longitude from the RH polarized emission observed inbound.

Power flux density

Figure 4 shows the average power flux density spectra of SKR at three different occurrence levels for both the inbound and outbound observations. The spectra have been averaged over all longitudes. The general trends evidenced in the occurrence probability distributions (Figs 2 and 3) can be seen. The flux densities observed during the outbound trajectory are lower at frequencies below 500 kHz than the corresponding values inbound. The equivalent isotropic powers for the three occurrence levels are shown in Table 1. Roughly three to four times as much power is received at observing position A than at B.

The frequency of peak flux for inbound observations is near 150-175 kHz. The peak outbound appears to be shifted to higher frequencies, in some cases as high as 400 kHz. However, comparison of the ratio of the corresponding inbound and outbound spectra reveals that this apparent shift towards higher frequencies is caused by a systematic decrease in observed intensities at lower frequencies for outbound observations. For example, for the 10% occurrence spectra, the received flux at 100 kHz is five times larger for the inbound case than for outbound. This ratio decreases to a factor of 2.5 at 250 kHz and approximately equal levels are observed at or above 500 kHz.

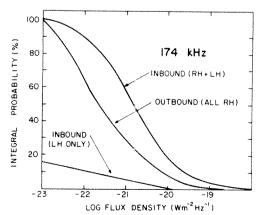


Fig. 3 The occurrence probability of SKR above a given flux level for a particular observing frequency for inbound, outbound and LH polarized events. The flux densities are normalized to a standard distance of 1.0 AU.

Discussion

The statistical SKR properties discussed above strongly constrain possible source locations. We know that SKR emanates from a source which is fixed relative to the Sun, perhaps much like the Earth's AKR. However, SKR would need to be emitted into an extremely sharp beam for the very small magnetic dipole tilt to account for the very large (more than a factor of 100) intensity modulation as a function of rotation¹³. Instead, we favour the concept that emission is generated when a particular longitude arrives at a particular solar hour angle (local time). From the flux level differences inbound and outbound, the radio beam is clearly oriented more towards the inbound trajectory. This is reinforced by the fact that the low frequency portion of each outbound SKR spectrum is depressed. Studies of the Earth's AKR angular distribution¹⁴ show that high frequencies

5%

20 %

35 %

50 %

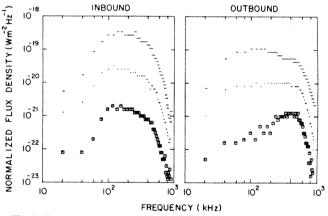


Fig. 4 Typical SKR flux density spectra observed 50% (□), 10% (•) and only 1% (bars) of the time are shown for inbound and outbound observations.

are beamed over a much wider angle than low frequencies due to refraction. If analogy with AKR is correct, a beam oriented more towards the inbound trajectory would tend to illuminate the outbound trajectory at high frequencies, but perhaps not at low frequencies.

From consideration of the inbound spacecraft trajectory and the polarization of SKR, Warwick et al. ¹³ concluded that the RH emission is emitted in the magnetoionic extraordinary mode from the northern hemisphere, and the LH emission is most likely from the southern hemisphere. This implies that SKR is most likely generated near the electron gyrofrequency in the source region. The magnetic field model ⁷⁻⁹, coupled with the observed SKR frequency range, $<20-\sim1200$ kHz, suggests that the emission source region is confined to field lines exiting the planet at rather high latitudes, probably >60°. At lower latitudes (<56°) the gyrofrequency does not extend as low as 20 kHz anywhere on the field line.

Additionally, because the SKR comes from a source fixed in local time, the observation¹³ that the sense of polarization remains RH inbound and outbound implies that both trajectories are illuminated by the forward lobe of the same beam. In other words, the initial wave direction must be predominantly

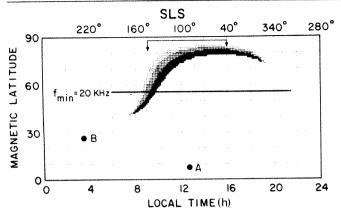


Fig. 5 The shaded region shows, in the saturnian local time-magnetic latitude plane, the locus of possible emission cone axes for frequencies near 175 kHz. The dark shading is for an assumed $\cos^{1/2} \theta$ beam pattern where θ is the angle between the ray and field line, the moderate shading for a $\cos \theta$ pattern, and the lightest shading for a $\cos^2 \theta$ pattern. Also shown is a line which corresponds to an equatorial electron gyrofrequency of 20 kHz. Only regions at higher latitudes than those indicated will have emission as low or lower than 20 kHz. The bracketed arrows show the region where similar plots for 20 kHz and 500 KHz intersect this plot. Because the SKR beam appears to be fixed in local time, we can approximately equate longitudes to local time and this scale is shown at the top . A similar analysis done with the cone axis parallel to the field gradient direction gave essentially the same result.

parallel to Saturn's magnetic field for both the A and B viewing locations. Thus, the SKR RH beam seems to emanate from the northern hemisphere in the sector containing A and B, and oriented more towards A.

We can speculate on the source location by making some assumptions concerning the shape and orientation of the SKR beam. The Earth's AKR beam near the frequency of peak flux

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- Kaiser, M. L. et al. Science 209, 1238-1240 (1980)
- Gurnett, D. A. J. geophys. Res. 79, 4227-4238 (1974). Alexander, J. K. et al. J. geophys. Res. 84, 5167-5174 (1979).
- Alexander, J. K. et al. J. geophys. Res. 86 (in the press). Desch, M. D. & Kaiser, M. L. J. geophys. Res. 85, 4248-4256 (1980).
- Boischot, A. et al. J. geophys. Res. 86 (in the press) Smith, E. J. et al. Science 207, 407-411 (1980).

density is very broad, closely resembling a cosine pattern with the beam axis oriented more or less parallel to the field15. If we assume that SKR is also emitted in a broad, symmetrical beam pattern, and that the axis of the pattern is also oriented parallel to the field, we can determine where the axis must be in local time and latitude so that intensities received at A exceed those at B by some factor consistent with Table 1. Figure 5 shows a plot similar to Fig. 1 indicating the locus of possible beam axes for three different broad beams where A would receive 2-4 times as much flux as B. The calculations were made at altitudes corresponding to 175-kHz electron gyrofrequency. The field lines yielding solutions were then traced to the surface (invariant latitude). Several source locations are possible, but most intriguing is the region at high latitudes near the noon meridian. This region most likely corresponds to the auroral zone 9.16, and, by analogy with the Earth² and Jupiter⁶, is a very good candidate for a region of enhanced particle precipitation required to produce radio emission. Additionally, the apparent confinement of the radio source to a limited dayside range of local times suggests an association with a dayside polar cusp in Saturn's magnetosphere17. Because SKR is fixed in local time, we can equate local time and sub-solar SLS as shown at the top of Fig. 5. The SLS corresponding to the proposed source region (40°-145°) may correspond to a region of anomalous magnetic field, although it is not clear that a precipitation producing field anomaly need be in the source region itself. The Voyager 1 close approach to Saturn was at high southern latitudes and displaced some 90-100° in longitude from the proposed source location, so fields and particle experiments were not able to make measurements on field lines threading this region. However, the Voyager 2 close approach on 25 August 1981 will be in a much better position to make measurements over this region.

- Acuña, M. H. & Ness, N. F. Science 207, 444-446 (1980).
- Ness, N. F. et al. Science 212, 211–271 (1981).
 Lang, G. J. & Peltzer, R. G. IEEE Trans. AES-13, 466–471 (1977).
- Lang, G. J. & Pettzer, R. G. IEEE Trans. AES-15, 400-4/1 (1977).
 Warwick, J. W. et al. Space Sci. Rev. 21, 309-319 (1977).
 Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 253-256 (1981).
 Warwick, J. W. et al. Science 212, 239-243 (1981).
 Gurnett, D. A. et al. Science 212, 235-239 (1981).

- Green, J. L. et al. J. geophys. Res. 82, 1825-1838 (1977). Broadfoot, A. L. et al. Science 212, 206-211 (1981).
- Behannon, K. W. et al. Nature 292, 753-756 (1981)

emissions Narrowband electromagnetic from Saturn's magnetosphere

D. A. Gurnett*, W. S. Kurth* & F. L. Scarf*

*Department of Physics and Astronomy, The University of Iowa, Iowa City, Iowa 52242, USA †TRW Defense and Space Systems, One Space Park, Redondo Beach, California 90278, USA

A series of narrowband electromagnetic emissions were detected by the plasma wave instrument on board Voyager 1 coming from the inner region of Saturn's magnetosphere in the frequency range 3-30 kHz. These emissions have many similarities to continuum radiation detected in the Earth's magnetosphere and narrowband kilometric radiation in the jovian magnetosphere. The observed frequency spacing suggests that the emissions are being generated near Tethys, Dione and Rhea, probably in regions of large plasma density gradients associated with boundaries of the plasma sheet.

During the Saturn flyby the Voyager 1 plasma wave instrument observed a series of narrowband electromagnetic emissions at frequencies between 3 and 30 kHz (ref. 1). These bands are remarkable because of their extremely complex spectral structure, consisting of a large number of nearly monochromatic emissions with frequency spacings corresponding to the equatorial electron gyrofrequencies near the moons Tethys, Dione and Rhea. Because of the possible association of the radio emissions with the moons of Saturn, we have analysed these emissions to determine their origin and to explore similarities to other planetary radio emissions. We describe here their characteristic and discuss models of the generation of these emissions (the plasma wave instrumentation is described in ref. 2).

Observations

The strongest and best resolved examples of the narrowband electromagnetic emissions occur in the inner region of the magnetosphere, near closest approach. The electric field intensities in this region are shown in Fig. 1. Each channel gives the electric field intensity on a logarithmic frequency scale at the centre frequencies indicated on the left of the plot. The electron gyrofrequency, f_g , and plasma frequency, f_p , are also shown for reference. The electron gyrofrequency $(f_g = 28B \text{ Hz}, \text{ where } B \text{ is})$ the magnetic field in nT) was determined from the Voyager 1 magnetic field instrument³, and the plasma frequency $(f_p =$ $9,000 \sqrt{n_e}$ Hz, where n_e is the electron number density cm⁻³) was obtained from the Voyager 1 plasma instrument⁴. The narrow-

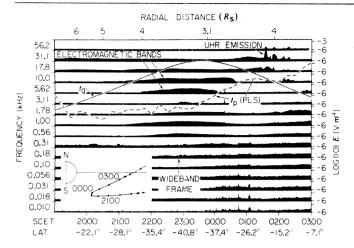


Fig. 1 The plasma wave electric field intensities in the region near closest approach. The narrowband electromagnetic emissions are confined to the low density region outside of the plasma sheet at $f > f_p$ (dashed lines). The plasma sheet is entered at about 00.00 to 01.00, as the spacecraft approaches the magnetic equator (see trajectory sketch).

band electromagnetic emissions consist of a series of smooth enhancements in the 3.11–31.1-kHz channels from about 21 h 00 min (spacecraft event time) on 12 November to 01 h 00 min on 13 November. These enhancements all occur at frequencies above the electron plasma frequency as determined by the plasma experiment. The sharp cutoffs evident in the 5.62- and 10.0-kHz channels shortly after closest approach seem to be directly associated with the local electron plasma frequency. The enhanced intensities extend across the electron gyrofrequency with no obvious propagation effect. These characteristics identify the mode of propagation as the free-space left-hand polarized ordinary (L,0) mode. For this mode propagation occurs for all frequencies $f > f_p$, and that no resonance or cutoff occurs at the electron gyrofrequency.

Another type of very intense narrowband emission occurs in the 31.1-kHz channel of Fig. 1 at about 01.30 on 13 November, coincident with the cutoff of the electromagnetic radiation. At peak intensity this narrowband burst has a field strength ~ 60 db larger than the adjacent radiation. Based on similar observations in the Earth's magnetosphere ⁵⁻⁷ and at Jupiter ⁸⁻¹⁰ this burst is identified as an electrostatic emission at the local upperhybrid resonance (UHR) frequency, $f_{\rm UHR} = (f_p^2 + f_g^2)^{1/2}$. The fact that the observed emission frequency is higher than the upper hybrid resonance frequency computed from the measured plasma frequency and gyrofrequency is attributed to uncertainties in the determination of the electron number density. The electron plasma frequency is a factor of ~ 3 higher than given by the plasma instrument at this particular time.

Because the intensity of the electromagnetic radiation is comparable in adjacent frequency channels, Fig. 1 implies that the spectrum is nearly continuous, possibly comparable with the continuum radiation detected in the Earth's magnetosphere⁵. Fortunately, one 48-s frame of wideband waveform data obtained in this region provides greatly improved frequency resolution. The high-resolution spectrum of the wideband data (Fig. 2) is not continuous, but rather consists of many narrowband emissions, some with bandwidths < 100 Hz. Three main bands can be identified at frequencies of ~1 kHz, ~6 kHz, and ~9.7 kHz. Because the low-frequency band at 1 kHz is below the electron plasma frequency, whereas the others are all above it, this band cannot be propagating in the same mode as the higher frequency bands. For $f < f_p$ the only possible modes are the whistler-mode and the z-mode. Based on the similarity to whistler-mode hiss emissions observed in the higher density regions of the plasma sheet, we concluded that this lowfrequency (~1 kHz) band is propagating in the whistler mode1.

The most definitive evidence that the high-frequency bands are propagating in the free-space electromagnetic mode is given in Fig. 3, which shows a series of wideband spectrograms

obtained over a 3-day period near and after closest approach. A single persistent band of emission can be seen at ~5 kHz extending over a range of radial distances from at least 3.26 to $58.3 R_{\rm S}$. Because the plasma parameters change over a wide range it is difficult to see how this band of emission could be observed over such a large range of radial distance without being a freely propagating electromagnetic wave. The last observation, Fig. 3 F, is in the magnetosheath where the local plasma frequency is ~3.8 kHz [J. Scudder, personal communication]. Although the electromagnetic bands can be detected in the wideband data over several days, the intensity and detectability vary considerably. The meridian plane plot in Fig. 4 summarizes the regions in which the bands were detected. Except for the brief interval near closest approach all of the emissions were confined to high latitudes on the outbound pass. None were found on the inbound leg and this is almost certainly due to the fact that the spacecraft was passing through the high density central region of the plasma sheet where the electron density is high enough to refract the radiation away from the equatorial region. In contrast, for the outbound pass $(R \ge 10 R_s)$ at high latitudes the electron density4 is always low enough to allow direct line-of-sight propagation to the spacecraft at frequencies above a few kHz. The strongest emissions were observed at position A near closest approach, suggesting that the radiation originates in the inner region of the magnetosphere at $R \leq$ $10 R_s$

Examination of the 16-channel survey plots shows evidence of a periodic modulation of the narrowband emission intensities at the 10 h 40 min rotation period of Saturn. Typically, the narrowband emission events last for ~ 3 or 4 h and tend to recur at intervals of ~ 11 h. The three intervals containing B, C-D, and E, in Fig. 4 show this periodicity. Maximum intensity occurs when the Saturn longitude system (SLS) longitude of the subsatellite point is near 290°. Event F in Fig. 4 is also in phase with this general rotational modulation, however, one cycle seems to have been missed between E and F, possibly owing to the very low intensity and difficulty of detecting these emissions far from the planet.

Relationship to other planetary radio emissions

Although the complex narrowband structure of these Saturn radio emissions appears unusual, very similar types of radio emissions have been identified in the magnetospheres of Earth and Jupiter. At Earth a relatively weak type of broadband radio emission called continuum radiation is generated within the magnetospere ^{5,11,12}. This has a relatively smooth steady temporal structure, and when analysed with spectral resolution comparable with that of the Voyager 16-channel analyser, the

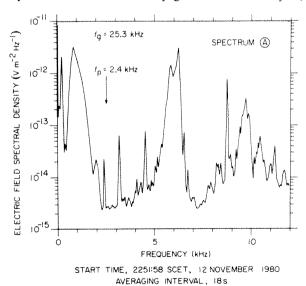
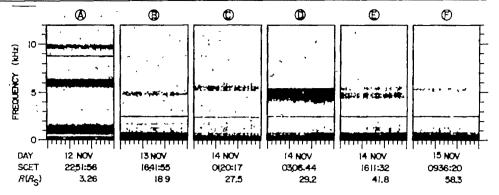


Fig. 2 A detailed frequency spectrum of the emission in Fig. 1, showing the occurrence of numerous bands with approximately harmonic frequency spacings.

Fig. 3 Prequency-time spectrograms obtained in various regions of the magnetosphere demonstrating that the band at ~5-6 kHz can be detected over a large range of radial distances. This series of observations provides the best evidence that the noise consists of freely propagating electromagnetic radiation.



overall appearance is very similar to the emissions shown in Fig. 1. When the terrestrial continuum radiation is analysed with better frequency resolution it becomes apparent that the spectrum is not continuous, but instead consists of a series of discrete narrowband emissions¹³. The detailed characteristics of the continuum spectrum depend on the frequency range. At frequencies below the solar wind plasma frequency, where the radiation is trapped in the low-density magnetospheric cavity, the spectrum tends to be nearly continuous with only occasional narrowband structure¹¹. At frequencies above the solar wind plasma frequency, where the radiation can freely escape from

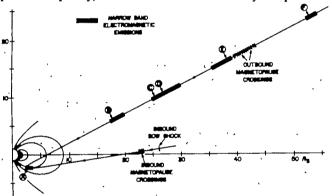


Fig. 4 A summary of regions where narrowband electromagnetic emissions were observed. The black bands indicate detections in the 16-channel spectrum analyser data, and the circles correspond to the wideband spectrograms shown in Fig. 3.

the magnetosphere, the frequency spectrum usually shows considerable narrowband structure. Typically, it consists of several relatively broad emission bands consisting of numerous narrowband features, some of which have harmonic frequency spacings.¹³ The spectral characteristics of the terrestrial narrowband emissions are remarkably similar to those observed at Saturn

At Jupiter narrowband electromagnetic emissions (narrowband kilometric radiation (nKOM)) with similar characteristics have been reported by Kaiser and Desch¹⁴. Although the frequency resolution available for the jovian nKOM observations is not sufficient to reveal the extraordinary fine structure evident in the terrestrial and saturnian emissions, the overall characteristics are quite similar. In particular, the jovian nKOM intensity is controlled by the rotation of Jupiter, very similar to the rotational control of the narrow-band emissions observed at Saturn. The period of the nKOM is slightly slower than the rotation period of Jupiter, and corresponds to the rotation period of the plasma in the outer region of the Io plasma torus¹⁴, at a radial distance of about $10\ R_J$.

The close similarities between the narrowband electromagnetic emissions at Earth, Jupiter, and Saturn strongly suggest that the same basic emission mechanism operates at all three planets. At Earth it is now widely believed that continuum radiation is generated by mode conversion from electrostatic waves generated by low-energy electrons. This general mechanism has a long history of experimental and theoretical development 5.7,15,16, including the observation of enhanced radiation intensities when low-energy electrons are injected into

the magnetosphere¹⁷. The electrostatic wave involved is an electrostatic mode which occurs near the upper hybrid resonance frequency, $f_{\rm UHR} = (f_{\rm p}^2 + f_{\rm g}^2)^{1/2}$. One of the specific conditions which must be satisfied for this mode to be unstable is that $(n+1/2)f_s \simeq f_{UHR}$ (refs 6, 7, 18). The physical situation in the Earth's magnetosphere is illustrated in Fig. 5, which shows a representative radial profile of f_{UHR} and f_g near the plasmapause. Intense electrostatic emissions occur whenever f_{UHR} crosses a half-integral harmonic of f_s (dashed lines). Kurth et al. 13 have demonstrated that the electrostatic emissions are directly converted to electromagnetic radiation with little or no frequency shift. The radiation is generated in the free space (L, 0) mode. The resulting radio emission spectrum therefore consists of a series of lines with a frequency spacing characteristic of the electron gyrofrequency in the source region. Kurth et al. 13 have proposed that the jovian nKOM radiation can be explained by essentially the same mechanism, with the density gradient in the outer region of the Io plasma torus having the same role as the plasmapause at Earth. Large plasma density gradients are an essential element of some theories for the electrostatic to electromagnetic mode conversion¹⁹.

Interpretation of Saturn observations

Because of the close similarities between the narrowband radio emissions at Earth, Jupiter and Saturn, the electrostatic to electromagnetic mode conversion mechanism probably also accounts for the narrowband emissions at Saturn. This view is supported by the fact that intense upper hybrid waves are observed at Saturn in the region where the narrowband electromagnetic radiation is most intense (see Fig. 1). Also, at Saturn these emissions are known to be propagating in the free space (L, 0) mode—the favoured mode for mode conversion. But the exact source location and the physical processes which lead to the generation of intense electrostatic waves remain uncertain.

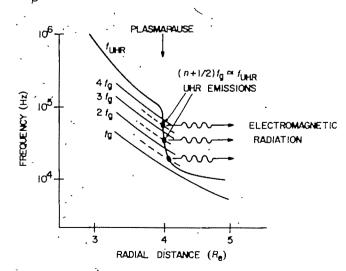


Fig. 5 A model illustrating the mechanism for generating narrowband electromagnetic emissions (continuum radiation) in the Earth's magnetosphere. Intense electrostatic upper hybrid resonance (UHR) emissions at $(n+1/2)f_a \simeq f_{\rm LHR}$ are believed to be converted to electromagnetic emissions in regions with steep plasma density gradients, such as near the plasmapause

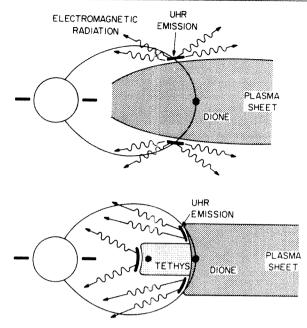


Fig. 6 Two possible models for generating narrowband electromagnetic emissions in Saturn's magnetosphere. a, The emissions are generated at the density gradient which exists at the north and south boundaries of the plasma sheet; b, the emissions are generated along the field-aligned boundaries of the plasma sheet.

Unfortunately, because of no appreciable tilt in the magnetic dipole axis of Saturn, there are no geometry-dependent propagation effects to help determine the source location. Probably the best clue is the frequency spectrum. If the mode conversion model is correct then the frequency spacing between the lines is approximately the electron gyrofrequency in the source region. Figure 2 shows that several half-integral harmonic frequency spacings can be identified in the radio emission spectrum. If we identify the main emissions at 6.0 and 9.7 kHz as a harmonic pair, then the gyrofrequency is 3.7 kHz. A second set of lines can be identified at 3.1, 4.7 and 6.1 kHz, which gives a gyrofrequency of 1.50 kHz. Finally, a third set of lines at 8.7, 9.6, 10.4 and 11.4 kHz gives a gyrofrequency of 870 Hz. Assuming that the source is located near the equatorial plane (as at Earth and Jupiter) the corresponding radial distances for these gyrofrequencies are 5.4, 7.3 and 8.8 Rs. based on a dipole moment of 0.21 G R_s^3 (ref. 3). Note that depending on the plasma density gradient in the source region and the location of the source relative to the magnetic equator, the observed emission frequencies may not occur at exact halfintegral harmonics. Therefore, these radial distances should be regarded as only a rough indication of the source position. The radial distances obtained are in the vicinity of Tethys, Dione and Rhea. Because at least one of Saturn's moons, Dione, is already thought to have an important role in controlling Saturn's kilometric radio emissions 1,20,21, this comparison strongly suggests that one or more of these moons is involved in generating the narrowband electromagnetic emissions.

The most likely way a moon could control nonthermal radio emission processes in the inner region of Saturn's magnetosphere is by injecting plasma much as Io controls radio emissions by injecting plasma into the jovian magnetosphere. Evidence from the Pioneer 11 and Voyager 1 plasma instruments^{4,22} suggests that plasma production by Tethys and Dione may be the source of the extended disk-shaped plasma sheet observed near the equatorial plane of Saturn. If nonequilibrium processes associated with the plasma injection are responsible for the narrowband electromagnetic emissions, then the analogies with Earth and Jupiter would suggest that the source is associated with a steep plasma density gradient near the edge of the plasma sheet. Figure 6a, b shows two extreme models for the source positions. In Fig. 6a the source is located near the north-south boundaries of the plasma sheet, and in Fig. 6b near the sharp

field-aligned inner boundary of the plasma sheet described by Bridge et al.4.

If the source is located as in Fig. 6a then it would have to be tightly confined to a specific L-shell to produce the narrow emission spectrum. Such a localization could, for example, be caused by a field-aligned current system flowing along the Dione L-shell. A source located on the Dione L-shell at the northsouth boundary of the plasma sheet $2 R_s$ from the equatorial plane would have an electron gyrofrequency in close agreement with the 3.7-kHz major frequency spacing evident in Fig. 2. On the other hand, if the source is located along a field-aligned inner boundary of the plasma sheet then the narrowband characteristic of the source would be easily explained as the gyrofrequency is, to first order, constant along the magnetic field line near the equator. This situation could be closely analogous to the generation of continuum radiation near the plasmapause at the Earth, except that the decreasing density gradient is facing towards rather than away from the planet. Note that the 7.3 R_s radial distance identified for the 1.5 kHz frequency spacing in Fig. 3 closely agrees with field-aligned boundary identified by Bridge et al. 4 at L = 7. This boundary occurs slightly beyond the orbit of Dione. Because of uncertainties concerning the plasma density distribution in the inner region of the magnetosphere, it is too early to decide which can best account for the characteristics of these emissions.

Conclusion

The Voyager 1 flyby of Saturn has revealed narrowband electromagnetic emissions originating from the inner region of the magnetosphere. At closest approach the broadband electric field strength of these emissions is $\sim\!50~\mu V~m^{-1}$, which corresponds to a total radiated power of about $2.5\times10^6~W$, assuming an isotropic source. Although this is small compared with the power radiated by Saturn at kilometric wavelengths, $\sim\!10^8-10^9~W$ (ref. 20), these emissions are of interest because of the close similarity to narrowband radio emissions from Earth and Jupiter.

Comparisons with similar emissions in the Earth's magnetosphere suggest that they are produced by mode conversion from electrostatic waves near the upper hybrid resonance. This radio emission mechanism has also been proposed to explain certain types of solar radio emissions2. Although the basic mechanism has been identified, the conditions in which the radiation can occur are poorly understood. Observations at all three planets suggest that distinct 'hot spots' occur from which the radiation is preferentially emitted. The association of the source with a plasma density gradient may be a requirement for the mode conversion process¹⁹. Unfortunately, the requirements for generating the electrostatic waves are not completely understood. The generation of these waves probably involves the presence of large fluxes of low energy (100 eV to 1 keV) electrons with a pronounced loss-cone or $\partial f/\partial v_{\perp} > 0$ feature and a suitably large cold-to-hot electron density ratio 18,24,25 The apparent control of the narrowband emission intensities by Saturn's rotation presumably provides an important clue to the processes involved. However, because of the very close alignment of the dipole axis with the rotational axis of Saturn³, it is not clear how this rotational control is imposed.

Finally, at Saturn no evidence was found for trapped continuum radiation comparable to the continuum radiation trapped in the low density magnetospheric cavity of Earth and Jupiter. As the Voyager 1 plasma measurements⁴ demonstrate the existence of a low-density cavity at Saturn with an electron plasma frequency well below the solar wind plasma frequency, it is surprising that no radiation is trapped in this cavity. The radio emission source at Saturn does not seem to extend down to sufficiently low frequencies to illuminate the cavity. The reasons for this marked difference in the radio emission spectrum are not understood.

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- 1. Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 235-239 (1981).
- Gurnett, D. A., Kurin, W. S. & Scart, F. L. Science 212, 237-239
 Scarf, F. L. & Gurnett, D. A. Space Sci. Rev. 21, 289-308 (1977).
 Ness, N. F. et al. Science 212, 211-217 (1981).
 Bridge, H. S. et al. Science 212, 217-224 (1981).
 Gurnett, D. A. J. geophys. Res. 80, 2751-2763 (1975).

- Christiansen, P. et al. Nature 272, 682-686 (1978) Kurth, W. S. et al. J. geophys. Res. 84, 4145-4164 (1979).
- Scarf, F. L. et al. Science 204, 991-995 (1979).
 Gurnett, D. A. et al. Science 206, 987-991 (1979).

- 10. Birmingham, T. J. et al. J. geophys. Res. (in the press)
- Gurnett, D. A. & Shaw, R. R. J. geophys. Res. 78, 8136-8149 (1973).
 Frankel, M. S. Radio Sci. 8, 991-1005 (1973).

- Kurth, W. S. et al. J. geophys. Res. 86, 5519-5531 (1981). Kaiser, M. L. & Desch, M. D. Geophys. Res. Lett. 7, 389-392 (1980).
- Jones, D. Nature 260, 686-689 (1976).
- Melrose, D. B. J. geophys. Res. 86, 30-36 (1981)
- Melrose, D. B. J. geophys. Res. 86, 30-36 (1981).
 Gurnett, D. A. & Frank, L. A. J. geophys. Res. 81, 3875-3885 (1976).
 Rönnmark, K. et al. Space Sci. Rev. 22, 401-417 (1978).
 Jones, D. Nature 288, 225-229 (1980); Adv. Space Res. 1, 333-336 (1981).
 Warwick, J. W. et al. Science 212, 239-243 (1981).
- Kurth, W. S. et al. Nature 292, 742-745 (1981). Frank, L. A. et al. J. geophys. Res. 85, 5695-5708 (1980).
- Kuijpers, J. Radio Physics of the Sun (eds Kundu, M. & Gergely, T.) 341-361 (Reidel, Dordrecht 1980)
- 24. Hubbard, R. F. & Birmingham, T. J. J. geophys. Res. 83, 4837-4850 (1978)
- 25. Ashour-Abdalla, M. & Kennel, C. F. J. geophys. Res. 83, 1531-1543 (1978)

Saturn's radio emissions: rotational modulation

C. K. Goertz*†, M. F. Thomsen*‡ & W.-H. Ip*

*Max-Planck-Institut für Aeronomie, 3411 Katlenburg-Lindau 3, FRG †Department of Physics and Astronomy, University of Iowa, Iowa City, Iowa 52240, USA ‡Los Alamos Scientific Laboratory, Los Alamos, New Mexico, 87545, USA

The unexpected rotational modulation of the Saturn kilometric radiation and Saturn electrostatic discharges, as revealed by Voyager 1 observations, are discussed in terms of a ring-current system and the geometry of the dipole field.

THE Saturn flyby observations of Pioneer 11 and Voyager 1 have revealed that the Saturnian ring system has important effects on the structures of the planetary ionosphere as well as the magnetospheric environment, such as the unexpected rotational modulation of the Saturn kilometric radiation (SKR) and the Saturn electrostatic discharge (SED) as discovered by the Voyager planetary radio astronomy experiment¹. The emissions of the bursty and highly polarized SKR (f < 1,200 kHz) and the broad banded and unpolarized SED ($f \sim 20.4 \text{ kHz}-40.2 \text{ MHz}$) of impulsive nature strongly peaks at the subsolar longitude (SLS) of ~110°. The rotational control of the SKR and SED is a clock-like effect-with radiations preferentially emitted when Saturn has a particular phase relative to the Sun 1,2. Although the sources of these radio emissions are not clear, Warwick et al.1 argued that the SED emissions most likely originate in the rings of Saturn.

The challenge then is to devise a mechanism which can produce electrodynamic coupling between the ring system and the planetary ionosphere and/or magnetosphere in which the associated SKR and SED radio emissions are subject to longitudinal control. One obvious candidate is the establishment of field-aligned current systems—connecting the rings and the ionosphere with longitudinal variation. Local acceleration processes in the vicinity of the rings are also needed. Although the solution will probably have to await further observations and more detailed data analyses, we now discuss the interplay between the ring-current systems and the geometry of the dipole field and the rings and describe some of the basic ingredients in the longitudinal modulations of SKR and SED.

The Pioneer 11 radio measurements³ and the Voyager 1 EUV experimental results⁴ reveal that a strong coupling between the neutral hydrogen atmosphere of the rings with a number density of 400-600 cm⁻³ (refs 4-7) and the ionospheric plasma. Assuming the ionospheric H⁺ ions have a number density of $n_i \sim 3 \times 10^3$ cm⁻³ near the ring plane, the pertinent chargeexchange loss time scale for the neutral H atoms can be estimated to be $\sim 10^6$ s. Thus, for a total volume of the ring atmosphere to be $\sim 3 \times 10^{30}$ cm³, the charge-exchange loss rate of the neutral atoms as a result of the $H^+ + H \rightarrow H + H^+$ process is $\dot{N} \sim 10^{27} \, \mathrm{s}^{-1}$. This value is perhaps the most direct estimate for the source strength of the ring atmosphere and it supports the assessment by Carlson⁸ that photo-sputtering of atomic hydrogen atoms from H₂O ice on the ring particles is responsible for supplying the observed H cloud of the rings, as other proposed effects9.10 are far less efficient.

Note that the acceleration of the new ions (from charge exchange and other ionization effects) to co-rotation with the

magnetosphere will necessitate the establishment of a so-called pickup current which flows radially outward outside the synchronous orbit (at 1.8 R_s) but inward inside this distance^{11,12}. An order-of-magnitude approximation can be obtained by using $I \sim \dot{M} \Delta v / B \Delta x$ for the total current. Here \dot{M} is the mass addition rate. B is the average magnetic field ($\sim 2,500 \text{ nT}$), $\Delta v \sim$ 1 km s⁻¹ is the difference between the corotating speed and the keplerian orbital speed, and Δx is the characteristic radial dimension of such a ring-current system. Even when the ionization rate of oxygen atoms is set equal to that of neutral H atoms, $I \le 10^3$ A for $\Delta x \sim 1 R_s$. This pickup current system is therefore relatively small, and not subject to rotational modula-

Another current system is more promising. The near-perfect dipole field of Saturn has a slight vertical displacement (h) of the dipole centre from the planet's centre along the axial direction 13-15. This enables charged particles to mirror just above the ring plane without being absorbed—a sort of plasma disk may be formed there. The longitudinal drifts (due to magnetic curvature force, gravity etc.) of the ions and electrons in this region would

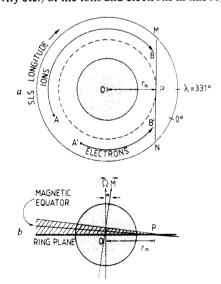


Fig. 1 a. Top view of the intersection between saturnian rings and the magnetic equator. The two planes intersect at \overline{MN} and $\overline{OP} = 1.64 R_s$ if the vertical displacement is $0.02 R_s$ and the tilt angle α is 0.7°. Inside the synchronous orbit r_{syn} at 1.8 R_s the ions drift west $(A \rightarrow B)$ and electrons east $(A' \rightarrow B')$. Beyond r_{syn} they move in opposite directions. b, Side view of the above system. Particles with near 90° equatorial pitch-angle in the wedgeshaped 'free-zone' are not subject to any absorption.

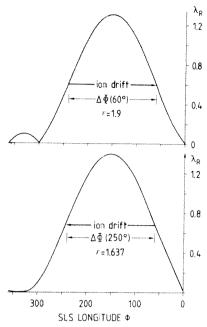


Fig. 2 The magnetic latitudes λ_r of the ring for h=0.02 and $\alpha=0.7^\circ$ for two radial distances: a, r=1.9 R_s ; b, r=1.64 R_s . Also indicated are longitudinal ranges of ions between ionization and ring absorption.

then constitute a current flowing in the azimuthal direction. At the same time, there is also a small tilt (α) of the dipole axis with respect to the spin axis of Saturn towards SLS longitude 331° (ref. 15). The important feature is that the magnetic equatorial plane and the ring plane intersect along a line which has its minimum distance from Saturn (r_m) at an SLS longitude of 331°. For $\alpha = 0.7^{\circ}$ and an offset of $h = 0.02 R_{\rm S}$ (refs 13-15), $r_{\rm m} =$ 1.64 R_s . This combination of the vertical displacement and the tilt would cause the azimuthal currents to be interrupted at certain positions in the rings for $r > r_m$ (see Fig. 1). (For $r < r_m$ the magnetic latitude of the ring plane is always non-zero and some charged particles will therefore escape absorption by the rings. This disk-population may suffer from pitch-angle scattering through wave-particle interactions and become unstable.) Assuming that the mirror-latitudes of the ions do not change over a time scale of one longitudinal drift around the planet, their drift paths $(r\Delta\Phi)$ can be determined by the difference between magnetic latitude (λ_r) of the ring plane and their mirror-latitudes (λ_m) . Once the charged particles drift into the region with $\lambda_m > \lambda_r$, they would be absorbed. The variations of $\Delta \Phi$ for two radial distances are sketched in Fig. 2.

Assuming current continuity $(\nabla \cdot j = 0)$, the longitudinal variation of the azimuthal current (j_{\perp}) due to the mirroring effect would mean continuous diversion of the currents into the field-aligned components (j_{\parallel}) . To estimate the field-aligned current at different longitudes (ϕ) the following relations are needed. First, the current continuity equation can be written as

$$j_{\parallel} = -\frac{1}{r} \frac{\partial}{\partial \phi} \int j_{\perp} \, \mathrm{d}z \tag{1}$$

and second, the continuity equation for mirroring particles as

$$\frac{1}{r} \frac{\partial}{\partial \phi} (n_i v_d) = \frac{n_H}{\tau_i} - L_i$$
 (2)

with $j_{\perp} \approx n_i e v_d$ where n_i is the ion number density at longitude ϕ , v_d is the drift velocity¹⁶, n_H is the neutral H atom number density of the ring atmosphere (say), τ_i the ionization time scale and L_i the loss rate. Assuming that the ions can only mirror along the field lines in the region where $\lambda_i > \lambda_m$,

$$n_{\rm i} \sim n_{\rm H} \left(\frac{\tau_{\rm d}}{\tau_{\rm e}}\right)$$
 (3)

where the longitudinal drift time scale between ionization and absorption is $\tau_d = r\Delta\Phi(\phi)/v_d$. Combining these relations, the field-aligned current density at longitude ϕ can be written as:

$$j_{\parallel}(\phi) \sim er \frac{\partial}{\partial \phi} \int_{0}^{\lambda_{r}(\phi)} \frac{n_{H}}{\tau_{i}} \Delta \Phi(\phi) d\lambda$$
 (4)

Note that in the corotating system $\Delta\Phi > 0$ if ions drift westwards towards increasing SLS longitudes (inside the synchronous orbit) and $\Delta\Phi < 0$ if ions drift in the opposite direction (outside the synchronous orbit).

Contour plots of the field-aligned current density (j_{\parallel}) inside the synchronous orbit are shown in Fig. 3. Two maxima are found at SLS longitudes 120° (upward current from ionosphere to the ring) and 250° (downward current from the ring to ionosphere). Such field-aligned currents are corotating with the planet and if the ionization rate in this system is subject to diurnal variation, rotational modulation may conceivably result. For example, the escape flux of the ionospheric electrons reaching the ring at L=1.6 may have large day-night variation due to absence of photoelectron emission and cooling of the exosphere in the night side. (Note that an important nightside heat source, precipitation of energetic particles, does not exist at the latitudes magnetically connected to the rings as the rings are extremely good absorbers of energetic charged particles.)

But note that the azimuthal currents and the associated field-aligned components would be maintained, to a certain degree, even in the shadow of Saturn. This is because the ions produced in the dayside would continue to drift into the shadow to replenish the drift currents. The actual ionization rate must be largely determined by charge exchange between the neutral ring atmosphere and the planetary ionosphere as photoionization and electron impact ionization all have very long ionization time scales ($\geq 10^9$ s). Using the minimum value of $\tau_i \sim 10^6$ s as appropriate for regions a fraction of a planetary radius from the ring plane, and a number density of 500 cm⁻³ for the hydrogen atoms) the peak flux (at SLS longitude 250°) can be estimated as $F_{\parallel} \sim 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ in the equator and $10^7 \text{ cm}^{-2} \text{ s}^{-1}$ near the top of the saturnian ionosphere. This is larger than the estimated photoelectron flux of 2×10^5 cm⁻² s⁻¹ from Saturn's ionosphere 10. Thus the downward field-aligned current near SLS longitude 250° could become supercritical, particularly when it moves into Saturn's shadow. In such conditions, an electric field parallel to the magnetic field must be set up accelerating the ionospheric electrons away to maintain the downward current. The parallel potential drop could reach an absolute maximum equal to the total potential drop ψ across the current generating region.¹⁷ This potential ψ arises from the fact that the fieldaligned currents must close through the saturnian ionosphere which has a finite conductivity. According to Hill et al. 18 the total

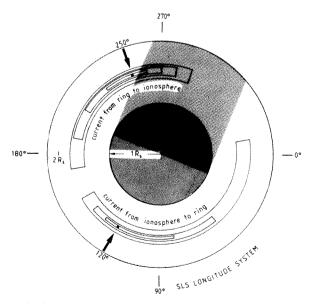


Fig. 3 Contour plot for the parallel electron flux (inside the synchronous orbit) associated with the field-aligned current system. Note that maximum flux from ionosphere to the ring occurs at SLS longitude 250° and the maximum flux from the ring to the ionosphere occurs at 120° SLS longitude.

potential drop across the longitude range $\Delta\Phi$ is

$$\psi = \frac{r\Delta\Phi}{\Sigma_{\rm p}} \int j_{\perp} \, \mathrm{d}z = \frac{I}{\Sigma_{\rm p}} \frac{r\Delta\Phi}{\Delta r}$$
 (5)

Here Δr is the radial range of the field-aligned currents at radial distance r, $\Delta \Phi$ is the corresponding longitude range and Σ_p is the height-integrated Pederson conductivity of the planetary ionosphere. With the total current going through the ring system estimated to be $\sim 10^4 - 10^5 \,\text{A}$, $\Delta \Phi \sim \pi$, $r \sim 1.5 \,R_J$ and $\Delta r \sim$ 0.3 R_1 ; $\psi = (0.15-1.5)/\Sigma_p$ (MV). Because Σ_p should be of the order of 1 S, if not less, the parallel potentials are conceivably quite large, that is of the order of a few keV.

Although the incorporation of SKR and SED radio emissions into this scheme is tempting but ambiguous a plausible scenario is offered below. We assume that a parallel potential of a few keV could be established in the shadow at SLS longitude 250° and that the ring particles are charged up to electrostatic potential of similar magnitude by the energetic electrons so generated. As soon as the ring particles move into the sunlight there will be immediate electrostatic discharge through emission of photoelectrons (accelerated to keV energies by the negative surface potential)¹⁹. The electrostatic energy stored on a 1-m radius ring particle charged to 10 keV is 10^{-2} J . Assuming no surface resistance the discharge time would be $\tau \sim 4 \times 10^{-8} \text{ s}$. The total

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- Warwick, J. W. et al. Science 212, 239 (1981).
- Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 235 (1981).
 Kliore, A. J. et al. Science 207, 446 (1980).
- Broadfoot, A. L. et al. Science 211, 206 (1981)
- Weiser, H., Vik, R. C. & Moos, H. W. Science 197, 755 (1977).
 Barker, E. et al. Astrophys. J. 242, 383 (1980).
- Judge, D. L., Wu, F. M. & Carlson, R. W. Science 207, 431 (1979).Carlson, R. W. Nature 283, 461 (1980).

- 9. Dennefeld, M. IAU Symp. No. 65, 471 (1974). 10. Ip, W.-H. Astr. Astrophys. 70, 435 (1978).

power emitted by each ring particle is 2.5×10^5 W and should be broadband extending to a frequency of roughly $1/\tau \sim 25$ MHz. The maximum intensity of SED should then occur when the spot at SLS longitude 250° emerges from the shadow at which time the SLS longitude 110° faces the Sun (see Fig. 3). This is almost exactly the longitude at which the SED and SKR activities peak. (It is not clear whether the formation of radial spokes in the B ring as observed by the Voyager Imaging experiment²⁰ is related to this effect.)

SKR events still have to be explained and to a certain extent SKR may also depend on the SED. That is, as the broadband and bursty SED radiation propagates outwards, it will be absorbed by the dense plasma sheet between 4 and 8 R_s (refs 20, 21). If pitch-angle scattering of trapped energetic particles into the atmospheric loss cone is enhanced by plasma wave absorption, rotational modulation of SKR (in the desired manner) would follow. As there are sharp rises in the proton $(E_p <$ 2 MeV) and electron (E_e <0.43 MeV) fluxes near the orbit of Dione²², precipitation events triggered by the SED radiation must involve principally particles from that region. It is perhaps no great surprise that the SKR emission—besides the 10-h rotational modulation—also seems to be affected by Dione².

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- 11. Ip, W.-H. & Axford, W. I. Nature 283, 180 (1980)
- Goertz, C. K. J. geophys. Res. 85, 2949 (1980). Smith, E. J. et al. J. geophys. Res. 85, 5651 (1980)
- Acuña, M. H., Ness, N. F. & Connerney, J. E. P. J. geaphys. Res. 85, 5675 (1980). Ness, N. F. et al. Science 212, 211 (1981).

- Siscoe, G. L. J. geophys. Res. 82, 1641 (1977). Smith, R. A. & Goertz, C. K. J. geophys. Res. 83, 2617 (1978).
- Hill, T. W., Dessler, A. J. & Maher, L. J. J. geophys. Res. (in the press). Mendis, D. A. & Axford, W. I. A. Rev. Earth planet. Sci. 2, 419 (1974)
- Frank, L. A. et al. J. geophys. Res. 85, 5699 (1980). Bridge, H. S. et al. Science 211, 217 (1981).
- 22. Trainer, J. H., McDonald, F. B. & Schardt, A. W. Science 207, 421 (1980).

Saturn's kilometric radiation: satellite modulation

M. D. Desch & M. L. Kaiser

NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Planetary Magnetospheres Branch, Greenbelt, Maryland 20770, USA

There is an episodic 66-h modulation of the Saturn kilometric radiation which is both frequency and Dione-phase dependent. The behaviour is significantly different from the way in which Io modulates the jovian emission.

THE discovery¹ of nonthermal (kilometric) radio emission (SKR) from Saturn by the Voyager planetary radio astronomy (PRA) instrument has raised the possibility of emission modulation by one or more of its satellites because Saturn, like Jupiter, contains satellites deep within a corotating magnetic field. However, examination of data extending from January to September 1980, yielded no Saturn analogue of the well-known Io-control phenomenon². Instead, short-term (10 day) modulation of the SKR has been reported^{3,4} at a period near 66 h, thus implicating satellite Dione as a possible agent. The nature of this modulation, if real, differs dramatically from the way in which Io controls the jovian emission. Using the PRA observations of Saturn, we here search for further evidence of episodic satellite control of SKR and examine the time evolution, frequency dependence and phase of the 66-h modulation. Results will be compared with the phenomenology of the jovian control by Io.

The earlier reports of modulation by Dione presented data in intensity/time format at Dione's revolution period. We will use the method of power spectral analysis to cast the results in a more objective light, although composite intensity-time dynamic spectra will also be used to illustrate graphically the material. The power spectrum method used is that of Deeming⁵, and has been applied to the PRA data as described elsewhere².

Results

Figure 1 shows the results of power spectral analysis of SKR observed at 174 kHz between 28 October and 18 December

1980. This period of time brackets the Saturn encounter of 12-13 November. The spectrum spans the range of periods from just under 10 h to just above 100 h, corresponding to the range of expected periods of revolution of Saturn's five major innermost satellites and the planetary rotation itself. Aside from the dominant peak near 11 h, which corresponds to modulation of the SKR at Saturn's rotation period, no other statistically significant peaks are evident in the spectra, and, in particular,

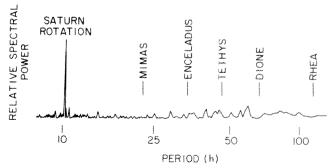


Fig. 1 The results of power spectral analysis of SKR observed at 174 kHz between 28 October and 18 December 1980. Only SKR events exceeding a flux density threshold of 2×10^{-21} W m⁻¹ (normalized to a standard observer-Saturn distance of 1 AU) were used to eliminate a severe inverse-R-squared bias during this period. The only statistically significant modulation occurs at the 10.66-h Saturn rotation period.

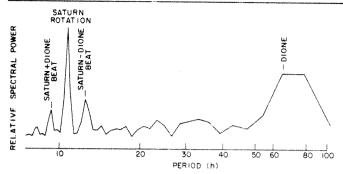


Fig. 2 Power spectral analysis like that described in Fig. 1 but for a shorter interval of time (10-18 November) at 59 kHz observing frequency. Statistically significant peaks occur at the Saturn rotation period, at 66 h, and at the two principal heterodynes between these periods.

there is no significant spectral power at any of the satellite periods. Several other radio frequencies in the SKR band, both above and below 174 kHz, show identical results.

The 66-h modulation

If we examine the PRA low frequencies for the time interval nearer encounter, significant modulation appears near a period of 66 h. To within the 10-h resolution limit inherent in the spectrum near the low-frequency end, this period corresponds to Dione's period of revolution of 65.68 h. In particular, Fig. 2 shows the power spectrum for the analysis interval 10-18 November at an observing frequency of 59 kHz. This 9-day period is equal to three Dione revolutions. The four principal peaks in the spectrum are Saturn's rotation period² (10.66 h). Dione (66 h), and the two principal heterodynes between the 10.66 and 66-h periods. A composite dynamic spectrum of the same time interval (Fig. 3a) shows the phase of this 66-h modulation. Here, it is clear that the predominant effect is an apparent quenching of the low-frequency SKR near 270° heliocentric phase, that is when Dione is above Saturn's dusk meridian. There is a pronounced gap in the emission occurrence levels at this phase, especially at frequencies below ~200 kHz. Above 200 kHz the emission has begun to fill into the gap, suggesting a frequency dependence of the effect. In addition, because this analysis interval spans the time of closest approach to Saturn, so that the emission is observed from two very different Sun-Saturn-spacecraft angles, the modulation would appear to be locked with respect to the Sun rather than the observer. Note also that there is an additional modulation apparent in Fig. 3. Peaks in the occurrence probability contours occur at 40°, 100°, 160°, 220°, and 340° Dione phase (filled triangles) and are due to the planetary-rotation modulation which is within 3% of an integral multiple of Dione's period. The phase of the expected, but missing, episodes of rotation-modulated SKR are also indicated (open triangle). During this 9-day

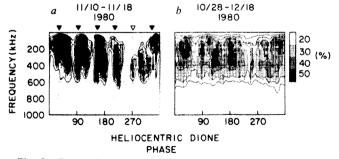


Fig. 3 Composite frequency-time dynamic spectra formed by stacking SKR events at Dione's heliocentric period of revolution for the time interval a 10–18 November 1980 and b 28 October-18 December. Grey-shaded contour intervals show the resultant per cent occurrence probability levels. The dynamic spectra cover the frequency range 1.2–1,000 kHz. ∇ in a show the phase of expected peaks in occurrence levels due to Saturn's rotation; \triangle shows the 'missing' events. Only events exceeding $2 \times 10^{-21} \, \mathrm{W \, m^{-2} \, Hz^{-1}}$ at 1 AU were used.

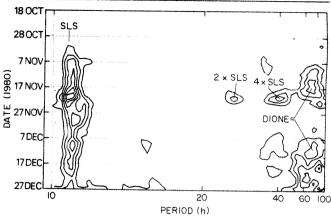


Fig. 4 Results of power spectral analysis on 12-day spans of data from 12 October to 31 December 1980. Spectra were stepped in 1-day increments. Observing frequency was 78 kHz. Contours show levels of relative spectral power as a function of spectral period and centre date of 12-day span. The contour intervals are 15, 25, 35, 45, 55 and 65% of the maximum spectral power. The modulation at Saturn's rotation period (indicated here as SLS, or Saturn longitude system) and two and four times the period are indicated. The broad spectral peak near Dione's period of revolution can be seen to occur in two episodes.

period, three such episodes fail to occur. It is this gap which produces the peak in the power spectrum at 66 h in Fig. 2.

Figure 3b supports the long-term power spectral result of Fig. 1. It shows again that when data are examined over extended periods, in this case 28 October to 18 December including the 9-day interval in Fig. 3a, the emission occurrence probability levels are distributed randomly in Dione phase. Modulating effects near Dione's period disappear over the long term.

Satellite modulation survey

Before exploring the 66-h modulation further, it is useful to investigate the possibility of additional episodic, short-term modulations. To do this we computed power spectra over 12-day intervals between 12 October and 31 December 1980. Successive spectra were stepped along in 1-day increments, thus yielding an independent spectral estimate on every twelfth point. Each spectrum covered the 90-h window in period spacing from 10 to 100 h (as in Fig. 1). The result is shown in Fig. 4. Contour intervals are proportional to spectral power. The dominant feature is, as might be expected, the Saturn rotation modulation at 10.66 h. It is statistically dominant over almost the entire analysis interval. Outside of this modulation, no significant power appears at periods ≤50 h, except for subharmonic power at 2 and 4 times 10.66 h which appear for a short interval of time over the computation window centred on 21 November 1980 when the modulation at the fundamental is strongest. Above 50 h significant modulation appears twice briefly near 66 h. A third island of significant spectral power slightly below 66 h is centred on 22 December; however, closer examination of the data shows it to be due to an accidental

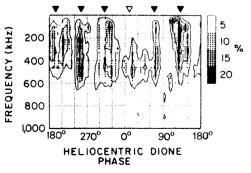


Fig. 5 As in Fig. 3 but for the period 2-14 December 1980. Only events exceeding a flux threshold of $8\times10^{-21}\,\mathrm{W}\,\mathrm{m}^{-2}\,\mathrm{Hz}^{-1}$ (at 1 AU) were used. The 'Dione gap' has shifted to about 30° phase. Note that the abscissa is shifted 180° for clarity.

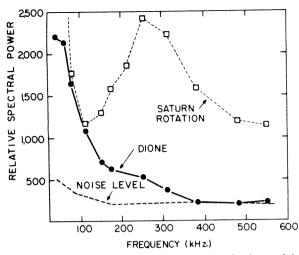


Fig. 6 The decline with increasing frequency in effective modulation at Dione's period of revolution. Spectral power at Dione's period falls to the spectrum noise level at 385 kHz. Modulation due to Saturn's rotation remains strong over the entire band. Analysis interval from 10–18 November 1981. Only events exceeding 2×10^{-21} W m⁻² Hz⁻¹ at 1 AU were included.

reoccurrence of data gaps at this period. At still longer spectral periods additional minor peaks appear; however, their significance is in doubt because they are so near the lowfrequency limit of the power spectrum. Identical surveys at several other radio frequencies in the SKR band have yielded identical results. Thus no evidence of short-term modulations of the SKR by any other satellites exists, at least during the late October to December period.

The two significant peaks in Fig. 4 labelled Dione correspond, first, to an interval of time around encounter, mentioned in the discussion of Figs 2 and 3, and, second, to an interval of time in early December. The activity for the period 2-14 December is shown in Fig. 5 in composite frequency-time dynamic spectrum format. The occurrence probability levels are again shown using gray-shaded contour intervals, and the data are organized in heliocentric Dione phase. The expected Dione phase of modulations due to planetary rotation are shown at 10.66-h intervals. The open triangle indicates the 'missing' rotation, in this case 5 missing rotations over the 13-day interval covered. Instead of occurring near 270° Dione phase as was the case near encounter (Fig. 3), the emission gap is now centred on about 30° Dione phase (note shifted phase scale for clarity). The gap is much more pronounced at the lowest frequencies, below 200 kHz, and has disappeared at 400 kHz and higher. This same frequency behaviour was noted with regard to the 66-h modulation observed near encounter.

Frequency dependence

Using power spectra computed at several observing frequencies, we have examined the dependence of the Dione modulation on frequency throughout most of the SKR frequency band. Both Figs 3 and 5 suggested a frequency dependence may exist. The analysis interval extends over the 9-day encounter period shown in Fig. 3 and the results are shown in Fig. 6. Here the power at 66 h is compared with the 10.66-h modulation of the SKR due to Saturn's rotation. The noise level inherent in the power spectra at each frequency is also shown. Note that the magnitude of the 66-h modulation falls by almost a factor of four between 40 and 200 kHz, and reaches statistically insignificant levels at frequencies exceeding ~385 kHz. In total this represents an order of magnitude drop in spectral power over a decade in frequency, f, thus corresponding to a 1/f decline. By comparison, the planetary rotation modulation is strong

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throughout the frequency band, although it also varies with frequency. The rotation modulation approximately follows the shape of the emission flux density spectrum which also has a peak near 200-300 kHz (ref. 6). Below 100 kHz, however, the Saturn rotation modulation increases sharply due, we think, to a possible low-frequency (f < 100 kHz) emission component⁴

Only those radio events which exceeded a constant flux density threshold, in this case $2 \times 10^{-21} \,\mathrm{W m^{-2} \, Hz^{-1}}$ normalized to an equivalent observer-Saturn distance of 1 AU, were used to derive the frequency dependence shown in Fig. 6. Because there is some dependence of spectral power on the choice of flux threshold used, we examined the effect on the frequencydependence curve of varying this threshold. We found that, over a two decade range of flux densities, the modulation is always greatest at low frequencies with no statistically significant modulation appearing at f > 400 kHz. The results shown for early December (Fig. 5) are clearly consistent with this frequency dependence. We conclude that the 66-h modulation is strongly and inversely dependent on frequency.

Discussion

In the two intervals of time during which significant modulation occurs near a spectral period of 66 h the modulation (1) varies inversely with frequency and (2) persists for short intervals limited to 3-5 cycles of the 66-h period. The phase of the modulation is not constant, however. The observed phase of the emission gap changes from about 270° Dione phase for the November episode to ~30° phase for the December episode.

Phenomenologically this modulation differs dramatically from the way in which Io is observed to control the jovian emission (DAM). Io enhances rather than quenches the emission level of DAM; the Io effect is, to first order, independent of frequency; the phase of the emission is fixed with respect to the observer, not the Sun; and, finally, Io control is not an intermittent phenomenon.

If Dione is responsible for the 66-h modulation of the SKR, it is difficult to imagine what mechanism could cause such an effect. Certain features, namely the cutoff in observed emission level and the 1/f frequency dependence, are consistent with preferential absorption or strong refraction of SKR at low frequencies due to a moderately dense plasma associated with Dione. In fact, Pioneer 11 (ref. 7) and Voyager^{3,8} observations seem to support the existence of a 15 to 20 cm⁻³ electron plasma in the vicinity of 4-8 R_s . Dione, at 6.3 R_s , is within this plasma. However, other observed features of the modulation are inconsistent with this simple picture. One might expect, for example, a more pronounced modulation on the inbound observing period when Voyager, at a saturnigraphic latitude of +8°, viewed the emission from a point closer to the equator plane. Instead, the outbound geometry seems more favourable, when Voyager was at +26° latitude. Also, during the first episode of the Dione modulations, one of the SKR gaps occurred near closest approach when Voyager 1 was at high southern latitudes. Furthermore, the variable phase of the modulation makes any simple absorption picture difficult to model.

An alternative method of cutting off the emission includes a more dynamic interaction between Dione and the radio source region. There is some evidence that Dione may be stimulating field-aligned currents, perhaps as Io does at Jupiter. If so, Dione may act, in some as yet unspecified way, to quench the emission process physically. The Dione flux tubes do intersect the modelled⁶ radio source region; however, the orbital phase of the intersection is near 120° Dione phase, not the preferred 270° or 30° gap locations. Thus neither the absorption nor dynamic quenching models are especially attractive, and we hope that the Voyager 2 observations of SKR near closest approach will shed some light on this perplexing problem.

Kaiser, M. L. et al. Science 209, 1238-1240 (1980)

Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 253–256 (1981). Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 235–239 (1981). Warwick, J. W. et al. Science 212, 239–243 (1981).

Deeming, T. J. Astrophys. Space Sci. 36, 137-158 (1975)

Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731-733 (1981). Frank, L. A. et al. J. geophys. Res. 85, 5695-5708 (1980).

Sittler, E. C., Scudder, J. D. & Bridge, H. Nature 292, 711-714 (1981).
Acuña, M. A., Connerney, J. E. C. & Ness, N. F. Nature 292, 721-724 (1981).

Control of Saturn's kilometric radiation by Dione

W. S. Kurth*, D. A. Gurnett*, & F. L. Scarf*

*Department of Physics and Astronomy, University of Iowa, Iowa City, Iowa 52242, USA †TRW Defense and Space Systems, One Space Park, Redondo Beach, California 90278, USA

Voyager 1 observations of Saturn's kilometric radio emissions reveal a strong but apparently transitory control by the orbital phase angle of Dione. This may be a geometrical effect and a time-variable plasma torus associated with Dione could explain most of the observed details of the Dione modulation by creating a shadow zone near the equatorial plane.

THE Voyager planetary radio astronomy investigation provided the first incontrovertible evidence of nonthermal radio emissions from Saturn¹. The radiation spectrum peaks in the kilometre wavelength regime near 200 kHz and periodicities in the occurrence of the radio bursts give an internal planetary rotation period of 10 h, 39.9 min (ref. 2). Observations of the radio emission at 56.2 kHz by the plasma wave instrument on Voyager 1 revealed a modulation of the intensity of the radio emission with a period very close to the orbital period of Dione indicating that some control of the emission was a function of the orbital phase of the moon³. This control was also reported by the planetary radio astronomy investigation4 with the maximum effect seen at lower frequencies⁵, but extending as high as 385 kHz. The magnitude of the Dione influence is not constant in time and was strongest near closest approach. (Dione has a radius of 560 km (ref. 6) and is 6.29 Rs from Saturn in an orbit whose eccentricity is 0.002.)

Here we analyse the effect of Dione's orbital phase on the emission of radio waves from Saturn to explain the apparent transitory nature and suggest a mechanism for the interaction with the moon.

Nature of Dione's influence

We begin by describing the saturnian kilometric radio (SKR) spectrum below $\sim\!56\,\mathrm{kHz}.$ Figure 1 is a typical event as seen by the plasma wave instrument as Voyager approached Saturn on 11 November 1980. The amplitude of radio signals as a function of time for the upper five channels of the plasma wave spectrum analyser are shown. The height of the solid black area represents the average power flux (W m $^{-2}$ Hz $^{-1}$).

The radio burst shown in Fig. 1 increases in amplitude with increasing frequency to a peak at least as high as the upper frequency limit of the instrument (56.2 kHz). The emission is highly time-variable, changing in amplitude by nearly an order of magnitude on time scales of a few minutes. While gross amplitude changes track fairly well from one channel to the next, many of the fine-scale features are totally uncorrelated with those of adjacent channels indicating narrowband elements in

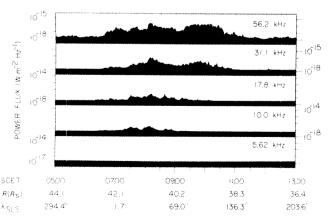


Fig. 1 A typical example of saturnian kilometric radiation extending from near 10 kHz to above 56 kHz.

the spectrum. Hence, the temporal variations of the SKR are similar in many respects to the auroral kilometric radiation from the Earth. The low-frequency cutoff of the saturnian emission has been reported to be frequently near 60 kHz; however, Fig. 1 shows that the emission is detectable at frequencies as low as 10 kHz.

We specifically consider the correlation between the orbital phase angle of Dione, ϕ_{Dione} , and the amplitude of the kilometric radio emission which was originally demonstrated by Gurnett *et al.*³, who showed that in addition to the 10 h 39.9 min modulation related to Saturn's rotation, the emission was strongest when Dione was in the local dawn sector. Figure 2 plots the hourly average power flux of the radio emission at 56.2 kHz as a function of radial distance; also shown is a dashed line

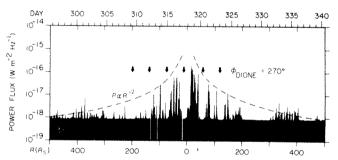


Fig. 2 A plot of hourly average values of the 56.2 kHz plasma wave receiver channel as a function of radial distance from Saturn. The narrow bursts are emitted at a period of $\sim 10 \text{ h}$ 39.9 min and generally rise in amplitude with decreasing R approximately as R^{-2} . The arrows indicate times when Dione is at local dusk and correspond closely to periods when there is little or no radio emission.

with a R^{-2} dependence. The arrows indicate times when Dione was at local dusk ($\phi_{Dione} = 270^{\circ}$) and correspond to periods when there is little or no kilometric emission. Figure 2 suggests that the effect of Dione is to suppress or attenuate the saturnian radio emission when $\phi_{\text{Dione}} = 270^{\circ}$. (The nearly-constant minimum signal strength of $\sim 8 \times 10^{-19} \text{ W m}^{-2} \text{ Hz}^{-1}$ corresponds to the receiver noise level.) The peaks of the 10 h 39.9 min bursts approximately follow a R^{-2} dependence except for periods within $\sim 200 R_s$ when the amplitude of the peaks fall well below the curve coincident with the passage of Dione through the dusk sector. Beyond $\sim 200 R_s$ on the inbound leg and $\sim 165 R_{\rm s}$ on the outbound leg the periodic suppression in phase with Dione's orbital motion is no longer evident. The Dione suppression might explain the apparent north-south asymmetry in the source strength at lower frequencies reported by Warwick et al.4 since Dione was near local dusk for most of Voyager 1's trajectory through southern latitudes.

Analysis of traces similar to that in Fig. 7 of ref. 3, and the present Fig. 2, shows that a clear signature of the Dione modulation is not present before day 308 or after day 325 of 1980. These dates correspond to Voyager-Saturn distances of 227 and $165 R_s$, respectively. To illustrate the transitory nature of the Dione effect further, we show the result of a statistical study of the occurrence of kilometric radiation as a function of Dione's

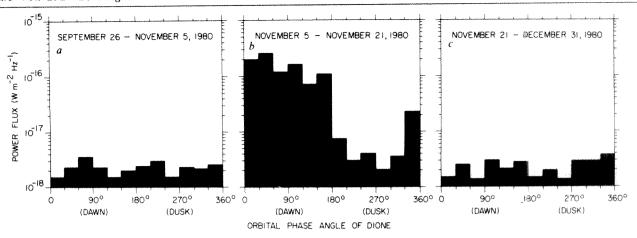


Fig. 3 Plots of the 98% amplitude as a function of Dione's orbital phase angle for the distant inbound, near encounter, and distant outbound Voyager 1 trajectories at 56.2 kHz. Note the strong Dione control near encounter and the complete lack thereof during the distant inbound and outbound passes.

orbital phase angle. The bar graphs in Fig. 3 show the power flux below which 98% of the samples fell for each of 12 phase angle bins. Recall that phase angle is measured positive eastward from a plane containing Saturn's rotation axis and the Sun, with 0° at local midnight. Figure 3b includes all data covered by Gurnett et al.³ when the Dione effect is qualitatively most apparent. Figure 3a, c present results from the inbound and outbound legs when little or no Dione control was apparent. It is clear the distant inbound and outbound data show no significant trend. On the other hand, the difference in amplitude at the 98% level between phase angles near 90° and 270° is about two orders of magnitude for the two-week period near closest approach. For this period, Dione clearly has an important role in determining the amplitude of Saturn radio emissions at 56.2 kHz.

Discussion

There are many possible interactions by which a moon may influence the generation of magnetospheric radio emissions. We shall attempt to narrow the list of likely candidates to one or two which are consistent with all or most of the observations. We assume the SKR originates at relatively low altitudes on high latitude field lines, presumably in the auroral region as suggested by Kaiser et al.⁸.

We first need to explain the transitory nature of the effect. A truly transient process which is active for two weeks and then inactive for long periods of time requires a mechanism to stimulate the control. The long-term studies show no Dione effect⁴ so the active periods must be quite rare. Hence, it is strange that the effect was seen coincidentally with closest approach and we shall argue that the effect is not transitory but depends on the location of the observer.

The simplest explanation of the transitory nature of the Dione control is geometric in nature and specifically related to Voyager's low latitude or small distance from the equatorial plane of Saturn around closest approach. The effect became apparent around day 308 when Voyager 1 was at 8.3° latitude and about $33~R_{\rm S}$ above the equatorial plane. On day 325, when the effect apparently disappeared, the spacecraft was at a latitude of 25.4° and $71~R_{\rm S}$ above the equatorial plane. A second episode of Dione control in early December has been reported by the planetary radio astronomy team⁵. The effect is visible in the plasma wave data at $56.2~{\rm kHz}$, however, we are not confident in interpreting our data as a genuine Dione effect at this time. Should this be a valid episode, however, our conclusion of a geometric effect would be in jeopardy.

Figure 2 showed Dione attenuated the saturnian radio emission as opposed to stimulating or amplifying it. The attenuation could be caused by a propagation effect or by a basic change in the source itself. In either case, the most likely interaction mechanism is a change in magnetospheric density associated with the satellite. A decrease in density could only be accomplished by a sweep-up effect and this is hardly a time-variable process capable of disturbing the inner magnetosphere on time

scales of a few days. An increase in magnetospheric density, however, is plausible as the result of a process which liberates particles from the moon making the moon a plasma source. This type of process occurs in the jovian system with Io being the primary source of plasma.

Frank et al.9 argued that Dione could be a reasonable source for an oxygen torus at Saturn. A peak in the ion density at Dione's orbit with density and temperature profiles reminiscent of those at Io in Jupiter's magnetosphere suggests that Dione, like Io, is a plasma source. Also, the water ice or frost surface of Dione opens the possibility of plasma production through dissociation and ionization. A ledge-like structure in the plasma torus near Dione's L-shell has been reported10, and similarities of this structure with that observed at Io in Jupiter's magnetosphere suggest a plasma source. Evidence from the Pioneer 11 magnetometer¹¹ also suggests a process involving plasma production. On the inbound Pioneer trajectory, significant magnetic field perturbation was seen at Dione's L-shell but no effect was observed on the outbound leg. Local time of Dione was $\sim 21 \text{ h} \ (\phi_{\text{Dione}} \sim 315^{\circ})$ and $\sim 1 \text{ h} \ (\phi_{\text{Dione}} \sim 15^{\circ})$ for the Pioneer inbound and outbound Dione L-shell crossing, respectively. Although there are several possible explanations, this magnetic field signature is consistent with a ring current associated with Dione which varies with ϕ_{Dione}

An increase in magnetospheric density could affect radio emissions by modifying conditions at the source of the radiation. There is some evidence that the Earth's auroral kilometric radiation is quenched by increasing the density in the source region $^{12-14}$, however, Gurnett and Anderson have stated that this is not very likely. Another difficulty with direct modification of the source is that the plasma produced at Dione would have to have easy access to the source. If the radio source were in the auroral region, it is unlikely that an equatorial plasma source at $L \sim 6$ could effectively modify the density in the auroral zone.

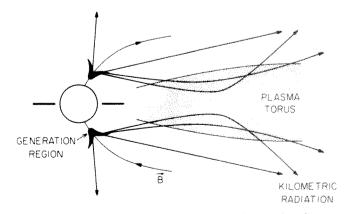


Fig. 4 How a Dione-related torus would refract radio waves from Saturn away from the equatorial region. A similar effect was found for broadband kilometric radiation from Jupiter.

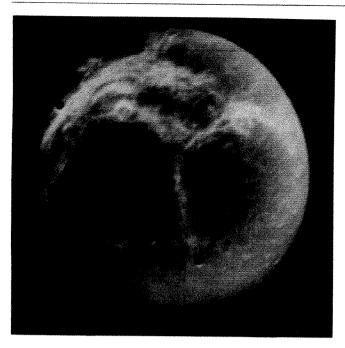


Fig. 5 An image of the trailing hemisphere of Dione taken by Voyager 1 when Dione was near local dusk. Note that this sunlit hemisphere is dominated by bright wispy features which are probably fresh outcroppings of water ice or frost. The frost may be a source of plasma for a torus whose density fluctuates with the same period as Dione's orbit.

The alternative is to assume that an increase in plasma density associated with Dione affects the propagation of radio waves through refraction or reflection as represented in Fig. 4. We used the model torus from ref. 3, although the model shown by Bridge et al.10 would give essentially the same effect. Waves with frequency less than or equal to the local plasma frequency in the torus cannot penetrate the torus. Waves of higher frequency will be refracted away from the equatorial region, although the effect will diminish with increasing frequency.

Many observations suggest that the model shown in Fig. 4 is viable. First, the density of the plasma torus as measured by Pioneer 11 (ref. 9) and Voyager 1 (refs 3, 10) is of the order of 40 cm⁻³ corresponding to a plasma frequency of 57 kHz. Waves below this frequency could not penetrate the torus and waves well above 100 kHz could undergo substantial refraction. This refraction is consistent with the frequency dependence of the Dione effect reported by Desch and Kaiser⁵, as lower frequencies tend to be refracted most. Second, because preliminary observations 3,10 suggest the plasma is confined near the equator, waves propagating near low latitudes would be most affected. Therefore, we expect a low-latitude shadow zone as was found for the jovian kilometric radiation 16,17. A shadow zone near the equator would explain the presence of a Dione control only near encounter when Voyager 1 was close to the equator. The asymmetry in the latitude at which the effect appears and disappears could well be a local time asymmetry in the thickness or density of the torus.

How then does the orbital phase angle of Dione modulate the emission? It is important first to establish that the modulating effect of the torus is longitudinally symmetric. The evidence is embodied in Fig. 7 of ref. 3 and Fig. 2 of this paper. Note that the phase of the Dione effect did not change as Voyager moved from near local noon before encounter to local early morning after the encounter. If there were a localized cloud of plasma which orbited Saturn, a phase shift would have been apparent as the viewing point changed. As the phase did not change, the modulation effect must be longitudinally symmetric. This longitudinal symmetry should be fairly easily achieved due to the rapid corotation of the plasma which will quickly distribute plasma in longitude even though the source may be localized.

To explain the dependence of the torus density on the orbital phase angle of Dione, we suggest that Dione may be a plasma

source only when it is near local dusk. Hence, the plasma cloud produced dissipates on a time scale of about a day so that by the time Dione is near local dawn the torus density has decreased by a factor of say, two. Because the trailing hemisphere of Dione is always the same, a hemispheric asymmetry in direct particle sputtering processes cannot explain the dawn-dusk asymmetry. On the other hand, there are obvious asymmetries in the surface features of Dione. Figure 5 shows Dione near local dusk. The trailing hemisphere shows a complex pattern of wispy features which do not appear on the opposite hemisphere of Dione. Smith et al.6 suggest the bright markings are controlled by a regional system of fractures or faults possibly formed or reopened by internally generated stresses. The bright material is probably water ice and it is tempting to speculate that this relatively fresh surface frost is photosputtered when the trailing hemisphere is sunlit as it is when Dione is near local dusk, or that photodissociated volatiles produce the plasma.

Carlson¹⁸ and Frank et al. 9 have discussed the rings as possible sources of plasma in the saturnian magnetosphere. Of processes such as photosputtering, ion sputtering, sublimation with subsequent photodissociation, and others, photosputtering seems to be the most efficient mechanism. It would be a convenient mechanism for an orbital phase-dependent source of plasma at Dione in view of the hemispherical asymmetry of the wispy features shown in Fig. 5. Taking the hydrogen atom flux derived by Carlson¹⁸ of $\sim 10^8 \text{ cm}^{-2} \text{ s}^{-1}$, Dione can produce $\sim 10^{24}$ atoms s⁻¹ (assuming the entire sunlit hemisphere is a source and photosputtering is applicable to Dione). If the time scales for both creation and dissipation of the plasma torus are ~ 1 day (to provide a 2.74-day modulation period), then the production/loss rate must be the order of $10^{28} \, \mathrm{s}^{-1}$. We have assumed a torus 4 Rs thick, centred at 6 Rs, which fluctuates between 20 and 40 cm⁻³. Obviously, photosputtering is not sufficient to produce a dense torus on the time scale of a day.

If we assume that the other processes considered by Carlson are no more efficient than photosputtering, we must consider a mechanism which is based on the release of volatiles from fractures in Dione's crust and subsequent photodissociation. Presumably the volatiles would be released into the exosphere at a constant rate but preferentially from the wispy hemisphere. Photodissociation of the molecules would occur only during the dusk sector of Dione's orbit when the wisps are sunlit. This mechanism is largely speculative and production rates depend on rate of volatile release, the type of volatile, and photodissociation rate. However, the injection rate at Io has been estimated 19 at $2 \times 10^{29\pm1}$ ions s⁻¹, about 200 times that required at Dione.

The proposed fluctuating torus model also requires a dissipation rate of the order of 10^{28} s⁻¹. Richardson et al. 19 point out that outward radial diffusion through flux-tube interchange is the predominant loss mechanism at Jupiter and given that oxygen or some other heavy ion is an important constituent of the Dione torus^{9,10} the centrifugally-driven interchange instability is likely to be important at Saturn, also.

We have arrived at a model which is similar in part to effects observed at Jupiter. We propose that Dione is responsible for the production of plasma when it is near local dusk which forms a longitudinally symmetric torus. The torus then casts a radio shadow on low latitudes so that the saturnian radio emission cannot be seen by a spacecraft near the equator. The torus decays with a time constant of about one day so that as Dione approaches local dawn the usual saturnian emission reappears. The possibility of an additional Dione-control episode⁵ in early December, 1980 at $\phi_{\text{Dione}} \sim 40^{\circ}$, however, presents an even more perplexing situation to be explained involving a shift in the phase of the control.

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- Kaiser, M. L., Desch, M. D., Warwick, J. W. & Pearce, J. B. Science 209, 1238-1240 (1980).
 Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 253-256 (1981).
 Gurnett, D. A., Kurth, W. S. & Scarf, F. L. Science 212, 235-239 (1981).
 Warwick, J. W. et al. Science 212, 239-243 (1981).
 Desch, M. D. & Kaiser, M. L. Nature 292, 739-741 (1981).

- Smith, B. A. et al. Science 212, 163-191 (1981).
- Smith, B. A. et al. Science 412, 103-191 (1901).
 Scarf, F. L. & Gurnett, D. A. Space Sci. Rev. 21, 289-308 (1977).
 Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731-733 (1981).
- Frank, L. A., Burek, B. G., Ackerson, K. L., Wolfe, J. H. & Mihalov, J. D. J. geophys. Res.
- 10. Bridge, H. S. et al. Science 212, 217-224 (1981).
- Rairden, R. L. thesis, Univ. Iowa, (1981).
 Benson, R. F. & Calvert, W. Geophys. Res. Lett. 6, 479-482 (1979)
- Wu, C. S. & Lee, L. C. Astrophys. J. 230, 621-626 (1979)
- 14. Calvert, W. Geophys. Res. Lett. (in the press).
 15. Gurnett, D. A. & Anderson, R. R. in Physics of Auroral Arc Formation (in the
- press). Kurth, W. S., Gurnett, D. A. & Scarf, F. L. Geophys. Res. Lett. 7, 61-64 (1980). Green, J. L. & Gurnett, D. A. Geophys. Res. Lett. 7, 65-68 (1980).
- Carlson, R. W. Nature 283, 461 (1980).
- 19. Richardson, J. D., Siscoe, G. L., Bagenal, F. & Sullivan, J. D. Geophys. Res. Lett. 7, 37-40

Pre-encounter distributions of Saturn's low frequency radio emission

T. D. Carr*, J. J. Schauble[†] & C. C. Schauble[†]

- * Department of Astronomy, University of Florida, Gainesville, Florida 32611, USA
- † University of Florida Graduate Center, Eglin Air Force Base, Florida 32542, USA

An analysis of Voyager 1 pre-encounter data is presented in which one-month averages of flux density from Saturn are determined as functions of both central meridian longitude and frequency. Comparisons of corresponding distributions for two one-month intervals seven months apart yield information on their stability, and a redetermination of the magnetospheric rotation period.

THE detection of a nonthermal component of Saturn's thermal radio emission has proved to be difficult. No synchrotron radiation from the planet has yet been found, and pre-Voyager searches for a nonthermal component at low frequencies were inconclusive. An unsubstantiated identification of such emission1 was made near 1 MHz, but not until Voyager 1 had approached to within 3 AU was a nonthermal radio component positively identified2. This radiation is not unlike the kilometric bursts from the terrestrial and jovian magnetospheres. Most of the activity is between ~70 and 300 kHz, and is polarized predominantly in the right hand sense²⁻⁴. The probability of occurrence tends to vary in a more or less repeatable way with

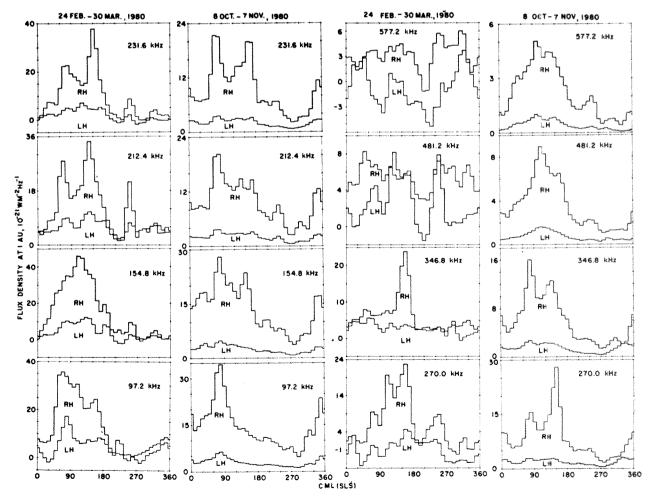


Fig. 1 Histograms of average flux density per 10° interval as a function of central meridian longitude for specified time intervals and frequencies. Averaging extends over inactive as well as active periods. Right hand (RH) and left hand (LH) polarized components are plotted separately. Small and negative flux densities result from background fluctuations when Saturn radiation is very weak, accentuated at times by slight errors in baseline determination

the rotation of the planet. From an analysis of this periodicity over several months before the Voyager 1 encounter, Desch and Kaiser³ arrived at a rotation period of 10 h 39 min $24(\pm 7)$ s, the presently accepted value for Saturn's magnetosphere. This period is the basis for the Saturn longitude system (designated SLS) in current use for specifying central meridian longitude (CML).

Observations

The data used in this analysis were obtained by Voyager 1 during two periods extending from 24 February to 30 March 1980, and from 8 October to 7 November 1980. Closest approach to Saturn was on 12 November 1980. The measurements were made with the low band receiver of the planetary radio astronomy experiment⁵, at 28 selected frequencies from 20.4 to 1,038 kHz. In calculating flux density as functions of CL and frequency, the averaging intervals covered inactive as well as active periods. Data reading and manipulation was computerized, except for preliminary editing when data containing interference from within the spacecraft, from the Sun, or from Jupiter were excluded. Solar bursts were easily identified but Jupiter bursts, which more closely resemble those from Saturn, were identified in most cases by locating the same event on the Voyager 2 record and noting the difference of onset times. As Voyager 2 was much closer to Jupiter than Voyager 1, nearly every jovian event appearing on a Voyager 1 record could also be seen on the corresponding Voyager 2 record with greater intensity and earlier onset time. For each of the two circularly polarized components at each frequency one or more short segments of undisturbed baseline were selected from each 24-h run (except for a very few runs in which no activity lull occurred). Zero-flux density baselines fitted to these segments were subtracted from the data points within that 24-h interval. Following the background subtraction and the application of an appropriate calibration factor, flux densities were normalized to a standard 1 AU spacecraft distance. Histograms of average flux density for 10° CML intervals formed the basis for subsequent analyses.

Results and discussion

Eight pairs of representative histograms are shown in Fig. 1. The two in each pair are for the same frequency, one for the first and the other for the second data period. Although the CML regions of maximum and minimum activity approximately coincide for histograms at the same frequency, the differences seen are probably due to statistical fluctuations. An extreme case of this is seen in the 481.2 and 577.2 kHz histograms, in which the radiation during the first data period is so weak that background fluctuations dominate. At the same two frequencies for the second data period, when the spacecraft was close to the planet, the distribution is very clearly defined.

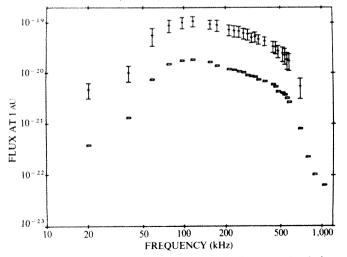


Fig. 2 Special distributions of average flux density (lower curve) and of 'peak' or 97 percentile flux density (upper curve). Error bars indicate 90% confidence range for the latter. Flux densities in Wm⁻² Hz⁻¹.

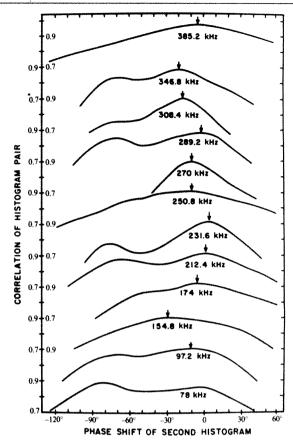


Fig. 3 Cross correlation of pairs of single-frequency histograms as a function of the longitude shift of the second histogram in the pair. Curve maxima are indicated by arrows.

In addition to statistical fluctuations, there seem to be systematic differences. For example, a secondary peak often appears near 360° in the second histogram which is not present in the first one. Such differences are unlikely to be latitude effects. The saturnigraphic latitude of the spacecraft on the median dates of the two data periods was 9.1° and 8.7°, respectively, unlikely to cause an observable effect: nor was the difference in saturnian local time at the spacecraft enough. The systematic differences are most likely due to temporal instability possibly caused by some alteration of the magnetospheric plasma distribution, and even of the multipole structure of the magnetic field, over the seven-month interval between the two data sets.

Figure 1 shows that the right hand (RH) circularly polarized component is much stronger than the left hand (LH) one. The histograms suggest that the ratio of LH to RH flux densities may be smaller in the vicinity of the main peak (near 100°) than elsewhere; however, this ratio would be subject to large errors

	Table 1	Rotation	period measu		
Frequency (kHz)	Shift (deg)	Mean	Correlation coefficient	Mean	Rotation period (10 h 39 min+)
97.2	-11		0.90		
154.8	-30		0.90		
174.0	-5		0.92		
		-15		0.91	21 s
212.4	1		0.91		
231.6	3		0.90		
250.8	-10		0.90		
270.0	-10		0.90		
		-4		0.90	23 s
289.2	-2		0.87		
308.4	-17		0.88		
346.8	-21		0.87		
385.2	5		0.96		
		-11		0.89	22 s

Weighted mean rotation period: 10 h 39 min 22 s. Maximum deviation of the three values from the mean: 1 s. SLS rotation period (Desch and Kaiser³): 10 h 39 min $24(\pm 7)$ s.

outside the main peak region because of the relatively low flux density values there.

The spectral distribution of average flux density (RH and LH combined), shown in Fig. 2, was obtained by integrating the histograms of average flux density against CML for each of the 28 frequencies for the second data set. This curve closely agrees with that corresponding to the first data set, except at those frequencies at which the signal-to-noise ratio in the first set was poor. Nearly all of the radiation occurs below 1 MHz, and the broad spectral peak has its maximum at ~150 kHz. Figure 2 also shows the spectral distribution of the 97-percentile level of the individual flux density measurements (RH and LH combined) in the second data set. This is the level which is exceeded 3% of the time. Each point on the 97-percentile curve is the mean of the 24-h 97-percentile values for the 1-month period.

Integration of the average curve of Fig. 2 yields the 1 AU equivalent of the average power per unit area (5.3× 10⁻¹⁵ Wm⁻²) reaching the spacecraft at all frequencies. The corresponding isotropic equivalent average power of the saturnian radio source is 1.5×10^9 W. This is $\sim 0.4\%$ of the isotropic power equivalent for all the jovian emission below 40 MHz, or about five times that of the jovian kilometric emission (below 300 kHz)⁶. A similar integration of the 97-percentile curve in Fig. 2 could not be used to calculate a 97-percentile isotropic equivalent power, because emission peaks do not occur simultaneously at all frequencies.

Single-frequency pairs of histograms such as those in Fig. 1 allow a more precise determination of Saturn's magnetospheric rotation period. Our method was previously used successfully to redetermine Jupiter's radio rotation period⁷. Each histogram in a single-frequency pair represents data from a period of about a month, and the interval between the medium dates of the two data periods was \sim 224 days (1.935 \times 10⁷ s). The histograms are based on the SLS rotation period3. From the measured shift of the second histogram in each pair with respect to the first, the correction to be applied to the SLS period was calculated. The histogram for the RH component was used in each case although the sum of the RH and LH components could have been used instead. The correct histogram shift was indicated by the principal maximum of the cross correlation function for the pair. The corrected rotation period, P_{new} , was then calculated from the formula

$$P_{\text{new}} = P_{\text{SLS}} \left(1 + \frac{P_{\text{SLS}} \Delta L}{360T} \right)$$

where P_{SLS} is the SLS period, ΔL is the longitude shift (in degrees) of the second histogram with respect to the first at the cross correlation maximum, and T is the time between the

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- Brown, L. W. Astrophys. J. 198, L89-L92 (1975).
 Kaiser, M. L., Desch, M. D., Warwick, J. W. & Pearce, J. B. Science 209, 1238-1240 (1980).
 Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 253-256 (1981).

median dates of the two data sets from which the histograms were plotted. The signal-to-noise ratio during the first data period was considered adequate for cross-correlation of 12 of the 28 originally selected frequencies. Plots of the regions about the principal peaks of the cross-correlation functions for the histogram pairs at these 12 frequencies are shown in Fig. 3. The principal peaks in 11 curves occur between -30° and 4°. Most of the curves also exhibit a secondary peak in the vicinity of -80°. For 78 kHz the positions of the principal and secondary peaks are interchanged; this curve was rejected. The longitude shifts at the cross-correlation maxima for the 11 remaining curves, and the correlation coefficients are presented in Table 1. These 11 measurements are not completely independent, because whenever activity is observed on a given frequency channel, it most likely occurs at about the same time on one or more nearby channels. Desch and Kaiser (personal communication) have found the 1/e decorrelation bandwidth of the Voyager preencounter Saturn emission to be ~115 kHz. We therefore averaged our 11 shift measurements in three groups, the centre frequencies of which are separated by nearly this amount, as indicated in Table 1. The resulting averages, which lie within -1 s of the mean (10 h 39 min 22 s), are believed to be largely independent. The mean differs from the SLS period by only 2 s The close agreement of our three measurements suggests that their statistical precision is better than the ± 7 s quoted for the SLS determination. However, Desch and Kaiser believe that this uncertainty is due to a basic instability in the averaged pre-encounter saturnian emission pattern. If this is true it suggests that the close agreement of our measurements is accidental. Further rotation period determinations over different time intervals should settle this question.

To approach maximum statistical precision using our method, a near-optimum combination should be sought for three intervals: (1) the data collection interval for the first histogram; (2) that for the second histogram (assumed to be as close to encounter as possible without producing excessive latitude change), and (3) the interval between the median dates of the two histograms. The latter should provide an adequate temporal baseline over which to measure the notation period, yet not make the Saturn events in the first interval too weak or too infrequent. We believe that a large part of the difference in the shapes of our first and second histograms in each pair results from the relatively low signal intensity relative to the background noise during the first interval. We should be able to improve our precision (and evaluate it) soon by using more recent Voyager 1 and 2 data.

We thank M. D. Desch and M. L. Kaiser for unpublished decorrelation bandwidth data, and for helpful comments.

- Warwick, J. W. et al. Science 212, 239-243 (1981).
- Lang, G. J. & Peltzer, R. G. IEEE Trans. AES-13, 466-471 (1977).

 Alexander, J. K., Carr, T. D., Thieman, J. R., Schauble, J. J. & Riddle, A. C. L. geophys. Res.
- 7. May, J., Carr, T. D. & Desch, M. D. Icarus 40, 87-93 (1979)

Plasma wave turbulence at planetary bow shocks

F. L. Scarf*, D. A. Gurnett & W. S. Kurth

* Space Science Department, TRW Defense and Space Systems Group, Redondo Beach, California 90278, USA † Department of Physics and Astronomy, University of Iowa, Iowa City, Iowa 52242, USA

Voyager 1 observations of plasma wave turbulence at Saturn's bow shock are discussed and compared with corresponding data from Jupiter, Earth, and Venus. The results suggest that the plasma instabilities that develop at the lower Mach number bow shocks of the terrestrial planets differ from those found at the high Mach number bow shocks of the outer planets.

As the solar wind streams out from the Sun, its flow speed remains constant, but its plasma density, ion and electron temperatures, and mean interplanetary magnetic field strengths all have distinct and varying radial profiles. Thus, the dimensionless interplanetary parameters that determine how microscopic plasma processes develop change with heliocentric distance. We therefore expect to find significant radial variations in several important interplanetary plasma phenomena. This is

very important to collisionless shock studies, in particular, because some combinations of plasma parameters occurring naturally in the solar wind have not yet been attained experimentally.

The planetary bow shock also forms in the solar wind, and this means that the characteristics of these very high Mach number discontinuities will vary across the Solar System as the expanding wind encounters each planet at a different radial position. As the processes that develop at the bow shock directly affect the properties of the post-shock plasma that actually impacts the ionopause or magnetopause, comparative planetary studies must include comparison of the bow shock turbulence spectra and associated wave-particle interaction phenomena. We now discuss recent Voyager 1 observations of plasma wave turbulence at Saturn's bow shock, and present an initial comparison of these wave measurements with corresponding data from Jupiter, Earth and Venus. The results suggest that the plasma instabilities that develop at the lower Mach number bow shocks of the terrestrial planets differ from those found at the high Mach number bow shocks of the outer planets. Thus the state of the post-shock plasma varies significantly from one planet to another.

Saturn bow shock crossings

As noted by Gurnett et al.¹, the solar wind upstream from Saturn was remarkably quiet in the 10 Hz-56 kHz range covered by the Voyager 1 plasma wave instrument. The only regular signals observed in this region were repetitive series of Saturn radio emissions^{1,2}. We also detected a single cluster of intense 3 kHz noise bursts between 17.05 and 17.20 on 11 November 1980, when the Voyager to Saturn distance was 32.4 R_s. We interpret these 3-kHz waves as electron plasma oscillations associated with suprathermal electrons streaming back from the shock, but when these emissions disappeared, Voyager was still in the solar wind. Terrestrial experience with similar sequences of observations suggests that the bow shock was actually near the

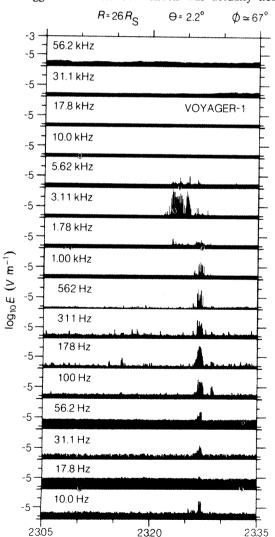


Fig. 1 Sixteen-channel measurements of plasma wave amplitudes at the inbound crossing of Saturn's bow shock. Several sporadic interference effects have been deleted. The 31.1-kHz and 56.2-kHz levels represent radio emissions from Saturn, and we interpret the 3.1-kHz waves as electron plasma oscillations.

spacecraft just after 17.00, but that the shock surface receded back towards the planet in response to a change in solar wind conditions. Similar 3-kHz bursts were detected again after 23.22, and these waves persisted until 23.27 when Voyager made its only inbound bow shock crossing at a radial distance of 26.1 $R_{\rm S}$. Figure 1 shows all of the 16-channel wave measurements for this event, and it can be seen that the shock was thin and well-defined in terms of the low frequency ($f \le 1$ kHz) wave activity. These bandpass channel amplitude profiles are very similar to those found when Voyager 1 approached Jupiter^{3.4}, and in both cases, the post-shock magnetosheath noise levels seem to be exceptionally low.

Just before 12.27, the wind speed (V) and density (N) were 420 km s⁻¹ and 0.11 ions cm⁻³, and the magnetic field strength (B) was about 0.3 nT. Here, the upstream proton and electron temperatures were $\sim 1.5 \times 10^4$ K and 2.3×10^4 K, respectively (J. Scudder, personal communication). For these parameters, the Alfvén speed, $V_A (= [B^2/4\pi N m_+]^{1/2})$, is only 20 km s⁻¹, and the magnetosonic wave speed, $V_{\rm ms} (= [V_A^2 + V_s^2]^{1/2})$, with $V_s = [(5kT_+/3 + kT_-)/m_+]^{1/2})$, is ~ 28 km s⁻¹. Hence, the Mach numbers for the collisionless shock of Fig. 1 are remarkably high $(M_{\rm ms} = V/V_{\rm ms} = 15)$, and $M_A = V/V_A = 21)$, and this event is probably one of the highest Mach number collisionless shocks for which any in situ observations are available.

Before comparing plasma wave measurements for the relatively well-defined dayside bow shock crossings at Saturn, Jupiter, Earth and Venus, we discuss characteristics of the outbound bow shock encounter with Saturn on the nightside

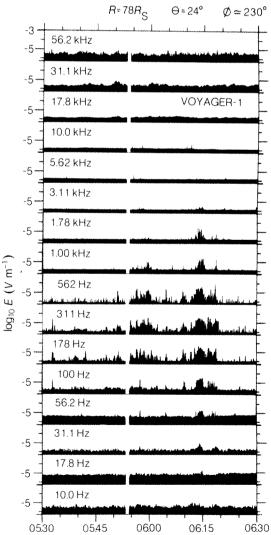
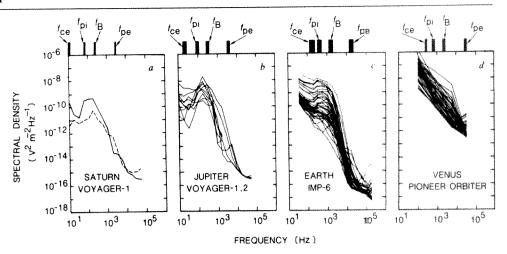


Fig. 2 Sixteen-channel measurements of plasma wave amplitudes at the diffuse outbound crossing of the bow shock. Here, the 10-56 kHz waves are Saturn radio emissions, and the gap between 05.53:23 and 05.54:11 corresponds to an interval when the Voyager tape recorder was acquiring waveform data.

Fig. 3 Multi-planet comparison of plasma wave spectra. a Was constructed amplitude 16-channel the measurements of Figs 1 and 2. The solid and dashed spectra represent the inbound and outbound crossings, respectively. The characteristic frequencies shown at the top are the electron cyclotron frequency (f_{∞}) , the ion plasma frequency (f_{pi}) , the Buneman mode frequency (f_B) , and the electron plasma frequency (f_{pe}) . Although the spectra for Earth (c), Jupiter (b) and Saturn (a) all have a primary or secondary peak in the noise spectrum between f_{pi} and $f_{\rm B}$, the intense low frequency enhancement which is evident in most terrestrial spectra and all the Venus observations is not found at Jupiter or Saturn.



almost midway between local midnight and dawn. During the interval 05.50 to 06.18 on 16 November when Voyager 1 was at $78 R_s$, the spacecraft traversed a relatively diffuse transition layer separating the magnetosheath from the solar wind. Figure 2 shows the relative thickness of the outbound shock and the variability in the plasma wave amplitude profiles; this is characteristic of a quasi-parallel shock configuration that is usually associated with an extensive region of upstream disorder, and it seems that after 06.18, much turbulence was present for an extended period. Ness et al.6 noted the elevated r.m.s. magnetic field noise levels which indicate that enhanced low frequency electromagnetic wave activity was present after 06.18. The plasma wave instrument was able to detect Saturn-associated bursts of ion acoustic waves and electron plasma oscillations out to at least early December more than 400 Rs away from Saturn. These results are readily understandable because at 9.5 AU, the Parker spiral model leads to an average interplanetary field oriented almost perpendicular to the Saturn-Sun line. Thus, the Saturn 'upstream region' or 'foreshock', which can develop when the B-field intersects the shock surface, was encountered after closest approach rather than before.

Planetary bow shock turbulence spectra

Pioneer Venus Orbiter provided the first data on plasma wave activity at the bow shock of a planet other than Earth in December 1978, and in March and July 1979, the Voyager spacecraft transmitted corresponding information from Jupiter's bow shock. Following the 1980 Voyager 1 encounter with Saturn, it is possible to present a preliminary comparison of wave-particle interactions at four planetary bow shocks, but a comprehensive analysis must await Voyager 2 Saturn data.

The IMP-6 study of Rodriguez and Gurnett⁷ serves as a reference for analysis of plasma waves at the terrestrial bow shock, and Fig. 3c shows the average electric field spectra detected at 36 representative crossings of the shock surface. For these crossings, the average solar wind density was 5.2 particles cm⁻³, and the average magnetic field strength was 7.5 nT; these values yield a mean electron plasma frequency ($f_{\rm pe} = 9,000 \sqrt{N}$) of 20.5 kHz, an ion plasma frequency ($f_{\rm pi} = 210\sqrt{N}$ for protons) of 480 Hz, and an electron cyclotron frequency ($f_{\rm ce} = 28B$) of 210 Hz. These characteristic frequencies, along with the 1.65 kHz value that is appropriate for the corresponding Buneman mode⁸ characteristic frequency, $f_{\rm B} = [m_{\rm e}/m_{\rm i}]^{1/3} f_{\rm pe}$ are marked at the top of Fig. 3.

Rodriguez and Gurnett studied how the plasma wave spectrum varied with changes in Alfvén and sonic Mach numbers, plasma β -value ($\beta = 8\pi NK[T_e + T_i]/B^2$), ion temperature, electron-to-ion temperature ratio, solar wind density and shock normal angle. Plots of the mid-frequency (200 Hz-4 kHz) Efield amplitude against T_e/T_i showed a strong positive correlation, and a weaker one when the low frequency (20 Hz-200 Hz) E-field amplitude was used, but the extreme shapes of the shock spectra shown in Fig. 3c were not discussed in association with any particular groups of upstream plasma

parameters. Indeed, as the upstream parameters associated with the Rodriguez and Gurnett data set varied over a huge range $(0.5 \le N \le 19 \text{ cm}^{-3}, 2 \le B \le 17 \text{ nT}, 1.5 \le M_A \le 26.6, 0.03 \le \beta \le 4.5)$, there was no a priori reason to expect to find characteristic changes in shock spectra as we moved inward towards Venus or out to Jupiter and Saturn. Nevertheless, Fig. 3a, b, d strongly suggests that the bow shock spectra at the other planets differ significantly from those detected at Earth.

The 48 Venus spectra on Fig. 3d have been discussed elsewhere⁹: these spectra are limited because the Pioneer Venus instrument has only four filter channels and it uses short antennas which are affected by spacecraft interference. Nevertheless, the shock processes at Venus seem to generate much higher levels of low frequency ($f \le f_{\rm pi}$) plasma wave turbulence than do the corresponding processes at Earth [we use $N({\rm Venus}) = (12 \pm 4)$ electrons cm⁻³ and $B({\rm Venus}) = (10 \pm)$ nT to compute the characteristic frequencies shown at the top of Fig. 3d].

Recently the nine jovian spectra shown in Fig. 3b have been compared with the IMP-6 data¹⁰. The Voyager 1 and 2 measurements were all obtained during the dayside inbound passes, and the characteristic frequencies shown at the top correspond to $N \approx (0.225 \pm 0.075)$ electrons cm⁻³ and $B \approx$ (0.8 ± 0.2) nT. The low frequency enhancement in electric field intensity, evident in most of the terrestrial wave spectra and all of the Venus observations, was not present at Jupiter. Assuming that these low frequency waves represent whistlers, it was speculated that whistler-mode noise may not be as important the jovian bow shock as at Earth and Venus. Alternatively, the Jupiter spectral peaks at higher frequencies could be attributable to plasma conditions in the distant solar wind that yield enhanced generation of ion acoustic or Buneman mode oscillations, rather than suppressed levels for the whistlers.

Figure 3a shows the Voyager 1 Saturn bow shock spectra derived from the measurements of Figs 1 and 2; the solid lines connect one-minute averages for the inbound crossing, and the dashed lines refer to the outbound case. The Jupiter and Saturn shock spectra are quite similar and both have intensity peaks near the $f_{\rm pi}$ and $f_{\rm B}$ characteristic frequencies. It is difficult to identify these wave modes, however, as ion acoustic waves have phase speeds small compared with the solar wind speed; thus, Doppler effects could account for large frequency shifts, and these waves may have $f \leq f_{\rm pi}$ in the solar wind rest frame. As the heliocentric distance increases, there appears to be a significant and consistent variation in the average bow shock turbulence, and we should therefore examine how some relevant interplanetary parameters vary from one planetary orbit to another.

A complete investigation of the variations in bow shock microstructure requires comparative study of the electron and ion temperatures, thermal anisotropies, heat flux moments, and distribution function shapes, as well as comparison of the macroscopic upstream parameters such as magnetic field vector, solar wind velocity, and wind density. Note that the average spectra shown in Fig. 3 can be associated with changes in average

Mach number. Specifically, using $M_A = V/V_A$ and $V_A =$ $(B/(4\pi Nm_+^{1/2}))$, the mean B and N values cited above give $M_A(\text{Venus}) = 6.3$, $M_A(\text{Earth}) = 5.6$, $M_A(\text{Jupiter}) = 11$, and $M_A(Saturn) = 21$. This suggests that there may be a relation between Mach number and spectral shape.

Support for this speculation comes from reanalysis of the IMP-6 bow shock observations. Although the result is not strongly evident in Fig. 3c, three of these spectra have 'outer planet' shapes in the sense that for these cases, the wave level at $f \approx 100 \text{ Hz}$ is much lower than the level at f = 100-1,000 Hz. These peaked terrestrial spectra were detected when the upstream conditions yielded a subset of high Mach numbers (P. Rodriguez, personal communication), and this tends to support a hypothesis that the Jupiter and Saturn shock spectra have unusual characteristics, primarily because the solar wind Mach numbers at the outer planets tend to be much higher than the customary values near 0.7-1.0 AU.

Discussion

On theoretical and experimental grounds it is known 11,12 that several distinct dissipation mechanisms develop in collisionless shocks and that the dominant interaction depends strongly on the Mach number. For low Mach numbers $(M \le M^* \approx 2-3)$, the shock is resistive, and for $M > M^*$, viscous dissipation becomes

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- Gurnett, D. A., Kurth, W. S. & Scarf. F. L. Science 212, 235 (1981).
- Warwick, J. W. et al. Science 212, 239 (1981).
- Scarf, F. L., Gurnett, D. A. & Kurth, W. S. Science 204, 991 (1979). Scarf, F. L., Gurnett, D. A., Kurth, W. S. & Poynter, R. L. Nature 280, 796 (1979).
- Bridge, H. S. et al. Science 212, 217 (1981).
 Ness, N. F. et al. Science 212, 211 (1981).

important. Above a second critical Mach number, $M^{**} \approx 6-7$, theory¹¹ requires other types of dissipation, but these very high Mach number shocks have not been intensively analysed. We now find that upstream conditions at the outer planets generally yield Mach numbers considerably higher than M^{**} , and thus it is of great interest to find that the Jupiter and Saturn bow shock crossings have plasma turbulence spectra that resemble those found at Earth when M is high. The Jupiter and Saturn characteristics are also unusual in that the jump in electron temperature across the shock is enormous $(T_e^2/T_e^1 \approx 15 \text{ at Jupiter}^{13})$ and the magnetosheath wave levels are extremely low.

In conclusion, our study of the bow shocks at Jupiter and Saturn has provided insight into collisionless shock microstructure for an important new plasma regime, and helps to understand shocked plasmas of non-equilibrium characteristics that seem to be different from those found in the terrestrial magnetosheath.

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- Rodriguez, P. & Gurnett, D. A. J. geophys. Res. 81, 2871 (1976). Buneman, O. Phys. Rev. Lett. 1, 8 (1958).
- Scarf, F. L., Taylor, W. W. L., Russell, C. T. & Elphic, R. C. J. geophys. Res. 85, 7599 (1980).
- 10. Gurnett, D. A. & Scarf, F. L. in Physics of the Jovian Magnetosphere (ed. Dessler, A. J.) (Cambridge University Press, in the press)
- Biskamp, D. Nucl. Fus. 13, 719 (1973)
 Formisano, V. J. Phys. 38, C6 (1977).
- 13. Scudder, J. D., Sittler, E. C. Jr & Bridge, H. S. J. geophys. Res. 86 (in the press).

Surface waves on Saturn's magnetopause

R. P. Lepping', L. F. Burlaga' & L. W. Klein[†]

* NASA/Goddard Space Flight Center, Laboratory for Extraterrestrial Physics, Greenbelt, Maryland 20771, USA † Computer Sciences Corporation, 8728 Colesville Road, Silver Spring, Maryland 20910, USA

Voyager 1 magnetometer data have shown that small-amplitude surface waves occurred on Saturn's dayside magnetopause, causing multiple inbound crossings of this boundary. These waves were travelling approximately parallel to Saturn's equatorial plane along the magnetopause ('tailward'), suggesting that they were driven by the rotation of Saturn's magnetosphere. Hydromagnetic waves (possibly slow mode) were observed in the adjacent magnetosheath.

ON 11 November (day 316) 1980 at 23.27 UT Voyager 1 crossed Saturn's bow shock on its inbound trajectory towards the planet. This was followed by a series of five, fairly regularly spaced magnetopause (MP) crossings ending at ≈ 02.47 UT of day 317, and discussed in a preliminary report¹. The analysis and interpretation of the magnetometer data taken over this ≈ 3.5-h period is now discussed in terms of the first observations of surface waves on Saturn's magnetopause, their characteristics, and a plausible connection between the magnetopause and hydromagnetic waves occurring in the magnetosheath.

Observations

The dual three-axis low field magnetometer system onboard Vovager 1 was in its most sensitive range of 0.0043 nT per quantization step for the entire period of interest. The measurements, at 0.060-s intervals, were probably accurate to ±0.03 nT per axis. The magnetometer experiment is described in detail elsewhere². Figure 1 presents 9.6-s averages of the magnetic field and shows the inbound bow shock, five magnetopause crossings, and the intervening magnetosheath. The inset shows the trajectory of the spacecraft projected on the orbital plane of Saturn; in the inbound magnetosheath the spacecraft was only about 10° below this plane (in a Saturn centred system). The two outstanding characteristics in the data are: (1) large quasi-periodic oscillations in magnitude (B) of the field in the magnetosheath that seem to grow in amplitude

from the bow shock to the MP; (2) fairly regularly spaced MP crossings.

Table 1 lists the centre times and durations (Δt) for the five MP crossings. The field latitudes are $\langle \delta \rangle \approx -75^{\circ}$, on the magnetosphere side of each crossing, as expected for Saturn's distant front-side magnetosphere 1.3, and the field shows , and the field shows moderate draping at medium (positive) latitudes in the magnetosheath near the MP. That is, the magnetosheath field near the MP boundary is apparently being constrained to lie parallel to the MP as it is convected along the boundary. At more distant locations from the MP the magnetosheath field is closer to $\delta \approx 0^{\circ}$ at $\langle \lambda \rangle \approx 285^{\circ}$. The directional changes of the field across the MPs were large, permitting an accurate estimation of the normal to the MP surface for each crossing.

Table 1 Inbound magnetopause crossings and normals

.,	day 317 centre time	Δt		Method 1			ethod 2		ethod 2'
No.	(h min s)	(min)	$\delta_{ m N}$	$\lambda_N - 180^\circ$	η	δ_n	$\lambda_n - 180^\circ$	$\delta_{ m eq}$	$\lambda_{eq} - 180^{\circ}$
1	01.54:45	2.50	-14°	17°	82°	4°	2°	-3°	4°
2	02.14:23	2.25	17°	-16°	78°	7°	3°	5°	6°
3	02.28:43	0.92	-11°	48°	89°	-11°	47°	8°	47°
4	02.42:28	1.08	30°	-20°	78°	23°	-4°	19°	-14°
5	02.46:51	0.43	-23°	38°	89°	-22°	40°	4°	45°
		Average	00	13°	83°	-1°	16°	5°	15°
		r.m.s.	23°	31°	6°	17°	25°	9°	29°

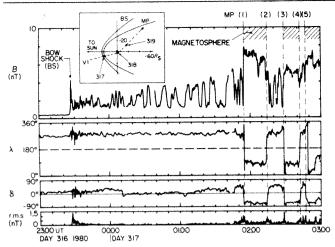


Fig. 1 Voyager 1 magnetic field (9.6 s) averages for 4 h around Saturn's inbound magnetosheath in terms of field magnitude B, longitude λ , latitude δ , and pythagorean r.m.s. deviation. The angles λ , δ are expressed in a heliographic, spacecraft centred, coordinate system, such that $\lambda = \tan^{-1} (B_T/B_R)$ and $\delta = \sin^{-1} (B_N/B)$, where: $\hat{\mathbf{R}}$ is radially away from the Sun; $\hat{\mathbf{T}}$ is parallel to the Sun's equator plane, normal to $\hat{\mathbf{R}}$, and positive in the general direction of Saturn's orbital motion; and $\hat{\mathbf{N}} = \hat{\mathbf{R}} \times \hat{\mathbf{T}}$. At this time $\hat{\mathbf{N}}$ is parallel to the ecliptic plane normal within $\sim 2^\circ$. The five inbound magnetopause crossings are labelled. The day numbers in the trajectory insert refer to spacecraft positions at the start of the referred day, and $R_s = 60,330$ km, Saturn's radius.

Analysis of the magnetopause

Two methods were used to estimate normals to the MP for comparison purposes. The first uses a variance analysis of difference fields (ΔB_i) across the discontinuity and assigns the normal direction (δ_N, λ_N) to the minimum variance direction; $\Delta \mathbf{B}_i = (\mathbf{B}_i - \langle \mathbf{B} \rangle)$, \mathbf{B}_i is one of the series of 1.92-s averages (in our case) occurring in the analysis interval across the MP, and $\langle \mathbf{B} \rangle$ is the average of the Bis. The second method is similar to the first but uses the full vectors, B_i (1.92-s average fields); then the minimum variance direction of the Bis is the estimated normal direction (δ_n, λ_n) (see Table 1). The angle η is defined as \cos^{-1} $(B_z/\langle B \rangle)$, where B_z is the component of $\langle B \rangle$ along the estimated normal, according to method (1), and $\langle B \rangle$ is $|\langle \mathbf{B} \rangle|$. When $\eta = 90^{\circ}$ the MP discontinuity is described as a tangential discontinuity. Within the expected errors on the η s all crossings can be considered as tangential discontinuities; this is also common for the Earth's dayside MP. When the MP is a tangential discontinuity and when magnetometer zero level errors can be ignored, method (2) yields more accurate normal estimates than method (1) (ref. 6). Only the results of method (2) will be used in our surface wave analysis. However, both methods qualitatively show the same main features: oscillations in both δ and λ , and almost the same overall averages.

Both methods indicate that surface waves were occurring on the MP surface, just as have been seen to occur on the Earth's MP^{7,8}. The nature of these waves is more evident in a more appropriate coordinate system. The variance analysis of method (2) applied to the normals shows that a maximum variance plane lies parallel to Saturn's equatorial plane within $\sim 10^{\circ}$. This suggested transforming the normals to a Saturn equatorial system, similar to the δ , λ (heliographic) system, except that Saturn's equatorial plane replaces the Sun's equatorial plane. This requires a 26° rotation about the Saturn-Sun line. Table 1 also shows the transformed method (2') normals (δ_{eq} , λ_{eq}). The oscillation in the latitude (δ) of the normals has now disappeared, as expected; by comparing method (2) and (2') normals in Table 1 we note how r.m.s. (δ) has decreased and r.m.s. (λ) has increased. Apparently the surface wave propagation vector is directed along the MP in Saturn's equatorial plane within $\approx 10^{\circ}$. The transformed estimated normal longitudes (λ_{eq}) are now used to analyse this surface wave⁷.

Magnetopause surface wave analysis

The surface wave model assumes that the wave is two-dimensional, transverse, and composed of two partial sinusoids which

must match in location, slope, and wave speed at their common point; the wave vector is locally parallel to the zeroth-order MP given by an earlier model1. A second assumption is that the observed MP crossings are due entirely to the passing wave, and not to large scale radial motion of the MP. The eight quantities to be estimated are: A_i , the amplitude of the i-part of the wave; α_1 , the phase of the *i*-part; λ_i , the wavelength of the *i*-part of the wave; $V_{\rm w}$, the phase speed of the wave, assumed constant for the full wave; and yo, the zero-displacement line of the wave, where i = 1, 2. The slopes and wave displacements at the two end points of the wave and at the common point between them must match observations, providing eight equations to solve. These three points occur at three successive MP crossings. The input quantities are: V_N , V_T , the components of the velocity of the spacecraft normal and tangential to the zeroth-order MP, respectively, in Saturn's equatorial plane; $\tan \theta$ (where $\theta = \lambda_{eq}$ - λ_0), the slope of the MP as estimated by the minimum variance analysis; and the times of the three successive MP crossings. The input (centre) times are given in Table 1, $V_N = 16.6 \text{ km s}^{-1}$ $V_T = 2.4 \text{ km s}^{-1}$. The components of the spacecraft's velocity (V_N, V_T) as well as the derived wave speed (V_W) are measured in a system fixed with respect to the Saturn-Sun line. The angle λ_0 is the longitude of the normal to an ideal zeroth-order MP in the locality of the three successive crossings. Three trial λ_0 s were used (197°, 200° and 202°) giving three trial sets of tan θ for the given λ_{eq} s. The λ_0 s are consistent with an estimated λ_0 of $200^{\circ} \pm 3^{\circ}$ based on a weighted average of a model normal (λ_0 (model) = 208°) and the average of the derived λ_{eq} s from Table 1 (195°), where the latter was favoured by 2-to-1 in the weighting. Three separate (but not independent) analyses were made of the wave characteristics using: MPs 1, 2 and 3 (Set I); MPs 2, 3 and 4 (Set II); and MPs 3, 4 and 5 (Set III).

Table 2 gives the results of the surface wave calculations, where A_e is the effective wave amplitude based on a weighted average of A_1 and A_2 , similarly for the effective wavelength λ_e and $\tau_e = \lambda_e/V_w$; (for the method of weighting see ref. 7). The five trial results not shown ($I_{1,2,3}$ and $I_{1,2,3}$) were not physically allowable. Regardless of the variability of V_w , A_e and λ_e , the effective period τ_e is consistently 23 \pm 2) min and λ_e/A_e is nearly constant at 10 (\pm 1). The latter fact indicates that the wave displacement is apparently of relatively small amplitude,

We now check the assumption that the MP crossings were primarily due to the surface wave and not to bulk normal motion of the MP. The time between centres of the first and last crossings is 52 min. Then $\langle A_e \rangle \approx \Delta T \times V_{\rm N}/2$, which is 26,000 km; the wave analysis gives a very favourable comparison of $\langle A_e \rangle = 25,600$ km (Table 2).

Simple geometrical considerations give an MP thickness

$$D = |\Delta t[(V_{\mathbf{W}} - V_{\mathbf{T}})\sin(\lambda_{\mathbf{eq}} - \lambda_0) + V_{\mathbf{N}}\cos(\lambda_{\mathbf{eq}} - \lambda_0)]|$$
 (1)

for each crossing. All quantities in equation (1) are fairly accurately estimated, measured, or known, except λ_0 and $V_{\rm W}$, which were moderately well estimated. We assume $V_{\rm W}$ to be $180~{\rm km~s^{-1}}$, the average of the four estimated cases (Table 2). The Δts and $\lambda_{\rm eq}s$ are given in Table 1. Consistent with our choice

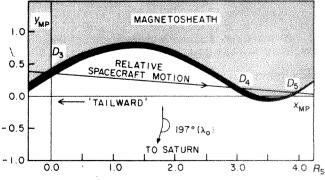


Fig. 2 An average example (case III_1) of Saturn's magnetopause surface waves. The unperturbed magnetopause is aligned with the X_{MP} -axis. $D_{3,4,8}$ refer to magnetopause thicknesses.

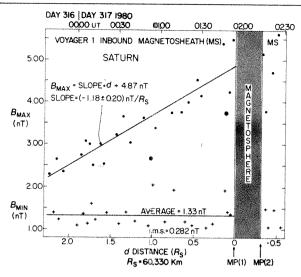


Fig. 3 The relative maximum $(B_{\text{MAX}}, \bullet)$ and minimum $(B_{\text{MIN}}, +)$ magnetic field magnitudes, based on 9.6-s averages, for the inbound magnetosheath regions, as a function of distance (d) from the first magnetopause crossing and time. \bullet , points not used in the straight line fit of B_{MAX} .

of $V_{\rm w}$ we assume that $\lambda_0=197^{\circ}$ and 200°, those giving intermediate wave characteristics. The resulting estimated MP thicknesses range from 2,300 km (crossing 5) to 8,500 km (crossing 2), giving $\langle D \rangle = 5.0 \pm 3.0 \times 10^3$ km, averaged over all five crossings and over both λ_0 s; $\lambda_0=197^{\circ}$ gave the smaller spread of values. Note that $\langle A_e \rangle$ is about five times greater than $\langle D \rangle$, for either λ_0 , consistent with another implicit assumption that the wave amplitude is significantly greater than the MP thickness.

Figure 2 shows the MP surface wave derived from trial III₁ (that is $\lambda_0=197^\circ$) chosen because it most closely resembles the average of all successful trials (Table 2). The variable MP thickness shown is based on $\lambda_0=197^\circ$ for crossings 3, 4 and 5. Such surface waves at this location on the MP (≈ 13.10 LT) near Saturn's equatorial plane are possibly due to a Kelvin–Helmholtz instability⁹ at the boundary, on either side of which plasmas are flowing parallel to it but at significantly different speeds. The difference in speed across the MP in Saturn's equatorial plane must be closely comparable with the wave speed $\langle V_w \rangle$, that is

$$\langle V_{\mathbf{w}} \rangle \approx V(\text{sphere}) - V(\text{sheath})$$
 (2)

if the Kelvin-Helmholtz instability is the causal mechanism. In our case the magnetosphere plasma speed, V(sphere), is faster than the magnetosheath plasma speed, V(sheath). Using an Earth-like magnetosheath model¹⁰ for $M_{\infty}=12$ and $\gamma=5/3$ at 13.10 LT and a typical solar wind speed of 400 km s⁻¹ (close to that actually measured for many hours before the inbound bow shock (J. Scudder, personal communication)), gives $V(\text{sheath})=0.16\times400=64$ km s⁻¹; this estimated magnetosheath speed is not very sensitive to M_{∞} , provided it is ≥ 5 . M_{∞} was calculated to be $\sim 13-15$ where the upstream proton and electron temperatures ($T_p\approx 2.3\times10^4$ K and $T_s\approx 1.5\times10^4$ K) were provided by the Voyager Plasma Science Team. For $\langle V_{\rm W}\rangle=180$ km s⁻¹ and this magnetosheath speed, equation (2) yields $V(\text{sphere})\approx 240$ km s⁻¹. If Saturn's magnetosphere rigidly corotates out to the frontside MP at ≈ 23 $R_{\rm S}$ (where $R_{\rm S}\equiv 60,330$ km, Saturn's

Table 2 Surface wave characteristics						
Quantity	$(\lambda_0 = 197^\circ)$ II_1	(197°) III ₁	(200°) III ₂	(202°) III ₃	Average	
$V_{\mathbf{w}} (\mathbf{km} \mathbf{s}^{-1})$	346	210	90	68	180	
$A_{\rm r} (\times 10^4 \rm km)$	4.97	2.70	1.37	1.18	2.56	
$A_{\bullet}[\times R_{\bullet}]$	[0.824]	[6.448]	[0.227]	[0.196]	[0.424]	
$\lambda_{\rm a} (\times 10^5 \rm km)$	5.06	2.80	1.30	1.02	2.55	
$\lambda_{\bullet}[\times R_{\bullet}]$	[8.39]	[4.64]	[2.15]	[1.69]	[4.22]	
$\tau_{\rm e}$ (min)	24	21	24	25	23.5	
λ_e/A_e	10.2	10.4	9.5	8.6	9.7	

radius) at this time, the corotation speed at the MP would be $230 \, \mathrm{km \, s^{-1}}$, close to the above result. As the uncertainty on our estimate of $V(\mathrm{sphere})$ is probably quite large, we cannot say that rigid corotation of the magnetosphere holds to $23 \, R_{\mathrm{s}}$, but only that this important necessary condition (equation (2)) for showing that the Kelvin–Helmholtz instability may be applicable is not obviously violated. The fact that the MP surface normals oscillate in Saturn's equatorial plane, rather than in a plane containing the local magnetosheath plasma flow (\approx the ecliptic plane), supports the view that it is primarily the magnetosphere motion which drives the instability.

Inbound magnetosheath waves

Here we briefly discuss the oscillations occurring in the inbound magnetosheath field, mainly in its magnitude, B, as shown in Fig. 1; these have an average period of ~ 6 min. as measured in the spacecraft frame of reference. The latitude $(\langle \delta_B \rangle \approx 0^\circ)$ and longitude $(\langle \lambda_B \rangle \approx 285^\circ)$ of the magnetosheath field agrees with an expected wrapped-up solar wind field at Saturn's orbit $(\lambda_B \approx 270^\circ \text{ or } 90^\circ)$. Oscillations in field direction, especially in longitude, are present, but dominated by the magnitude changes. The values of the local maxima and minima of the field in the magnetosheath are plotted in Fig. 3 against normal distance from the first MP(1), assuming a stationary zeroth-order MP at this time and using the spacecraft speed normal to the MP of $V_N = 16.6 \text{ km s}^{-1}$. Straight line fits were made to the B_{MAX} and B_{MIN} points; the B_{MIN} set was assumed to have zero slope. The wave has apparent amplitude (A) growth given by

$$A = (B_{\text{MAX}} - B_{\text{MIN}})/2 = \text{slope} \times d/2 - 1.77 \text{ nT}$$
 (3)

where slope = (-1.18 ± 0.20) nT/ $R_{\rm S}$ according to the straight-line fits. The marked growth in amplitude as the MP is approached suggests that the MP may be the source of these waves; notice the highest $B_{\rm MAX}$ amplitudes were maintained in the magnetosheath even when the spacecraft returned there between ≈ 02.15 and 02.30 UT. Similar waves in the inbound Pioneer 11 magnetosheath data were reported by Smith et al.³. The Pioneer observations, however, differed in one respect: the $B_{\rm MIN}$ s did not remain nearly constant but grew approximately in line with the $B_{\rm MAX}$ s, yielding $A\approx$ constant. The apparent difference is either a different phenomenon or simply due to different conditions, such as possibly an outward moving MP in the Pioneer 11 case and a static (excluding surface waves) MP for Voyager. In any case, a gradual increase in B is probably not needed to produce the waves.

Examination of the Voyager plasma science electron data (E. Sittler, personal communication) for the period shown in Fig. 3 indicates that the electron density was $\sim 180^\circ$ out-of-phase with B for these waves, suggesting that they are slow mode magnetosonic waves, which have their most rapid phase velocity along the ambient magnetic field. If the MP is the source of these waves, they must have a component of their propagation vector upstream, and it must be greater than the incoming convected magnetosheath plasma velocity; this is possible, because at this location the magnetosheath plasma is subsonic. Although the waves in B resemble solitons³, β in the magnetosheath is large, so a large perturbation in B does not necessarily imply a correspondingly large change in density.

Summary and discussion

Voyager 1 magnetometer data have revealed that surface waves occur on Saturn's dayside MP. Estimates of Saturn's magnetopause wave properties show that: the wave speed is 180 ± 90 km s $^{-1}$, amplitude $12-50\times10^3$ km (or $\sim0.5~R_{\rm S}$), wavelength $1-5\times10^5$ km (or $\sim5~R_{\rm S}$), and a well determined period 23 ± 2 min. Most estimates yielded a consistent ratio of wavelength-to-amplitude of 10 ± 1 , and a typical magnetopause thickness of $5.0\pm3.0\times10^3$ km or $\sim0.1~R_{\rm S}$ was determined.

The success of the model was partly due to the large-scale stationarity of the MP during the analysis period, probably the result of apparently persisting quiet upstream solar wind conditions, which was the case before the inbound bow shock cross-

ing. The occurrence of only a single bow shock crossing shortly before the multiple MP crossings is consistent with our basic premise that the surface waves caused the multiple crossings at a time when the solar wind was fortuitously quiet.

Hydromagnetic waves occurring in magnetosheath and having an average period in the spacecraft frame of reference of 6 min grew markedly in amplitude as the magnetopause was approached. This suggests the MP as a

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- 1. Ness, N. F. et al. Science 212, 211-217 (1981).

- Ness, N. F. et al. Science 212, 211-217 (1981).

 Behannon, K. W. et al. Space Sci. Rev. 21, 235-257 (1977).

 Smith, E. J. et al. Science 267, 407-410 (1980); J. geophys. Res. 85, 5655-5674 (1980).

 Sonnerup, B. U. Ö. & Cahill, L. J. J. geophys. Res. 72, 171-183 (1967).

 Siscoe, G. L., Davis, L. Jr., Coleman, P. J. Jr., Smith, E. J. & Jones, D. E. J. geophys. Res. 73, 61-82 (1968).

possible source of these waves unless the amplitude growth is due to a geometrical effect. These waves have been tentatively identified as slow mode magnetosonic waves. Recently such waves have been observed in the Earth's magnetosheath by the ISEE spacecraft (J. Scudder, personal communication).

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- Burlaga, L. F., Lemaire, J. & Turner, J. M. J. geophys. Res. 82, 3191-3200 (1977). Lepping, R. P. & Burlaga, L. F. J. geophys. Res. 84, 7099-7106 (1979).
- Fairfield, D. H. in Magnetospheric Particles and Fields (ed. McCormac, B. M.) 67-77 (Reidel, Hingham, 1976).
- Lee, L. C., Albano, R. K. & Kan, J. R. J. geophys. Res. 86, 54-58 (1981).
 Spreiter, J. R., Alksne, A. Y. & Summers, A. L. NASA TND-4482 (National Aeronautics and Space Administration, Washington DC, 1968).

Saturn's magnetic tail: structure and dynamics

K. W. Behannon, J. E. P. Connerney & N. F. Ness

Laboratory for Extraterrestrial Physics, NASA Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

Voyager 1 magnetic field observations have provided evidence of a saturnian magnetic tail. Tail current system distributions are inferred through comparison of the observations with a realistic magnetotail current system model. Temporal variations observed in the tail were probably produced by solar wind variations.

SATURN'S magnetic tail was first observed by Voyager 1 as it tranversed the saturnian magnetosphere outbound at 03.40 LT. The observed magnetic field directions clearly indicated the sweeping back of field lines into a tail configuration pointing away from the Sun¹ and led us to conclude that Saturn, like Earth and Jupiter, possesses a magnetotail that develops in the interaction of the solar wind with the planetary magnetic field. Closest approach for Voyager 1 at Saturn occurred at 23.45 UT on 12 November 1981. Between 17.29 and 21.52 UT on 14 November, it crossed the magnetopause outbound five times at an average distance from Saturn of 45 R_s (24 R_s antisolar distance, where R_s = Saturn radii = 60,330 km) and at a latitude ~24° (ref. 1). The encounter trajectory characteristics, together with the length of time (~45 h) spent in the night-side magnetosphere, gave ample opportunity to study the geometry of the tail. We estimated the tail diameter to be $\sim 80 R_{\rm s}$, less than that of the jovian tail by a factor of 4-6. From the observed size of the tail and the average magnitude of the distant tail lobe field that was measured (~3 nT), a conservation of flux argument gave an estimate of 11°-15° for the half-angular size of the saturnian polar cap auroral zone, smaller than that of Earth¹. Because of the high latitude of the outbound pass, there were no traversals of the tail current sheet. Only the northern lobe field (directed away from the planet) was measured. The near alignment of Saturn's magnetic dipole and rotational axes made the outbound traversal a nearly constant magnetic latitude path through a planetary magnetosphere.

Although there was no direct tail current sheet observation, plausible current distributions and geometry can be inferred through comparison of measurements with the fields calculated from simple but realistic magnetosphere models that include magnetotail current systems. The preliminary results of the study of one such model are described below.

Model magnetosphere

We have studied a model of Saturn's magnetosphere which is consistent with the Voyager 1 magnetic field observations but which must also be non-unique. A noon-midnight meridian projection of the model field and the Voyager 1 outbound hourly averaged magnetic field observations is shown in Fig. 1. The closure of the tail field lines is very sensitive to the detailed current distribution in the more distant (and unexplored) tail and is not a direct result of the fit to the magnetic field observations. It is, rather, a boundary condition imposed on this model determined, in part, by the observed electron pitch angle distributions² which are indicative of closed field lines. The closure of field lines in the tail is uncertain. Observations of solar cosmic rays to 9 R_s by the Pioneer 11 cosmic ray experiment suggest open field lines in the magnetotail, although Saturn's magnetosphere was in a disturbed state during the Pioneer 11 encounter.

The model field has the following components: an internal dipole field of $0.21 \,\mathrm{G} - R_{\mathrm{S}}^3$ aligned with z_{SM} ; a distributed near-equatorial disk or ring current in the region $8 < \rho < 16 R_s$; cross-tail currents extending from $x_{SM} = -16$ to $-100 R_S$, closing on the magnetopause boundary; and confinement of the day-side magnetosphere by image dipoles. The azimuthal ring current at Saturn is modelled on a similar structure at Jupiter and will not be discussed here. The field of the cross tail currents

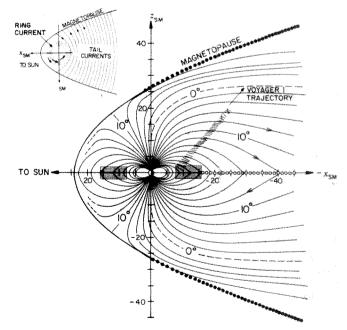


Fig. 1 Conceptual and quantitative model of Saturn's magnetosphere in the noon-midnight meridian plane. Field lines are drawn for 2° increments of invariant latitude. Azimuthal disk or ring current (stippled) is indicated, as well as model cross-tail currents closing on the magnetopause boundary. Projections of Voyager 1 outbound hourly-averaged magnetic field observations onto the solar magnetospheric (SM) x-z plane are also shown. Insert, model cross-tail currents in the SM x-y plane. Current flows eastward in parabolic paths to the magnetopause (MP) pause boundary and closes in planes of constant x equally on both the northern and southern lobe MP

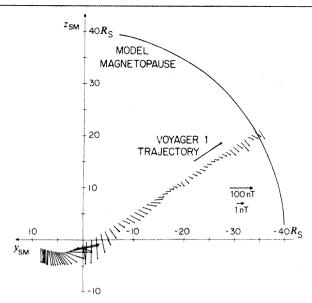


Fig. 2 Projection of hourly-averaged magnetic field components in the saturnian magnetosphere onto the SM y-z plane along the projected Voyager 1 trajectory. A logarithmic scale has been used to represent field magnitude as indicated.

is computed numerically by summation of the field due to a system of individual closed current loops similar to that used in models of the Earth's magnetosphere ^{5.6}. Individual loops are spaced at $1-R_{\rm S}$ intervals and carry an eastward current in the solar magnetospheric (SM) equatorial plane as indicated in Fig. 1. The SM coordinate system is a right-handed, orthogonal, non-rotating system defined such that $\hat{x}_{\rm SM}$ is directed from the planet to the Sun and the $\hat{z}_{\rm SM}$ axis lies in the plane formed by $\hat{x}_{\rm SM}$ and \vec{M} , the magnetic dipole of the planetary field. The current has a $1/(x_{\rm SM})^{1/2}$ dependence, decreasing from $\sim 5~{\rm A~km}^{-1}$ at $x_{\rm SM}$ of $-16~R_{\rm S}$, which is about half the (linear) current density of the distributed ring currents at their outer edge.

In this view of Saturn's magnetic tail, the high latitude field lines observed along most of the Voyager 1 trajectory remain closed, while the observed magnetic field orientation along the trajectory remains essentially parallel to the SM equatorial plane. The estimated size of the polar cap¹ based on the conservation of flux using an estimate of 3 nT for the tail field at $x_{\rm SM} = -25$ may represent an upper limit on the size of the polar cap. As indicated in Fig. 1, to the extent that field lines cross the equatorial plane at greater $-x_{\rm SM}$, Saturn's polar cap may be smaller and is not symmetric with respect to the dusk meridian.

A consequence of the confinement of Saturn's magnetosphere to a parabolic magnetopause boundary is the very Earth-like day-side cusp at $\sim 80^{\circ}$ invariant latitude. The inferred source region of Saturn kilometric radiation (SKR) is centred about the intersection of the noon-midnight meridian and 80° invariant latitude. This suggests that SKR is related to particle precipitation in the cusp, analogous to the terrestrial kilometric radiation associated with the Earth's polar cusp⁸.

Tail dynamics

Variations of the magnetotail magnetic field were observed by Voyager 1 during the outbound traversal¹; they appear as moderately small amplitude, long-period excursions in field direction and magnitude. The directional variations can be seen in the hourly average SM y-z plane vector projections of Fig. 2. The x-y and x-z plane projections have been presented previously¹. Assuming that the observed changes are most likely temporal and not spatial, is the source of these perturbations of the tail lobe field internal or external to the magnetosphere? Quasiperiodic variations of the flux of low energy charged particles were also observed during this period², and a Saturn-related magnetic anomaly-type feature suggested as the cause.

The magnetic field measurements have been analysed to try to elucidate the source location of the perturbations producing the

observed variations. To test the possibility of an internal, planet-associated (and hence periodic) source, hourly values of the magnetic field direction angles λ and δ have been plotted against sub-spacecraft Saturn longitude in superposed epochs for the 36-h period when Voyager 1 was at a radial distance $r > 10~R_{\rm S}$ (Fig. 3). Here λ and δ are the SM azimuth and latitude angles, respectively, of the field. Because the dipole tilt relative to the rotation axis is $\sim 1^{\circ}$, SM coordinates are defined for Saturn such that λ is measured anticlockwise in the magnetic dipole equatorial plane, as viewed from north of (above) the plane, with the $\lambda=0^{\circ}$ meridian plane at local noon.

The data suggest a recurrence tendency at roughly the planetary rotation rate, but with changes evident in the amplitude and phase of the variation. Note that Voyager 1 was in the tail lobe for only $\sim 2-1/2$ planetary rotation periods, and it is therefore difficult to establish a recurrence pattern. If recurrence is assumed, a period of 12.1 h is obtained for the field variation, significantly greater than the 10.66 h planetary rotation period deduced by the Voyager 1 Planetary Radio Astronomy experiment. The longer period seen at Voyager 1 may be caused by Doppler-shifting of the perturbation signal as it 'propagates' like a wave from Saturn. Taking into account the velocity of Voyager 1 relative to Saturn, a radial wave speed $V_r \approx 140 \text{ km}^{-1}$ is obtained.

This wave speed can be tested for consistency with the Alfvén speed $V_A = B/(4\pi mn)^{1/2}$ expected in the tail lobe plasma. The ion number density, n, is assumed to be equal to that reported for the plasma electrons¹⁰. At distances $r = 10 R_s$ and $40 R_s$, respectively, field magnitudes of 21 and 3 nT and densities of 0.3 and $0.003\,\mathrm{cm^{-3}}$ were measured. Assuming the ions are protons gives $V_A = 836 \text{ km s}^{-1}$ in the near tail, increasing to 1,196 km s⁻¹ in the distant tail. If the ions were nitrogen, these values would decrease to 316 and 452 km s⁻¹, respectively. The V_A values for a nitrogen plasma are factors of more than 2-3 higher than the Doppler-shift predicted wave speed of 140 km s⁻¹ and factors of 6-8 too high in the case of hydrogen. Conversely, assuming $V_A = 140 \text{ km s}^{-1}$ predicts densities for hydrogen and nitrogen of $n_H = 10.7 \text{ cm}^{-3}$ and $n_N = 1.5 \text{ cm}^{-3}$ at $r = 10 R_{\rm s}$ compared with the observed $n_{\rm e} = 0.3 \,{\rm cm}^{-3}$, and $n_{\rm H} =$ 0.22 cm^{-3} and $n_N = 0.03 \text{ cm}^{-3}$ at $r = 40 R_S$ compared with $n_e =$ 0.003 cm^{-3} .

These results indicate an inconsistency between the observations and the hypothesis of a planet-associated propagating wave. Furthermore, because the magnetic dipole axis tilt relative to the axis of rotation is estimated to be in the order of only 1°, the dipole wobble is unlikely to produce the observed variations.

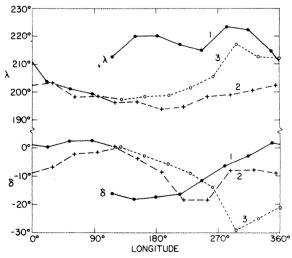


Fig. 3 Superposed epoch presentation of hourly values of magnetotail magnetic field azimuthal (λ) and latitude (δ) angles as functions of planetary longitude for three successive rotations of the planet (1, 2 and 3). The data do not demonstrate clearly a longitude-based recurrence pattern.

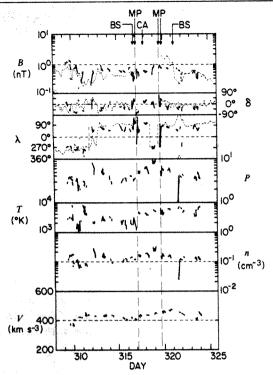


Fig. 4 Predicted solar wind parameters and interplanetary magnetic field during the Voyager 1 encounter period. These data were derived from the solar wind measurements by Voyager 2 upstream of Saturn (see text). Plasma parameters shown include bulk speed (V); number density (n); temperature (T); and plasma pressure (P). The magnetic field is given in terms of its magnitude, B, and the heliographic latitude and azimuthal angles, δ and λ . Actual Voyager 1 measurements are also given (dashed curves). Voyager 1 magnetopause (MP) crossing times are given by arrows at top and further delineated by vertical lines. Bow shock (BS) crossings and closest approach (CA) times are also indicated by arrows at the top.

An investigation of the solar wind during the encounter period indicates that perturbations outside the saturnian magnetosphere may have been the source of the observed variations. The solar wind and interplanetary magnetic field (IMF) conditions at Saturn during the encounter have been predicted by applying a transformation to Voyager 2 interplanetary hourly average field and plasma observations (J. D. Sullivan and H. S. Bridge, personal communication). This projects the observations in two-dimensional space and in time assuming that each parcel of plasma expands radially outward at constant speed. The hourly averaged data propagated in this way from Voyager 2 to Saturn are shown in Fig. 4. Time is given as day-of-year number (day 317 = 12 November). The distorted and fragmented appearance of the data is due to effects of the variable speed correction applied in time-shifting each parcel and the intermittent tracking and data retrieval from Voyager 2. The sizeable gaps (~14 h) mean that a one-to-one correlation between changes in these data and those seen by Voyager 1 inside the saturnian magnetosphere is impossible. However, the data show substantial changes in the solar wind and IMF during the encounter. The lighter dashed data curves superimposed on the predicted data in the top three panels are the actual Voyager 1 IMF observations for comparison. The field data are in spacecraft-centred heliographic coordinates, a spherical system with azimuth $\lambda = \tan^{-1} (B_T/B_R)$ and $\delta = \sin^{-1} (B_N/B)$. where \hat{R} is radially away from the Sun; \hat{T} is perpendicular to \hat{R} and parallel to the solar equatorial plane, positive in the general direction of Saturn's orbital motion; and $N = R \times T$.

An examination of λ shows a prediction of an IMF sector transition occurring ~6 days before closest approach (CA) and a similar, return transition occurring between 7 and 16 h after CA. These are accentuated by vertical dashed lines at the midpoints of the gaps in the λ data where the transitions occurred. In the latter transition, which would have taken place while Voyager 1 was in the tail, the new state of the medium only persisted for between 10 and 32 h and thus was either a very narrow sector or a filamentary-type structure. The field magnitude increased within this region, accompanied by a plasma density increase of about a factor of two and a corresponding increase in solar wind pressure. The leading edge of this region is predicted to have passed between the middle and the end of day 318—the period when the temporal excursions in the magnetotail began.

The major uncertainty is that the Voyager 2 data are being extrapolated over a distance of ~1.7 AU, and in that distance the characteristics of plasma streams and associated IMF structures can be modified significantly by dynamical processes. A comparison of the projected Voyager 2 data with measurements from Voyager 1 before and after passing through Saturn's magnetosphere (included in Fig. 4) confirms the essential features of the prediction. However, the sector transition predicted to occur at the end of day 311 was actually observed to begin ~12 h later. A similar shift in the onset of the perturbations during tail passage would start their effect at a later time but with Voyager 1 still in the magnetotail. Different transformation conditions (higher solar wind speed) could have produced a closer agreement in this case between predicted and observed features than was found during the earlier, pre-encounter period.

While the direct consequences for a magnetotail orientation and field strength of changes in external solar wind direction and pressure on the boundary are generally understood, how changes in the IMF configuration influence a magnetotail is not completely understood. Studies of the terrestrial magnetosphere show that this influence is probably exerted in most cases indirectly through a chain of events controlled by the IMF-tomagnetospheric field reconnection efficiency and including the process of flux transfer within the magnetosphere 11,12. Because the observations at Saturn are limited to one tail lobe, and because of the supporting interplanetary measurements, we can show only that plausible conditions existed in the interplanetary medium for stimulating the observed tail perturbations, with the precise mechanism remaining a matter of speculation. As an internal source mechanism for the perturbations seems inconsistent with measured physical parameters, we conclude that an external source, the solar wind, is responsible.

We thank our Voyager plasma investigation colleagues at MIT for providing the projected Voyager 2 plasma and magnetic field data for use as a prediction of solar wind conditions at Saturn during the Voyager 1 encounter.

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- Ness, N. F. et al. Science 212, 211-217 (1981)
- Krimigis, S. M. et al. Science 212, 225-231 (1981). McDonald, F. B., Schardt, A. W. & Trainor, J. H. J. geophys. Res. 85, 5813-5830 (1980).
- Connerney, J. E. P., Acuña, M. H. & Ness, N. F. J. geophys. Res. 86 (in the press); Natur 292, 724-726 (1981). Olson, W. P. J. geophys. Res. 79, 3731-3738 (1974).
- Walker, R. J. in Quantitative Modeling of Magnetospheric Processes ed. Olson, W. P. 9-34 (American Geophysical Union, Washington DC, 1979).
- Kaiser, M. L., Desch, M. D. & Lecacheux, A. Nature 292, 731-733 (1981). Alexander, J. K., & Kaiser, M. L. J. geophys. Res. 82, 98-104 (1977).

- Alexander, J. A., & Kaiser, M. L. J. geophys. Res. 84, 98-104 (1927).
 Desch, M. D. & Kaiser, M. L. Geophys. Res. Lett. 8, 251-256 (1981).
 Bridge, H. S. et al. Geophys. Res. Lett. 8, 217-224 (1983).
 Holzer, R. E. & Slavin, J. A. J. geophys. Res. 84, 2573-2578 (1979).
 Holzer, R. E. & Slavin, J. A. J. geophys. Res. 86, 675-680 (1981).

ARTICLE

Total synthesis of a human leukocyte interferon gene

Michael D. Edge, April R. Greene, Gillian R. Heathcliffe, Peter A. Meacock*, Wolfgang Schuch[†], Denis B. Scanlon, Thomas C. Atkinson, Clive R. Newton & Alexander F. Markham

ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK
* ICI/University Joint Laboratory, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, UK
† ICI Corporate Laboratory, The Heath, Runcorn, Cheshire WA7 4QE, UK

A 514-base pair fragment of double-stranded DNA coding for human interferon- α_1 (166 amino acid residues), and containing initiation and termination signals plus appropriate restriction enzyme sites for plasmid insertion, has been totally synthesized. The synthesis involved preparation of 66 oligodeoxyribonucleotides, ranging in size from 14 to 21 residues, plus 1 deoxydecanucleotide, by rapid, solid phase procedures, and enzymatic ligation of the oligonucleotides. After ligation of the synthetic gene to a plasmid vector and transformation of Escherichia coli, clones containing the anticipated gene sequence were obtained.

THE antiviral^{1,2}, cell proliferation inhibition³ and immuno-modulating properties⁴ of human interferons (IFNs) have generated great interest because of their potential clinical value in the treatment of viral infections and malignancies. Thorough investigation of the efficacy of both leukocytic (IFN- α) or fibroblastic (IFN- β) interferons has, however, been hampered by an inability to purify large amounts of these proteins from human cells.

The recent application of recombinant DNA techniques has dramatically changed this situation. Several groups have obtained cDNA clones prepared using mRNA from a variety of human cells induced to synthesize interferon(s)⁵⁻¹⁰. DNA sequencing has allowed the amino acid sequences of a number of IFN- α s¹¹ and IFN- β ^{12,13} to be deduced (as well as their putative signal sequences) and various elegant genetic manipulations have generated expression plasmids which direct the bacterial synthesis of biologically active interferons 14-21. A second phase of this research has seen eight or more distinct human leukocyte interferon genes²² identified in human DNA libraries and neither these IFN- α genes^{23,24}, nor the IFN- β gene^{25,26}, apparently contain introns. In some of the above studies the availability of specific oligonucleotides, designed on the basis of limited amino acid sequence data^{27,28}, was invaluable for the identification of cloned IFN cDNA. Furthermore, synthetic oligonucleotides have been generally useful in the various gene editing procedures used for the construction of expression plasmids.

On the basis of the nucleotide sequence of the first cloned human IFN-α cDNA published by Weissmann and co-workers (IFN- α_1), we designed a DNA fragment which would encode the same protein and be amenable to total chemical synthesis (Fig. 1). Such a synthetic gene has several potential advantages, which are discussed further below. However, it is obviously desirable that such a synthesis be accomplished in a comparable time scale with that required for isolation of the gene from natural sources. Various other synthetic genes, including those for human somatostatin²⁹, human insulin A and B chains³⁰ and N^{α} -desacetylthymosin α_1 (ref. 31), have previously been constructed but these molecules are all considerably smaller than the IFNs. Total synthesis of an IFN gene would have been unrealistic using standard solution phase phosphotriester methodology as applied in the above examples³². Recently, we have developed a novel solid phase phosphotriester approach for the synthesis of oligonucleotides^{33,34}. This increases the rate of synthesis by an order of magnitude and is routinely reliable, the inherent hazards of repeated chromatographic purification being largely avoided.

Using these methods, 67 different oligonucleotides containing around 15 nucleotide residues were synthesized. They were separately purified and 5' phosphorylated, then sequentially ligated to yield a 514-base pair (bp) fragment. After insertion in a hybrid plasmid and cloning in *Escherichia coli*, the synthetic

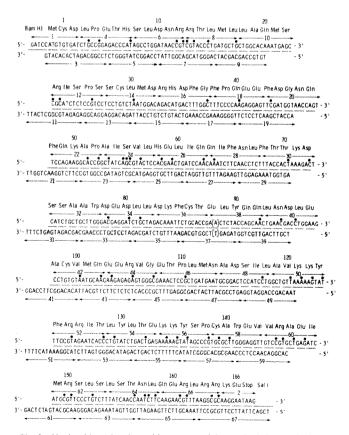


Fig. 1 Nucleotide and amino acid sequence of the synthetic human leukocyte interferon gene. Individual oligonucleotides (1-67) are arrowed. Dots above bases in the coding strand indicate changes with respect to the native sequence⁷. Numbers above each line refer to amino acid position.

gene was characterized by restriction enzyme digests and total sequence determination. Biological activity of material expressed by these clones will be described elsewhere (E. De Maeyer, manuscript in preparation).

Design of the synthetic gene

Initially, the published DNA sequence⁷ coding for mature human IFN-α, was scanned with a computer program designed to detect both complementary and repeated sequences (D. J. Gilman, unpublished results). A number of such sequences were identified. For example, bases 75-85 in the native sequence, d(TCCTTCCTCCT), are complementary to bases 487-497, d(AGGAGGAAGGA), and bases 32-40, d(ACAGGAGGA) are complementary to bases 79-87, d(TCCTCCTGT). Similarly, there are 4 separate complementary pairs of octamers and 13 complementary pairs of heptamers. The sequence d(GAAGAAATAC) (bases 359-368) is repeated (bases 399-408). There are seven separate repeated heptamers, five repeated octamers and one repeated nonamer. Numbering here is with respect to the first base in the triplet coding for Nterminal cysteine (T=1) in the mature peptide (see Fig. 3 in ref. 7).

We reasoned that elimination of these features as far as possible within the constraints of the genetic code would make correct ligation of synthetic oligonucleotides more straightforward. The target sequence is illustrated in Fig. 1. Dots above bases in the coding strand indicate changes with respect to the native sequence (71 in total). By so modifying the natural sequence, the longest self-complementary regions are four heptamers, all separated by more than 100 bp. There are two repeated nonanucleotides; d(AATCTTCAA) in blocks 28 and 64/66 and d(AAAAAGTAT) in blocks 50 and 56. There are three repeated heptamers and one repeated octamer.

The gene was divided (Fig. 1) into a series of oligonucleotides of ~ 15 residues so as to give overlaps of at least 7 bp on each side of all ligation points. It is convenient to use oligonucleotides of this size as the synthesis and purification of significantly larger molecules may not always be straightforward³³. Where possible, changes in the native sequence are such as to introduce codons which may be preferred for high levels of expression in bacteria^{35–37}. The codon usage profile is compared with that of the native strand in Table 1.

Outline of chemical synthesis strategies

The basic chemistry involved in our synthetic approach is summarized at the top of Fig. 2 and in its legend and we have previously described the methodology in some detail^{33,34}. During this study several minor changes have been made which considerably improve both the rate and yields of synthesis. In our hands, use of mesitylenesulphonyl-3-nitro-1,2,4-triazole³⁸ as condensing agent increases yields in dimer coupling reactions and allows individual reaction times to be reduced to ~1 h. We have also reduced the deprotection/washing cycle to ~ 0.5 h so that the overall time for addition of each dimer block to a growing chain is ~1.5 h. With a semi-automated solvent delivery system it is convenient to perform two or three such assemblies concurrently so that up to two pentadecanucleotides can be constructed per man-day. Previously we have not attempted to dry the support between coupling cycles but co-evaporation of the polydimethylacrylamide resin with anhydrous pyridine before each condensation reaction again significantly improves the final yields of purified deprotected oligonucleotides. Thus (Fig. 2a, c), in the syntheses of blocks 24 and 65 (Fig. 1) the desired oligonucleotide product is the major peak in ion-exchange HPLC on Partisil 10-SAX.

Further resolution by reverse-phase HPLC on μ -Bondapak C_{18} columns (Fig. 2b, d) gives pure material in most cases, as judged by standard 5'-[32 P]-phosphorylation and sizing on 20% polyacrylamide gels in 7 M urea (for examples see Fig. 2e, f) 39 . In our previous work 33,34 , oligonucleotides synthesized by this approach were characterized by complete sequencing. During this synthesis, the oligonucleotides were characterized by sizing

(above) and 5' residues were confirmed after total snake venom phosphodiesterase digestion of 5'-[³²P]-labelled aliquots. In cases where unequivocal identification of product by HPLC and sizing was not possible, complete sequencing was undertaken.

A more complete description of improved methodology for the synthesis of these oligonucleotides will be published elsewhere. The oligomers 5, 7, 17, 44, 58 and 63 could not be purified by standard Partisil 10-SAX ion-exchange HPLC with phosphate gradients in a 5% ethamol system at ambient temperature. Elevated temperature and increased ethanol concentration or electrophoretic techniques were necessary³³. We note that all these oligomers contain three or more consecutive dG residues. In retrospect, these features could have been avoided in oligomers 5, 7, 17, 44 and 63 by alternative choices of codons but it is obviously desirable to be able to synthesize such molecules so as to have the widest possible latitude in codon usage. Furthermore, this sequence cannot be avoided in oligomer 58 given the amino acid sequence Trp-Glu [d(TGGGAPur.)].

Ligation strategy

Eleven groups of oligomers (A-K) were initially ligated separately (Fig. 3a). 5' Phosphorylation with low specific activity ³²P-ATP and polynucleotide kinase was performed on

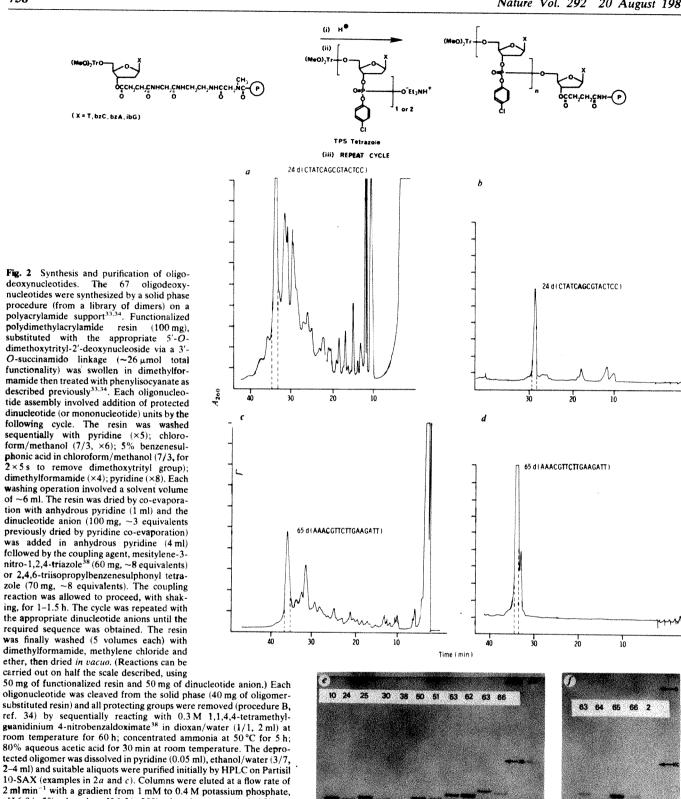
Table 1 Codon usage in synthetic (and natural) human IFN- α

	U	С	Α	G	
U	Phe $\begin{cases} 3(4) \\ 5(4) \\ 2(2) \\ 0(4) \end{cases}$	Ser $ \begin{cases} 3(4) \\ 4(4) \\ 2(2) \\ 0(0) \end{cases} $	Tyr $\begin{cases} 3(1) \\ 1(3) \end{cases}$ Ochre $1(1)$ Amber $-$	Cys \begin{cases} 3 (4) \ 2 (1) \ Opai & - \ Trp & 2 (2) \end{cases}	U C A G
С	Leu	$ Pro \begin{cases} 0 (3) \\ 1 (2) \\ 0 (1) \\ 5 (0) \end{cases} $	His $\begin{cases} 2(2) \\ 1(1) \end{cases}$ Gln $\begin{cases} 7(2) \\ 3(8) \end{cases}$	Arg	U C A G
A	$\begin{array}{c} \text{Ile} & \left\{ \begin{array}{l} 0(0) \\ 7(7) \\ 0(0) \end{array} \right. \\ \text{Met} & 6(6) \end{array}$	Thr $ \begin{cases} 3(2) \\ 6(4) \\ 0(3) \\ 0(0) \end{cases} $	Asn $\begin{cases} 2(2) \\ 4(4) \\ 4(4) \end{cases}$ Lys $\begin{cases} 4(4) \\ 4(4) \end{cases}$	Ser $\begin{cases} 0(0) \\ 4(3) \end{cases}$ Arg $\begin{cases} 3(6) \\ 1(5) \end{cases}$	U C A G
G	Val	Ala $ \begin{cases} 6 (4) \\ 1 (3) \\ 2 (2) \\ 1 (1) \end{cases} $	Asp $\begin{cases} 4(5) \\ 7(6) \\ 7(6) \\ 6 \end{bmatrix}$ Glu $\begin{cases} 7(6) \\ 8(9) \end{cases}$	Gly (1 (0) 2 (1) 0 (2) 0 (0)	U C A G

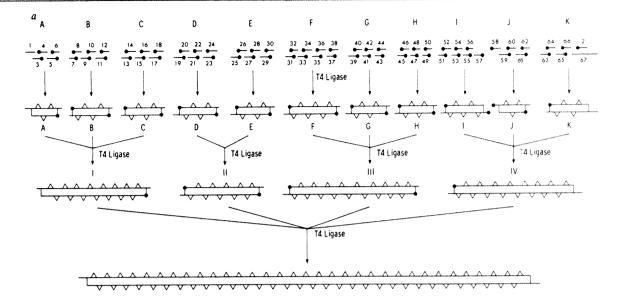
individual oligomers which were then isolated by elution from 20% polyacrylamide gels in 7 M urea (oligomers 1 and 67 were not phosphorylated at this stage). After denaturation, slow annealing and ligation, reaction mixtures were examined on polyacrylamide gels in both native and denaturing conditions (Fig. 3b, c). Ligated products of the expected size were isolated by preparative polyacrylamide gel electrophoresis in non-denaturing conditions, elution and ethanol precipitation. Product recovery was estimated by counting aliquots dried separately on GFC filter disks. Aliquots of the 11 fragments were combined, annealed and again ligated with T4-induced DNA ligase to give the four blocks shown in Fig. 3d. Ligation of these four blocks in a similar manner gave a discrete band of approximately the expected size as identified by 3.5% polyacrylamide native electrophoresis with appropriately sized markers (Fig. 3e). This fragment was isolated either by excision and elution from the gel or by gel permeation chromatography of the reaction mixture. 5' Ends were phosphorylated by standard techniques for plasmid insertion.

Construction of recombinant plasmids

The synthetic gene contains single-stranded cohesive ends to facilitate ligation to a *BamHI-SalI* plasmid vector. The plasmid, pPM50, chosen for cloning the synthetic gene, is a derivative of plasmid pAT153 (ref. 40) in which the region of the tetracycline



oligonucleotide was cleaved from the solid phase (40 mg of oligomersubstituted resin) and all protecting groups were removed (procedure B, ref. 34) by sequentially reacting with 0.3 M 1,1,4,4-tetramethyl-guanidinium 4-nitrobenzaldoximate¹⁸ in dioxan/water (1/1, 2 ml) at room temperature for 60 h; concentrated ammonia at 50 °C for 5 h; 80% aqueous acetic acid for 30 min at room temperature. The deprotected oligomer was dissolved in pyridine (0.05 ml), ethanol/water (3/7, 2-4 ml) and suitable aliquots were purified initially by HPLC on Partisil 10-SAX (examples in 2a and c). Columns were eluted at a flow rate of 2 ml min⁻¹ with a gradient from 1 mM to 0.4 M potassium phosphate, pH 6.8 in 5% ethanol or pH 6.5 in 30% ethanol, over a period of 50 min at ambient temperature. The most retained peak(s) were collected, evaporated, redissolved in water (5 ml) and applied to a μ -Bondapak C₁₈ reverse-phase column. The oligonucleotide was further purified by first eluting with 0.1 M ammonium acetate, then with a gradient of 10-25%~0.1~M ammonium acetate/acetonitrile (1/1) over 40~min with a flow rate of 2 ml min⁻¹ at ambient temperature (examples in b and d). After addition of excess triethylamine, oligonucleotide-containing fractions were lyophilized and redissolved in water (1 ml). Aliquots of each solution (80 pmol of oligonucleotide) were evaporated and phosphorylated with 1.1 units of T4-induced polynucleotide kinase in 10 μ l of a solution containing 160 pmol [γ -32P]ATP (12.5 Ci mmol⁻¹), 0.1 mM spermidine, 20 mM dithiothreitol (DTT), 10 mM MgCl₂, 50 mM Tris-HCl (pH 9.0) and 0.1 mM EDTA for 45 min at 37 °C. Reactions were terminated by addition of 270 µl of 50 mM Tris-borate (pH 8.3), 1 mM EDTA in 80% formamide and heating to 90 °C for 5 min. A 7-µl aliquot was examined on a 20% polyacrylamide gel in 7 M urea (examples in e and f).



Synthetic Interferon Gene

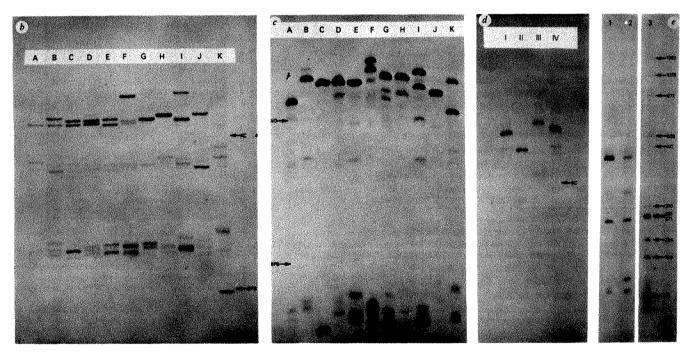


Fig. 3 Ligation of chemically synthesized oligonucleotides. a, Ligation strategy. Aliquots of the chemically synthesized oligonucleotides, 2-66 (40 pmol of each), were phosphorylated with 6.5 units of T4-induced polynucleotide kinase in 25 μl of a solution containing 80 pmol [γ-²²P]ATP (8.5 Ci mmol⁻¹), 100 μM spermidine, 20 mM DTT, 10 mM MgCl₂, 50 mM Tris-HCl (ρH 9.0) and 0.1 mM EDTA for 30 min at 37 °C. Each oligonucleotide was ethanol precipitated and fractionated by electrophoresis on a 20% polyacrylamide gel in 7 M urea. The ²²P-labelled oligomers were sliced from the gel and eluted⁴⁶. Eleven separate T4 ligase-catalysed joining reactions were performed to give fragments A to K. In groups A and K, oligonucleotides 1 and 67, respectively, were not phosphorylated. For the construction of each fragment, the terminal oligonucleotides were present in excess over the internal oligonucleotides. For example, fragment B was constructed by annealing, in 50 μl water, 3.8 pmol of oligonucleotides 7 and 12, 3.6 pmol of 8 and 11 and 3.4 pmol of 9 and 10, heating to 100 °C and cooling slowly to room temperature. Each mixture was dried in vacuo and ligated in 20 μl of a solution containing T4 DNA ligase (26 units), 74 mM Tris-HCl (ρH 7.6), 6.6 mM MgCl₂, 10 mM DTT, 2.9 mM ATP, 1.6 mM mercaptoethanol and 1.6 μg bovine serum albumin for 24 h at 22 °C. b, Aliquots (1%) of the reaction mixtures were examined on a 15% polyacrylamide gel in 7 M urea. c, The DNA from each of the remaining mixtures was ethanol precipitated and fractionated on a 15% polyacrylamide gel in the absence of urea. The DNA from each band was eluted from the gel. An aliquot of each band was re-run in denaturing conditions. In each group, the most slowly migrating band had oligonucleotide strands of the correct size. d, Fragments I-IV were constructed from 0.1-pmol of fragments A-K. Amealing was carried out at 45 °C and the mixtures were ligated as described previously. The fragments were isolated by electrophoresis on a 10% polyacrylamide gel i

resistance determinant between the EcoRI and BamHI sites has been replaced by a 95-bp AluI-generated fragment from the promoter region of the E. coli lactose operon. The construction of this plasmid, as shown in Fig. 4A, was arranged so that insertion of the AluI fragment resulted in the regeneration of both the EcoRI and BamHI recognition sequences. Intro-

duction of the synthetic oligonucleotide between the BamHI and SalI cleavage sites of the pPM50 vector plasmid should therefore place the interferon coding sequence under the transcriptional control of the lac promoter in an orientation such that the ATG codon forms a 'hybrid ribosome binding site' with the lac promoter Shine-Dalgarno (S-D) sequence⁴¹. Thus, the

sequence around the BamHI site will be

S-D Bam HI Met Cys 5'----AGGAAACAGGATCCATGTGT----3'

A construction of this type should achieve active transcription and translation leading to expression of the interferon coding sequence. Such promoter fragments containing an EcoRI cleavage site in place of the BamHI site have been used to promote expression of the streptomycin resistance gene of the plasmid R300B (P.M., unpublished data). Similarly, plasmid vectors incorporating the analogous AluI fragment from a mutant lac promoter (UV5) have been used to promote high level expression of various heterologous genes (see, for instance, refs. 42-44).

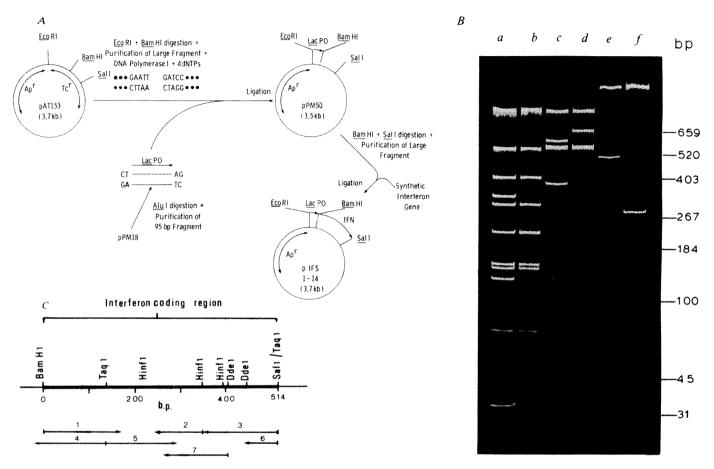


Fig. 4 A, Construction of the recombinant pIFS plasmids. DNA (7 μg) of the parental plasmid, pAT153 (ref. 40), was digested sequentially for 60 min at 37 °C with restriction endonucleases *Eco*RI and *Bam*HI in 10 mM Tris-HCl (pH 7.6), 6 mM MgCl₂ and 1 mM DTT containing 50 mM (*Eco*RI) or 150 mM (*Bam*HI) NaCl. Reactions were terminated by extraction with phenol/chloroform (3/1) and ethanol precipitation. The DNA was resuspended in 30 µl DNA polymerase I buffer (6 mM Tris-HCl (pH 7.4), 7 mM MgCl₂, 1 mM DTT) containing 40 µM each of dATP, dGTP, dCTP and dTTP to synthesize 3'-oligonucleotide extensions complementary to the protruding 5'-oligonucleotide ends generated by the endonuclease treatment. The reaction was initiated by addition of DNA polymerase I-Klenow fragment (1.5 units) and incubated at 11 °C for 90 min. 3 H-dTTP (3 μ Ci; 16 Ci mmol $^{-1}$) was added so as to follow the extent of reaction by trichloroacetic acid precipitation of 5-µl samples. The reaction was terminated by extraction with phenol/chloroform (3/1) and ethanol precipitation. Plasmid pPMR is a derivative of the vector plasmid pACYC184 (ref. 49) which has a 6.6-kb EcoRI fragment derived from \(\rho\)plac5(ref. 50) inserted at the EcoRI site in the plasmid chloramphenical resistance gene. The λplac5 fragment carries the promoter region of the E. coli lactose operon and part of the β-galactosidase gene⁵¹. Plasmid pPM18 DNA (15 μg) was digested with restriction endonuclease AluI in 6 mM Tris-HCI (PH 7.6), 6 mM MgCl₂, 50 mM NaCl, 6 mM β -mercaptoethanol and the 95-bp fragment containing the E. coli lactose operon promoter/operator region isolated by electroelution³² from a 5% polyacrylamide gel⁵³ and purified by chromatography on DE52 DEAE-cellulose⁵². The 'filled in' *EcoRI/BamHI* cleaved pAT153 DNA was blunt-end ligated to the 95-bp *AluI* fragment in a volume of 50 µl in 20 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 10 mM DTT, 5 mM ATP, with T4 DNA ligase (5 units) at 10 °C for 16 h. The ligated mixture was transformed to *E. coli* strain C600 (ref. 54) and ampicillin-resistant transformant colonies selected on antibiotic (25 µg ml⁻¹) minimal X-gal (20 µg ml⁻¹) medium⁵⁵. Colonies containing recombinant plasmids with the *lac* promoter/operator sequence were identified by their blue coloration on the X-gal medium, due to constitutive expression of the chromosomal \(\beta\)-galactosidase gene. Plasmid DNA, isolated from eight independent blue colony transformants, was screened by digestion with restriction endonucleases EcoRI and BamHI and one found to contain a single cleavage site for each enzyme separated by ~100 bp. Nucleotide sequence determination of the small (100 bp) fragment generated by digestion with these two enzymes showed that it contained the lac promoter/operator sequence flanked by EcoRI and BamHI sites and orientated such that transcription would proceed towards the BamHI site (P.M., unpublished data). This plasmid was named pPM50 and was used for the subsequent cloning of the synthetic interferon gene. DNA (4 µg) of plasmid pPM50 was digested with restriction endonucleases *Bam*H1 and *Sal*1 in 10 mM Tris-HCl (pH 7.6), 6 mM MgCl₂, 150 mM NaCl and 1 mM DTT at 37 °C for 60 min. The reaction was terminated by extraction with phenol/chloroform and the products subjected to electrophoresis through 1% agarose in 40 mM Tris-HCl (pH 7.8), 6 mM sodium acetate, 1 mM EDTA buffer. The larger 3.2-kb fragment was isolated and chromatography on DE52 DEAE-cellulose. The purified BamHI-Sal1 3.2-kb vector fragment (1 µg) was ligated to the synthetic oligonucleotide preparation with T4 DNA ligase (0.4 units) in a total reaction volume of 30 µl containing 20 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 10 mM DTT at 12.5 °C for 16 h. The reaction mixture was used to transform competent cells of E. coli K-12, strain MRC 8 (F⁻, dap 103, hsd R, met B1, glm 533, upp 1, dap 101, sup E, rec A) and ampicillin-resistance transformants selected on L-agar⁵⁵ medium containing N-acetylglucosamine (0.02%) and diaminopimelic acid (50 µg ml⁻¹). Transformant colonies containing recombinant plasmids which carried the synthetic oligonucleotide (pIFS plasmids) were identified by Grunstein-Hogness⁴⁵ colony hybridization using a radioactively ³²P-labelled oligonucleotide from within the synthetic sequence (coding for amino acids 78-97) as hybridization probe. B, Structure of plasmids containing the synthetic oligonucleotide. Restriction endonuclease-digested plasmid DNAs were analysed by electrophoresis through 6% HinfI digests of pIFS 1 (a) and pPM50 (b); TaqI digests of pIFS 1 (c) and pPM50 (d); BamHI and SalI double digests of pIFS 1 (e) and pPM50 (f). Reaction conditions were as suggested by the enzyme suppliers. C, Strategy for sequence determination of the synthetic interferon gene. Top line shows the positions of relevant restriction endonuclease cleavage sites within the synthetic oligonucleotide insert (heavy line) of the recombinant plasmid pIFS1. The nucleotide sequence was determined by use of the Maxam-Gilbert method⁴⁶ on fragments labelled at the 3' end by use of DNA polymerase I (Klenow) with an appropriate $[\alpha^{-32}P]$ -labelled deoxynucleoside triphosphate. The arrows (1-7) indicate the direction and extent of the sequence determined from each labelling position. Fragments sequenced are as follows (the numbers refer to the arrows and the labelled ends of the restriction fragments are designated by an asterisk); 1, BamHI-Sall; 2, HinfI-HinfI*; 3, BamHI-SalI*; 4, EcoRI-TaqI*; 5, *TaqI-DdeI; 6, DdeI-TaqI*; 7, TaqI-DdeI*

Recombinant plasmids of this type were constructed by ligation of purified BamHI-SalI-cut pPM50 vector DNA to the synthetic oligonucleotide preparation. Two cloning experiments were performed. In one case, plasmid DNA was ligated to a gel-purified 514-bp product of the oligonucleotide ligation mix; in the other, plasmid DNA was mixed with the total oligonucleotide ligation mix and ligation allowed to continue. The ligation mixtures were used for transformation of E. coli strain MRC8 and the resulting ampicillin-resistant transformant colonies screened for the presence of the synthetic oligonucleotide insert by Grunstein-Hogness colony hybridization⁴⁵ using as hybridization probe a radioactively labelled synthetic oligonucleotide, 60 residues long, coding for amino acids 78-97 of the interferon sequence. Two positively responding colonies were detected among the transformants arising from the ligation of the vector plasmid to the purified 514-bp fragment, and a further 12 from the other ligation. This corresponded to a 10% yield of recombinant plasmid transformants.

Analysis of the plasmid DNA isolated from these colonies showed that all 14 contained equivalent-size plasmids (pIFS 1-14). On digestion with BamHI and SalI endonucleases and examination of the products by gel electrophoresis, all these plasmid DNAs produced an identical 514-bp fragment in addition to the 3.2-kilobase (kb) vector fragment. Four of these plasmids were selected for more detailed analysis (pIFS 1, 2, 4,

Inspection of the theoretical nucleotide sequence of the 514bp synthetic oligonucleotide (Fig. 1) showed that the d(TCGA) sequence, which is cut by endonuclease TaqI, is present twice (at amino acid residues 43/44 and at the SalI restriction site) and the d(GANTC) sequence which is cut by the endonuclease HinfI is present three times (at amino acid residues 72/73, 115/116 and 126/127). Therefore, cleavage of this sequence by these endonucleases should yield an internal 375-bp fragment in the case of TaqI and 129-bp and 34-bp internal fragments in the case of Hinfl. Examination by acrylamide gel electrophoresis of the DNA fragments produced on treatment of the four selected pIFS plasmid DNAs with these two endonucleases showed that all produced these predicted fragments, whereas the vector plasmid pPM50 produced no such fragments. Figure 4B shows these digestion patterns for one of the recombinant plasmids, pIFS 1. We therefore conclude that these four pIFS plasmids must contain a 514-bp insert between the BamHI and SalI cleavage sites which has several widely spaced restriction endonuclease recognition sequences in the positions predicted from the theoretical sequence of the interferon gene oligonucleotide. To confirm that the correct interferon gene sequence was present in these clones, the 514-bp BamHI-SalI fragment from one plasmid, pIFS 1, was subjected to complete nucleotide sequence determination⁴⁶ on terminally ³²P-labelled DNA fragments according to the strategy shown in Fig. 4C. The nucleotide sequence so determined is in complete agreement with the theoretical sequence shown in Fig. 1.

Discussion

The rapid synthesis of genes of this size should allow several interesting problems to be examined. There has been some speculation about the mechanisms by which the expression of human genes cloned into bacteria is controlled (see discussion in ref. 47). Obviously, regulation at the level of transcription or translation initiation will be of fundamental importance and a number of elegant promoter systems have been devised. These have allowed high levels of 'eukaryotic' protein synthesis to be achieved, presumably as a result of the presence of abundant copies of their mRNAs. However, studies by Grantham et al. 35-37 comparing the mRNA sequences of highly expressed and weakly expressed genes in bacteria suggest that highly expressed genes have a special strategy for the selection of codons, particulary at position III. This may reflect an optimization of codon-anticodon pairing energies. We have attempted to reproduce this pattern of codon usage in the synthetic gene (Table 1). Others⁸ have noted the rarity of the dinucleotide CG

in human IFN mRNA, which reflects the strong preference for AGA and AGG as codons for arginine. Whereas the native sequence contains 3 CG residues, 2 of which occur within codons, the synthetic sequence contains 22 CG residues, 14 of which occur within codons. The synthetic sequence also has a marked increase in the incidence of CUG for leucine and of CCG for proline. Insertion of either the natural human DNA sequence or the sequence described herein (or further variations) into the same vector and comparison of expression levels may shed some light on these questions. Examination of, for example, the effect of using a particular codon twice when the same amino acid occurs consecutively in the protein awaits synthesis of further gene variants (see below). It would also be of interest to determine whether any of the complementary sequences within the natural gene (or within the synthetic gene) affects expression. This could involve either formation of internal DNA cruciform structures 48 with resultant influence on transcription or formation of secondary structures at the mRNA level with a resultant influence on translation.

An exciting recent development concerning interferon has been the construction of hybrid genes. Cleavage at a convenient common BglII site within the coding sequences of a number of the IFN- α genes^{23,24} allows fusion of the N-terminal sequence from one gene with the C-terminal sequence from another. Thus, in addition to the range of natural IFN- α molecules with their different pharmacological profiles¹¹, a potentially large number of hybrid interferons with further distinct properties are accessible. The availability of a large pool of synthetic fragments clearly extends the range of interferon analogues which can be prepared. It should be possible to compare rapidly the relative activities of proteins generated by this approach in a common vector system and it will be interesting to determine whether the natural interferons can be improved upon. The wider implication is that classical medicinal chemistry structure-activity analysis should be possible in relatively large peptides. This may yield considerable benefits in a number of important pharmaceutical areas.

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- Isaacs, A. & Lindenmann, J. Proc. R. Soc. B147, 258-267 (1957). Stewart, W. E. II The Interferon System (Springer, New York, 1979).
- Gresser, I. & Tovey, M. G. Biochim. biophys. Acta 516, 231-247 (1978). Bloom, B. R. Nature 284, 593-595 (1980)
- Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. & Muramatsu, H. Gene 16, 11-15 (1980).
- Mantei, N. et al. Gene 10, 1-10 (1980). Dervnck, R. et al. Nature 285, 542-547 (1980).
- Taniguchi, T. et al. Nature 285, 547-549 (1980)
- Weissenbach, I. et al. Proc. natn. Acad. Sci. U.S.A. 72, 7152-7156 (1980). Weissenbach, J. et al. Proc. nam. Acad. Sci. Os. A. Ing. 1722-7130 (1980).
 Streuli, M., Nagata, S. & Weissmann, C. Science 209, 4343-1347 (1980).
 Houghton, M. et al. Nucleic Acids Res. 8, 1913-1931 (1980).
- Houghton, M. et al. Nucleic Acids Res. 8, 2885–2894 (1980) Goeddel, D. V. et al. Nature 287, 411–416 (1980).
- Nagata, S. et al. Nature 284, 316-320 (1980)
- Derynck, R. et al. Nature 287, 193-197 (1980). Goeddel, D. V., Shepard, H. M., Yelverton, E., Leung, D. & Crea, R. Nucleic Acids Res. 8, 4057-4074 (1980)
- Taniguchi, T. et al. Proc. natn. Acad. Sci. U.S.A. 77, 5230-5233 (1980).
 Yelverton, E., Leung, D., Weck, P., Gray, P. W. & Goeddel, D. V. Nucleic Acids Res. 9,
- 731-741 (1981).
- Stewart, W. E. II et al. Gene 11, 181-186 (1980)
 - Masucci, M. G. et al. Science 209, 1431-1435 (1980). Allen, G. & Fantes, K. H. Nature 287, 408-411 (1980).
- Nagata, S., Mantei, N. & Weissmann, C. Nature 287, 401-408 (1980). Goeddel, D. V. et al. Nature 290, 20-26 (1980).
- Houghton, M. et al. Nucleic Acids Res. 9, 247-266 (1981).
 Tavernier, J., Derynck, R. & Fiers, W. Nucleic Acids Res. 9, 461-471 (1981).
- Knight, E., Hunkapiller, M. W., Korant, B. D., Hardy, R. W. F. & Hood, L. E. Science 207, 525-526 (1980).
- Zoon, K. C. et al. Science 207, 527-528 (1980). Itakura, K. et al. Science 198, 1056-1063 (1977)
- Goeddel, D. V. et al. Proc. natn. Acad. Sci. U.S.A. 76, 106-110 (1979), Wetzel, R. et al. Biochemistry 19, 6096-6104 (1980).
- Itakura, K. & Riggs, A. D. Science 209, 1401-1405 (1980). Markham, A. F. et al. Nucleic Acids Res. 8, 5193-5205 (1980).
- Gait, M. J. et al. Nucleic Acids Res. 8, 1081–1096 (1980).
 Grantham, R., Gautier, C., Gouy, M., Mercier, R. & Pavé, A. Nucleic Acids Res. 8, r49–r62
- 36. Grantham, R., Gautier, C. & Gouv, M. Nucleic Acids Res. 8, 1893-1912 (1980)

- 37. Grantham, R., Gautier, C., Gouy, M., Jacobzone, M. & Mercier, R. Nucleic Acids Res. 9,
- Reese, C. B., Titmas, R. C. & Yau, L. Tetrahedron Lett., 2727-2730 (1978). Frank, R. & Köster, H. Nucleic Acids Res. 6, 2069-2087 (1979).
- Twigg, A. J. & Sherratt, D. Nature 283, 216-218 (1980). Shine, J. & Dalgarno, L. Nature 254, 34-38 (1975).
- 42. Backman, K., Ptashne, M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 73, 4174-4178
- 43. Roberts, T. M., Kacich, R. & Ptashne, M. Proc. natn. Acad. Sci. U.S.A. 76, 760-764 (1979).
- Goeddel, D. V. et al. Nature 281, 544-548 (1979).
- 45. Grunstein, M. & Hogness, D. S. Proc. natr., Acad. Sci. U.S.A. 72, 3961-3965 (1975).
- 46. Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560-564 (1978).
- 47. Morgan, J. & Whelan, W. J. Recombinant DNA and Genetic Experimentation, 141-143 Morgan, J. & Whelan, W. J. Recombinant DNA and Genetic Est. (Pergamon, Oxford, 1979).

 Panayotatis, N. & Wells, R. D. Nature 289, 466-470 (1981).

 Chang, A. C. Y. & Cohen, S. N. J. Bact. 134, 1141-1156 (1978).

 Shapiro, J. et al. Nature 224, 768-774 (1969).

- Sinapro, J. et al. (Value 224, 706-7/4 (1909)). Helling, R. B., Goodman, H. M. & Boyer, H. W. J. Virol. 14, 1235-1244 (1974). Smith, H. O. Meth. Enzym. 65, 371-380 (1980).
- Maniatis, T., Jeffrey, A. & Van deSande, H. Biochemistry 14, 3787-3794 (1975). Bachmann, B. J. Bact. Rev. 36, 525-557 (1972)
- 55. Miller, J. H. Experiments in Molecular Genetics (Cold Spring Harbor, New York, 1972).

Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey

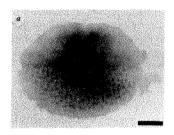
Jonathan C. Horton & David H. Hubel

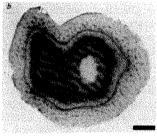
Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115, USA

In two preliminary studies, normal macaque¹ and squirrel-monkey^{2,3} striate cortex cut parallel to the surface and stained for cytochrome oxidase (a mitochondrial enzyme) showed a striking pattern of regularly spaced patches. This was surprising. since until then no physiological or anatomical studies had suggested such a patchy organization. In the present study in the macaque we found that the patches were arranged in rows spaced about 350 µm apart. When one eye was injected with tritiated proline the rows of patches in layers II and III lay in register with the ocular-dominance bands seen autoradiographically in layer IVc. Removing one eye caused the patches in every other row to shrink and blanch. The rows of patches are therefore centred on the ocular dominance columns. Regions labelled by 2-deoxyglucose autoradiography after stimulating one eve with black-and-white stripes in all orientations consisted of rows of patches that lay in register with the cytochrome oxidase patches in every other row. On stimulating monkeys with stripes of a single orientation, the deoxyglucose-labelled regions formed a lattice that included the cytochrome oxidase patches but was more extensive. Thus either the deoxyglucose is not labelling the orientation columns at all, or the orientation columns coalesce in the areas marked by the cytochrome oxidase stain.

Wong-Riley has used a stain for cytochrome oxidase to demonstrate a pattern of alternating light and dark bands in layer IV of a monocularly deprived kitten, the lighter bands presumably reflecting a lowered metabolic activity in the set of columns belonging to the closed eye4. Our original purpose was to examine cytochrome oxidase activity in the different layers of the striate cortex of the normal macaque monkey. Sections (50 μm) from glutaraldehyde-paraformaldehyde fixed tissue were cut on a freezing microtome and processed for cytochrome oxidase activity following a procedure modified from Seligman et al.5. In sections cut perpendicular to the cortical surface, cytochrome oxidase staining was darkest in layers IVa and IVc, which receive the major direct projections from the lateral geniculate body⁶. Layers II and III were lightly stained but showed periodic fluctuations in density. To examine the pattern more closely we sectioned the cortex tangentially. Cytochrome oxidase staining showed an array of dark oval patches about 150 × 200 µm which were most obvious in Layers II and III but were also present, though faint, in layer VI. Although varying to some extent from animal to animal, the patches were generally aligned in rows spaced $\sim 350 \,\mu m$ apart, with the long axis of the patches parallel to the rows. Within a row the patches were ~550 µm apart, but in some places became confluent. Occasionally, patches in neighbouring rows seemed to be aligned, forming a square or hexagonal array over small regions of cortex.

The rows of cytochrome oxidase patches resembled ocular dominance columns in their spacing and in intersecting the 17-18 border at right angles (Fig 1a). It was obviously important to learn whether these rows were really related to the ocular dominance columns, and, if so, whether the patches lay along the dominance columns or straddled the borders between them. We therefore removed one eye of a macaque monkey, killed the animal 10 days later and stained the visual cortex for cytochrome oxidase. Sections through layer IVc showed ocular dominance columns visable as alternating light and dark bands (Fig. 1b), in striking contrast to the uniformly deep staining of this layer for cytochrome oxidase in normal monkeys. In sections passing through layers II and III there were parallel





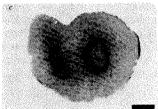


Fig. 1 a, Tangential section through the superficial layers of the visual cortex of the normal macaque monkey; cytochrome oxidase stain. The section passes through the 17-18 border, which runs horizontally in the figure with area 17 below and 18 above. The patches in 17 are aligned in rows perpendicular to the 17-18 border. b, Cytochrome oxidase section tangential to area 17 in a macaque monkey that had its right eye removed 10 days previously. Section grazes layer V, which forms a pale oval near the centre, surrounded from within out by layers IVc (dark stripes), IVb (pale and narrow), IVa (dark, very narrow) and II, III (containing patches). Dark and pale alternating stripes in layer IVc are typical of ocular dominance columns, in their regularity, direction and spacing. c, Same monkey as in b. This section is more superficial, grazing layer IVb. Cytochrome oxidase patches lie in rows that are alternately dark and pale: the pale rows lie over the light stripes in layer IVc corresponding to the injured eye. Scale bar, 2 mm.

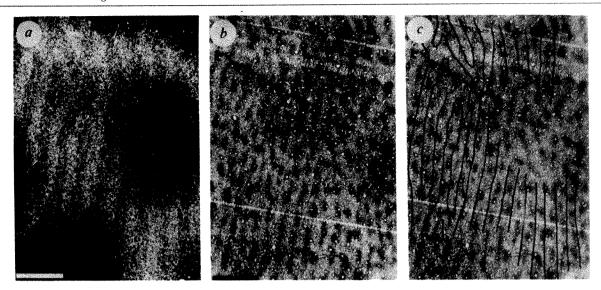


Fig. 2 a, Dark-field autoradiograph of striate cortex in a normal monkey whose right eye was injected with ³H-proline 2 weeks earlier. The section is tangential, grazing layer V (dark ovals) but passing mainly through layer IVc. Here typical ocular dominance columns are seen as light bands of label corresponding to the injected eye separated by darker gaps. b, More superficial section from the same block as a, cytochrome oxidase stain. The rows of cytochrome oxidase patches in the superficial layers follow the pattern of the ocular dominance patches. This is shown in c by drawing the borders of the columns from a directly onto b. Scale bar, 1 mm.

rows of patches as in normal monkeys, but in every other row the patches were paler and smaller (Fig. 1c). When one of these sections was aligned with a section through layer IVc, using as a guide small blood vessels (which generally run normal to the cortex through its full thickness), the alternating rows of dark and light patches fell into precise register with the dark and light bands in layer IVc. This indicated that the rows of patches in normal monkeys lie centred on the ocular dominance columns rather than along the borders separating them. All the cells in any given patch are thus likely to be strongly dominated by the same eye.

In a second monkey we injected one eye with 2 mCi 3 H-proline to label the ocular dominance columns in layer IVc by transneuronal autoradiography. Figure 2a, a dark-field autoradiograph of a section tangential to layer IVc, shows typical ocular dominance columns in which the light bands represent the injected eye. A more superficial section stained for cytochrome oxidase shows the expected array of patches (Fig. 2b). When aligned with the autoradiograph, again using radial blood vessels as a guide, the rows of patches follow the course of the ocular dominance columns and are centred on them (Fig. 2c).

In a third monkey we used the 2-deoxyglucose method to label regions of cortex activated by stimulation of one eye. We anaesthetized the animal with sodium thiopental injected 14C-2deoxyglucose (100 µCi per kg), and stimulated the visual field of the right eye with a set of black-and-white stripes of irregular width and spacing, moved slowly back and forth and steadily rotated so as to expose the animal to all orientations about once every minute. After 45 min the monkey was killed, the brain perfused, frozen and sectioned and the dried sections pressed against X-ray film. Alternate sections were stained for cytochrome oxidase. A pattern representing ocular dominance columns was visible in the deoxyglucose autoradiographs of tangential sections through layer IVc. An adjacent section stained for cytochrome oxidase showed no trace of ocular dominance columns in layer IVc, indicating that the brief period of monocular stimulation was insufficient to affect cytochrome oxidase levels. When a cytochrome oxidase section through layers II and III (Fig. 3a) was superimposed on a deoxyglucose autoradiographs of layer IVc, the rows of cytochrome oxidase patches were again centred over the ocular dominance columns, confirming the results of the eye removal and transneuronal autoradiography experiments.

Deoxyglucose sections adjacent to Fig. 3a showed an array of patches of increased deoxyglucose uptake (Fig. 3b) which lay

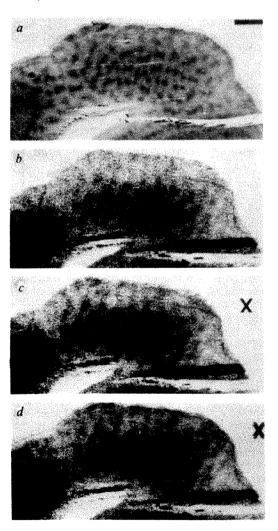


Fig. 3 a, An array of cytochrome oxidase patches in layers II, III of striate cortex of a normal macaque monkey. b, This 2-deoxyglucose autoradiograph of an adjacent section shows patches of uptake of label after stimulation of one eye with black-and-white stripes in all orientations. c, When a and b, photographed on film, are overlapped in precise register the two patterns reinforce. d. When shifted out of alignment by 150 μm (see Xs) the patterns tend to cancel. Scale bar, 1 mm.

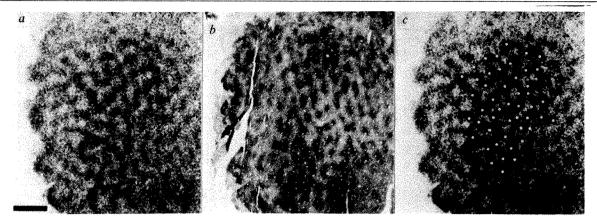


Fig. 4 a, Complex pattern of ¹⁴C-2-deoxyglucose label in layers II, III of macaque striate cortex after stimulation of both eyes with vertical stripes. b, An adjacent section stained for cytochrome oxidase shows an array of patches. c, The cytochrome oxidase patches fall within the lattice of deoxyglucose label, as shown by representing each patch in b, with a small white dot, and placing them directly on a. Scale bar, 1 mm.

over the deoxyglucose-labelled ocular dominance columns in layer IVc. A similar patchy pattern from stimulating one eye with all orientations has been observed by Kennedy et al. and by Hendrickson and Wilson⁸. The deoxyglucose pattern resembled the cytochrome oxidase pattern, but the patches were larger and the rows more widely spaced. When Figs 3a and 3b were superimposed, in and out of register (Fig. 3c, d), the patches of increased deoxyglucose label matched the cytochrome patches lying in every other row.

Attempts to learn whether there was any relationship between the cytochrome exidase patches and orientation columns produced unexpected results. In two experiments we stimulated with vertical black-and-white stripes after injecting 2-deoxyglucose. As shown in Fig 4a, the pattern in tangential sections through layers II and III was periodic and yet highly complex, forming a lattice of stripes, rosettes and patches, as reported previously9. The patches seen in adjacent sections stained for cytochrome oxidase (Fig. 4b) fell along the lattice of deoxyglucose label (Fig. 4c). Where the deoxyglucose pattern appeared patchy, the patches tended to coincide with the cytochrome oxidase patches; the deoxyglucose pattern thus included the cytochrome patches but seemed to extend beyond and between them. We first thought that the cytochrome oxidase patches might have some special relationship to vertical orientation columns², but two similar experiments using deoxyglucose and horizontal stripes gave the same result, with the cytochrome oxidase patches lying along the lattice formed by the deoxyglucose label. Thus all orientations of line stimuli probably activate cortex in the regions of the patches, as indeed is suggested by the deoxyglucose experiment in which one eye was stimulated with all orientations. A combination of physiological recordings and a double-label deoxyglucose technique 10 will probably help clarify the relationship between the patches and the orientation columns.

In squirrel monkeys Humphrey and Hendrickson³ have shown that the deoxyglucose pattern obtained when both eyes are stimulated with all orientations is identical to the cytochrome oxidase pattern. Similarly, we have found in two squirrel monkeys a strong correlation between the cytochrome oxidase patches and the pattern after stimulation with horizontal stripes in both eyes. In the human and the galago, a prosimian primate, the striate cortex likewise shows cytochrome oxidase patches, we find that the cat and tree shrew lack such patches. A patchy distribution of cytochrome oxidase staining may reflect a system of inputs, outputs and intrinsic connections unique to the visual cortex of primates.

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- Hubel, D. H. & Wiesel, T. N. J. Physiol., Lond. 160, 106-154 (1962), 165, 559-578 (1963), 195, 215-243 (1968); J. Comp. Neurol. 158, 267-294 (1974).
 Horton, J. C. & Hubel, D. H. Soc. Neurosci. 113, 5 (1980).
- Humphrey, A. L. & Hendrickson, A. E. Soc. Neurosci. 113, 6 (1980).
- Wong-Riley, M. Brain Res. 171, 11-28 (1979).
- Seligman, A. N. et al. J. Cell Biol. 38, 1-14 (1968)

- Hubel, D. H. & Wiesel, T. N. J. comp. Neurol. 146, 421-450 (1972).
 Kennedy, C. et al. Proc. natn. Acad. Sci. U.S.A. 73, 4230-4234 (1976).
 Hendrickson, A. E. & Wilson, J. R. Brain Res. 170, 353-358 (1979).
- Hubel, D. H., Wiesel, T. N. & Stryker, M. P. J. comp. Neurol. 177, 361-380 (1978).
 Livingstone, M. S. & Hubel, D. H. Nature 291, 554-561 (1981).

Effect of chemical environment on levels of substance P and somatostatin in cultured sensory neurones

Anne W. Mudge*

Departments of Physiology and Pharmacology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115, USA

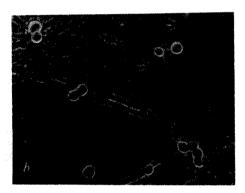
The neurotransmitter produced by autonomic neurones is influenced by the environment in which they develop1. For example, Le Douarin² and her colleagues have shown that regions of the neural crest which normally give rise to cholinergic ganglia can be induced to develop into adrenergic cells by transplanting them into a region of the crest which normally gives rise to adrenergic ganglia. Similarly, sympathetic neurones grown in culture express either adrenergic or cholinergic properties depending on whether they are grown in the absence or presence of certain types of non-neuronal cells 1,3-5. Patterson et al.6.7 demonstrated that this is not due to selective survival of a population of neurones but that the transmitter choice of individual neurones can be altered by soluble factors produced by non-neuronal cells. Like autonomic neurones, sensory neurones derive from the neural crest and it therefore seems likely that the type of transmitter they produce could also be influenced by the environment in which they develop. Here I demonstrate that when sensory neurones from embryonic chick dorsal root ganglia are grown together with ganglionic nonneuronal cells or with medium 'conditioned' by incubation with such cells, they produce increased amounts of somatostatin (SOM). This increase is neither accompanied by an increase in substance P (SP) content nor a detectable change in neuronal survival and thus differs from the effect of nerve growth factor (NGF), which increases survival of sensory neurones without affecting the relative levels of SOM and SP.

^{*} Present address: MRC Neuroimmunology Project, Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK.

Although the transmitters used by sensory neurones are unknown, several peptides, namely substance P, somatostatin, cholecystokinin (CCK) and vasoactive intestinal peptide (VIP), are present in small-diameter neurones of the DRG (dorsal root ganglia) and in small-diameter fibres that project into the spinal cord dorsal horn⁸. Although SP and SOM seem to be in separate populations of neurones⁹, it is not known which subclasses of small fibres contain these peptides or whether they coexist with other transmitters. As both SP and SOM can be released from sensory neurones¹⁰⁻¹³ and both can alter neuronal firing rates in the dorsal horn^{14,15}, it seems likely that they (and probably also CCK and VIP¹⁶) are involved in synaptic transmission in the spinal cord, although their functions remain unclear.

DRG from 10-day-old chicken embryos were dissociated to single cells and plated on to collagen-coated tissue culture dishes





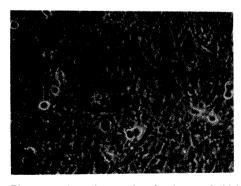


Fig. 1 Phase contrast micrographs of cultures of chick DRG. Dissociated cells were plated on to collagen-coated 35-mm culture dishes (Falcon). The growth medium used was Eagle's minimum essential medium (MEM) supplemented with 2 mM glutamine, 50 μg ml⁻¹ penicillin, 50 U ml⁻¹ streptomycin, 10% horse serum (all Gibco), 5% chick embryo extract (CEE) plus nerve growth factor (NGF). a, Neurones plus ganglionic non-neuronal cells 24 h after plating. b, Neurones plus ganglionic non-neuronal cells 15 days after plating. The dividing non-neuronal cells were confluent 6 days after plating, at which time the cultures were irradiated with ^oCo to arrest further cell division. c, Neurones-alone cultures 15 days after plating. These cultures were treated with $5 \times 10^{-6} \,\mathrm{M}$ cytosine arabinoside for the period between 12 h and 5 days after plating to kill almost all the non-neuronal cells. Cultures from the same plating as in a, b and c provided the data shown in Fig. 2. Scale bar, 50 µm

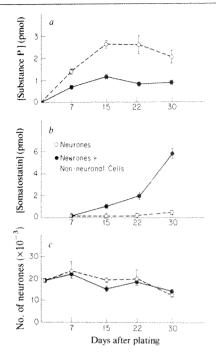


Fig. 2 SP (a) and SOM (b) content of DRG cultures grown for the number of days indicated in either the presence (\bullet) or virtual absence (\bigcirc) of ganglionic non-neuronal cells. The number of neurones present in each culture is shown in c. Data are expressed as mean \pm s.e.m. of either three or four cultures; all cultures were from the same plating.

to give two types of culture: (1) cultures of 'neurones alone' in which almost all the non-neuronal cells were eliminated by treatment with cytosine arabinoside; (2) neurones grown together with dividing ganglionic non-neuronal cells. Within 24 h after plating, both neurones and non-neuronal cells had attached to the collagen substratum and phase-bright, neurone-like cells had extended processes (Fig. 1a) which grew during the next 2 weeks to form a dense network (Fig. 1b, c).

At various times after plating, the number of phase-bright neuronal somata was estimated by counting about 30 fields (×200 magnification) chosen at random and normalizing for the total area of the dish. In typical platings there were 15-20 neurones per field and these were evenly distributed throughout the dish. The cultures were then extracted using 2M acetic acid and the extracts lyophilized. The amount of SP and SOM present in a culture was assayed by radioimmunoassay. Antibody R6P was used for the SP assay as previously described 17 The SOM assay used was a modification of that described elsewhere 18. Briefly, the SOM assay used antibody M4 with ¹²⁵I-tyrosinyl-SOM as a tracer. The incubation and separation procedures were essentially the same as those used for the SP assay. SOM standards were prepared using cyclic-SOM (Boehringer Mannheim) and the concentrations checked by amino acid analysis. When extracts of neurones plus nonneuronal cell cultures were chromatographed on a gel-filtration column (Sephadex G-25-80; Sigma), over 85% of both the SPand SOM-immunoreactive material eluted as single peaks in the same volume as either synthetic SP or synthetic SOM. (The fact that only one peak of SOM is present in the extracts of DRG cultures is noteworthy, as a variety of tissues, including adrenal , contain SOM-immunoreactive material of several different molecular weights when assayed using the same antibody).

The peptide content of cultures containing either neurones alone or neurones plus non-neuronal cells is shown in Fig. 2. The cells plated on to each dish (from five ganglia) contained 35 fmol of SP and 120 fmol of SOM when freshly dissociated. During the next 2 weeks, the content of SP in the cultures of neurones alone increased ~75-fold and then reached a plateau, while the content of SOM remained low; the ratio of SOM/SP was 0.03 at

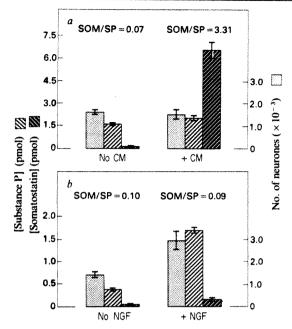


Fig. 3 Effect of conditioned medium and NGF on levels of SP and SOM and on DRG neuronal survival. a, Neurones-alone cultures were fed every day for 11 days with medium containing 33% conditioned medium (CM); control cultures received only fresh medium (all media contained 1 µg ml⁻¹ NGF). Results are mean ±s.e.m. of triplicate cultures. Ganglionic non-neuronal cells without neurones were obtained by removing from the cultures neurones, together with non-neuronal cells, using trypsin, filtering the mixture through lens paper, re-plating and irradiating the cells when confluent; neurones do not survive this treatment. Medium incubated for 2 days with such cultures provided the conditioned medium. Aliquots of conditioned medium and the ganglionic non-neuronal cells were also assayed for SP and SOM. b, Neurones-alone cultures were fed for 11 days with medium either with or without the addition of NGF at a protein concentration of $1 \mu g \text{ ml}^{-1}$. NGF was prepared from mouse salivary glands²⁶. Results are mean ±s.e.m. of triplicate cultures.

2 weeks and 0.13 at 4 weeks. However, when the neurones were grown together with ganglionic non-neuronal cells, the content of SOM steadily increased after the first week of culture (the time at which the non-neuronal cells are confluent) and after 2 weeks and 4 weeks, the ratio of SOM/SP was 0.96 and 6.34. respectively (Fig. 2a, b). This increase in SOM content could not be attributed to a general effect on neuronal growth, because it was not associated with an increase in SP content. Indeed, the content of SP in the presence of non-neuronal cells was suppressed by $\sim 50\%$ at all time points (Fig. 2a). The number of neurones surviving in culture remained constant from the first day after plating, for 3 weeks in culture, while in the fourth week there was a 20% loss of neurones (Fig. 2c). There was no detectable difference within the limits of the counting procedure in neuronal survival in the presence or absence of non-neuronal cells.

To determine whether the non-neuronal cells had to be in contact with the neurones to influence the production of SOM, cultures of neurones alone were fed with medium that had been conditioned by incubation with ganglionic non-neuronal cells without neurones. Such conditioned medium markedly increased the SOM/SP ratio without detectably influencing neuronal survival (Fig. 3a). This experiment also showed that it was the neurones which increased their SOM content, as neither the non-neuronal cells nor the conditioned medium contained detectable amounts of SOM or SP.

Although NGF is known to affect the development 20,21 and SP content^{22,23} of sensory neurones and is produced by cultured non-neuronal cells²⁰, it is unlikely to be the agent responsible for the change in relative amounts of SOM and SP in these experiments. When NGF was added to cultures of neurones alone, it doubled the number of neurones which survived and increased

both SOM and SP proportionately, but did not significantly alter the ratio of SOM/SP (Fig. 3b). Moreover, all the experiments demonstrating the effect of non-neuronal cells or conditioned medium on the SOM/SP ratio were done in saturating levels of NGF.

The survival of sensory neurones depends on additional factors distinct from NGF which are present in brain extracts and secreted by a glioma cell line²¹. In my experiments, the 5% chick embryo extract (CEE) present in the medium presumably provided some factor which supported the survival of neurones in the absence of added NGF. As CEE was present in all the experiments reported here and, again, because the change in SOM/SP ratio occurred without a change in neuronal survival, the 'survival factor' demonstrated by Barde et al.21 is also unlikely to be the factor which increased SOM content. Furthermore, when an extract of embryonic chick brain² $(100 \ \mu g \ ml^{-1} \ protein)$ was added to cultures of neurones alone for 2-10 days after plating, SP content was increased ~two-fold and SOM rather less; thus there was little effect on the SOM/SP ratio.

These results demonstrate that, like sympathetic neurones. sensory neurones respond to a factor from non-neuronal cells that dramatically influences peptide synthesis. This change in the relative content of SOM and SP is distinct from previously described influences on neuronal survival in the ganglia. It is not known whether, analogous to the switch in adrenergic-cholinergic properties of sympathetic neurones¹, a single population of developing sensory neurones is capable of making either SP or SOM. It is also unknown whether the levels of CCK and VIP can be similarly influenced, nor is it clear what part this effect plays in normal development of sensory neurones. However, in view of the diversity of peptides present in sensory (and autonomic) ganglia, it is possible that the soluble factor described here may represent one of the differentiation signals required for development of neural crest cells.

As DRG non-neuronal cells consist of two major cell types, glial cells and fibroblasts, it is of interest to know which type of cell is influencing the relative levels of SOM and SP. Experiments using purified cultures of either Schwann cells or fibroblasts from DRG²⁵ or autonomic ganglia should establish this point and provide a well defined system for extending these observations.

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- Patterson, P. H. A. Rev. Neurosci. 1, 1-17 (1978).
 Le Douarin, N. M. Nature 286, 663-669 (1980).
 Varon, S. S. & Bunge, R. P. A. A. Rev. Neurosci. 1, 327-361 (1978).
 Furshpan, E. J., Macleish, P. R., O'Lague, P. H. & Potter, D. D. Proc. natn. Acad. Sci. U.S.A. 73, 4225-4229 (1976).
- Landis, S. C. Devi Biol. 77, 349-361 (1980). Patterson, P. H. & Chun, L. L. Y. Devi Biol. 56, 263-280 (1977).
- Reichardt, L. F. & Patterson, P. H. Nature 270, 147-151 (1977
- Hokfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J. M. & Schultzberg, M. Nature 284, 515 (1980). Hokfelt, T. et al. Neuroscience 1, 131-136 (1976).
- Mudge, A. W., Leeman, S. E. & Fischbach, G. D. Proc. natn. Acad. Sci. U.S.A. 76, 526-530 (1979).
 Yaksh, T. L., Jessell, T. M., Gamse, R., Mudge, A. W. & Leeman, S. E. Marrier, 286
- 12. Jessell, T. M., Mudge, A. W., Leeman, S. E. & Yaksh, T. L. 9th. A. Meet. Soc. Neuroscience
- Abstr. (1979) Mudge, A. W., Fischbach, G. D. & Leeman, S. E. 7th A. Meet. Soc. Neuroscience Abstr., 410 (1977).

- Henry, J. L. Brain Res. 114, 429-451 (1976).
 Randic, M. & Miletic, V. Brain Res. 152, 196-202 (1978).
 Phillis, J. W. & Kirkpatrick, J. R. Can. J. Physiol. Pharmac. 57, 887-899 (1979).
 Mroz, E. A. & Leeman, S. E. in Methods of Hormone Radioimmunoassay, 2nd edn. 121-137 (Academic, New York, 1979).
- Arnold, M. A. & Fernstrom, J. D. Neuroendocrinology 31, 194-199 (1980).
- Role, L., Leeman, S. E. & Perlman, R. Neuroscience (in the press).
 Greene, L. A. & Shooter, E. M. A. Rev. Neurosci. 3, 353-402 (1980).
 Barde, Y. A., Edgar, D. & Thoenen, H. Proc. natn. Acad. Sci. U.S.A. 77, 1199-1203
- 22. Kessler, J. A. & Black, I. B. Proc. natn. Acad. Sci. U.S.A. 77, 649-652 (1980).

- Otton, U., Goodert, M., Mayer, N. & Lombeck, F. Nature 287, 158-159 (1980)
 Iossell, T. M., Seggel, R. E. & Fuchbach, G. D. Proc. natn. Acad. Sci. U.S. A. 76, 5397-5401 (1979)
- Brockes, J. P., Freids, K. L. & Raff, M. C. Brezs Res. 165, 105-118 (1979)
 Varon, S., Nomura, J. & Shooter, E. M. Brockementry 6, 2202-2209 (1967)

Inability of tolerant males to sire tolerant progeny

R. N. Smith

Department of Reproductive Biology, Case Western Reserve University, Cleveland, Ohio 44106, USA

Recent data of Gorczynski and Steele suggested that male mice made tolerant of an allogeneic strain by the neonatal injection of hybrid lymphomyeloid cells may, by a presumably genetic mechanism, pass on this operationally similar form of hyporesponsiveness to their progeny 1,2—the progeny have inherited an acquired characteristic. Their theory, proposed to account for the inheritance of an acquired characteristic, uses a RNA tamour virus that transfers to the spermatogenic germinal epithelium DNA coding for the gene products of the foreign alloantigens. This is possible because the tolerant mouse is chimaeric and contains small numbers of foreign lymphomyeloid cells. Thus, the virus transfers genetic information for transplantation antigens from the chimaeric foreign cells to the germinal epithelium of the host. The progeny of the tolerant male are themselves tolerant because they have received DNA coding for the foreign antigens from paternal sperm. The progeny are tolerant because foreign is now 'self'. I have now repeated these experiments in a strain combination of rats, DA tolerant of PVG, and found no hyporesponsiveness to the PVG strain in the progeny of tolerant DA males.

Neonatally induced transplantation tolerance requires the acceptance of an allogeneic skin graft³, and such tolerance seems coincidental with the inability of lymphocytes from these skingraft-tolerant animals to generate cytotoxic lymphocytes against alloantigens of the tolerated strain^{4,5}. In addition, the lymphocytes of some tolerant animals are incapable of proliferation in the mixed lymphocyte culture (MLC) when stimulated by alloantigens of the tolerated strain^{4,6}. These lymphocytes are also able to transfer adoptively specific tolerance to sub-lethally irradiated syngeneic recipients^{4,7}. These four criteria were used to analyse the tolerant state of DA males made neonatally tolerant to PVG, and to analyse the relative responsiveness to PVG alloantigens of the DA progeny of these tolerant males.

DA neonates from three litters (20 pups) were made tolerant of PVG by the injection into the tail vein of 1×10° spleen and bone marrow cells from (PVG×DA)F₁ hybrid female donors. Injections of 0.1 ml using a 30-gauge needle were given within 24 h of birth. At 6 weeks of age, all putatively tolerant DA rats—18 of the surviving animals—were grafted with PVG ear skins, and of these, 14 accepted PVG grafts for 60 days without evidence of rejection. The skin-graft-tolerant rats were also analysed to determine if their peripheral blood leukocytes (PBL) could be stimulated by PVG alloantigens in the MLC, a sensitive assay used to detect reactivity to allogeneic determinants^o. DA males that accepted PVG skin grafts for 60 days and had PBL which were not stimulated by PVG alloantigens in the MLC, were allowed to sire, one litter with a normal DA female. The tolerant males were cannulated and their thoracicduct lymphocytes (TDL) analysed in quantitative MLC and cytotoxic lymphocyte culture (CTL) assays for their ability to respond to PVG and third party Lewis alloantigens. In addition, 1×10° TDL from these tolerant males were transferred adoptively to sub-lethally irradiated (500 rad) DA recipients to determine if the TDL from tolerant males could transfer unresponsiveness to secondary recipients against PVG antigens^{4,7}. Six of the eight males met the four criteria of tolerance to transplantation alloantigens—acceptance of skin grafts, unresponsiveness in the MLC and CTL assays to PVG

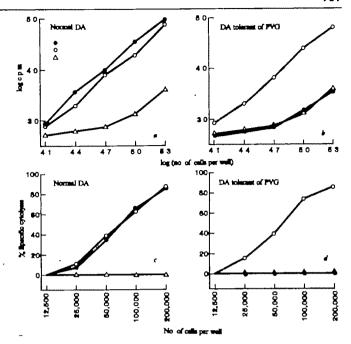


Fig. 1 A representative experiment of the mixed lymphocyte culture (MLC) and cytotoxic lymphocyte culture (CTL) in which responding cells are titrated against a constant number of stimulating cells. The relative estimates of frequency in Table 3 are derived from this type of experiment. Stimulators and targets: Δ, DA; •, PVG; O, Lewis. The MCL was used as a quantitative assay following procedures described elsewhere 10 in which serial dilutions of responder TDL were added to an excess of irradiated stimulator spleen and lymph node cells in a constant volume of 0.2 ml per round-bottomed microtitre well. Cells were cultured in RPMI 1640 containing 5% fetal calf serum (FCS), 2 mM ghitamine, M 2-mercaptoethanol and antibiotics. The FCS was selected for its ability to support the generation of cytotoxic lymphocytes without high spontaneous proliferation. Stimulator cells were spleen and lymph node cells pooled from one donor and were irradiated with 1,000 rad. The MLC was incubated for 4 days at 37 °C in a humidified CO2 incubator. During the last 4h of incubation each well contained 1.0 μCl of ³H-methylthymidine (15 Ci mmol⁻¹), added in 0.01 ml of phosphate buffered saline. The cells were collected on a semi automatic harvester. The same quantitative approach was used to assay the potential cytotoxic activity of a responder population. Cytotoxic activity was measured in wells from titrated MLC responses on day 5. 0.1 ml of culture supernatant was removed from each well and replaced with 0.1 ml of target cells (10⁵ per ml). The plates were spun at 500g at room temperature for 10 min and incubated at 37 °C for 4-6 h in a humidified CO2 incubator. To terminate the cytotoxic experiment, plates were placed on ice and 0.1 ml removed for counting. Target cells were 3-day Con A blasts prepared from lymph node cells in complete medium with 2.5 μg ml $^{-1}$ Con A. Target cells $(2-3\times10^{7})$ were labelled for 1-2 h with 50-100 μ Ci of 51 Cr sodium chromate (Amersham), washed and then resuspended in complete medium.

alloantigens and the ability to transfer suppression (Table 1, Fig. 1). Responses to the Lewis strain, the third party control, were normal, indicating that the tolerance and suppression were specific for PVG antigens.

Half of the progeny (21 DA rats) of the six tolerant males were analysed by quantitative MLC and CTL assays¹⁰, and half were skin grafted with (PVG×DA)F₁ ear skin. Males were grafted with male skin and females with female skin. The data on skin graft rejection (Table 2) indicate that the progeny of the tolerant DA males rejected PVG grafts and third party Lewis grafts at the same rate as did normal DA rats. Thus, there was no evidence of tolerance by this assay.

As the rate at which skin grafts are rejected might not detect subtle and weak hyporesponsiveness, the remaining progeny were analysed to estimate their response to PVG and Lewis alloantigens, as measured by MLC and CTL assays¹⁰. The number of DA TDL responding to PVG and Lewis alloantigens

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was titrated to generate a dose-response curve. The advantage of this type of analysis is that displacement along the x-axis represents the relative change in the frequency of cells reactive to the stimulating antigen¹⁰. The relative frequency between control and experimental groups may be related by comparing the required number of cells, determined by regression, needed to give a certain response. For the MLC assays the number of cells required to give 10,000 c.p.m. ³H-thymidine incorporation was chosen to relate the relative frequency between animals, and the number of cells required for 33% specific cytolysis was chosen as the estimate of frequency for the CTL assay. The data in Table 3 indicate that the relative response of DA TDL from either normal DA rats or DA rats that were the progeny of tolerant males is the same with respect to PVG alloantigens.

These results suggest that, in this strain combination of rats, tolerance or any weak hyporesponsiveness to transplantation antigens cannot be transmitted to the progeny of tolerant rats. Thus, acquired tolerance could not be transmitted genetically. Although these results clearly cannot confirm those of Gorczynski and Steele^{1,2}, they do not falsify their theory that a virus, which includes in its genome some DNA coding for the tolerated transplantation antigens, could infect spermatogenic germinal epithelium. The resulting sperm would render progeny tolerant because the foreign alloantigen is now self. Presumably, genetic information for other immunological phenomena, for example,

Table 1 Adoptive transfer of tolerance by thoracic-duct lymphocytes from tolerant males

Status of DA male donor	Adoptive transfer assay* (graft survival, days)			
	PVG	Lewis		
Rat 1 Tolerant of PVG	14; 48; >100; >100	8; 9; 9; 10		
2 Tolerant of PVG	17; >100; >100; >100	9; 9; 9; 10		
3 Tolerant of PVG	18; 91; >100; >100	9; 10; 10; 11		
4 Tolerant of PVG	21; 53; >100; >100	8; 9; 10; 11		
5 Tolerant of PVG	25; 63; >100; >100	9; 9; 10; 10		
6 Tolerant of PVG	20; >100; >100; >100	8; 10; 10; 10		
Normal DA	9; 10; 10; 11	8; 10; 10; 11		
Normal DA	8; 9; 9; 11	9; 9; 9; 10		
No cells transferred	17; 19; 24; 24	13; 15; 18; 19		
No cells transferred	17; 21; 23; 25	14; 16; 19; 20		
No cells transferred	18; 19; 21; 22	15; 18; 19; 19		

At the time of cannulation for thoracic-duct lymphocytes (TDL), each DA male tolerant of PVG was 3-4 months old and had not yet rejected a PVG graft 60-90 days after grafting.

According to Dorsch and Roser7. Four DA rats, irradiated with 750 rad (a sublethal dose), were grafted with both PVG and Lewis ear skin and injected intravenously with 5×10^7 TDL from the indicated donor.

anti-idiotypic antibodies, could also be transmitted, and in this way account for the tolerant progeny.

Because viruses show host specificity, that is, preferentially infect certain species or even subgroups of a species, it is possible that mice could transmit genetically acquired tolerance, but that other species, for example, rats, could not.

An additional experiment relevant to the interpretation of the experiments of Gorczynski and Steele is the backcross $(A \times B)F_1$ male \times A female. The hybrid male, $(A \times B)F_1$, and the male, A, made tolerant of B are similar in that both have acquired a state of tolerance to B. If such tolerance to B can be transmitted genetically by sperm haploid for A, then less than 50% of the progeny of the backcross should respond to B. As far as I am aware, this is not the case—50% of such progeny are histoin-

Table 2 Skin graft rejection of the progeny of tolerant male rats

Group	No. of animals	Rejection (days) (MST ± 1 s.d. (Range))	
Normal DA rats	20	PVG 7.75±0.72 (7-10)	Lewis 7.9 ± 0.85 $(7-10)$
Progeny of DA males tolerant of PVG	21	7.95 ± 0.87 $(7-10)$	7.81 ± 0.87 $(7-10)$

All rats were doubly grafted with ear skin from PVG (left flank) and Lewis (right flank)

Table 3 Relative response to PVG alloantigens by thoracic-duct lymphocytes

		MLC assay	
N	Status of DA rats	Slope (mean ± s.d. (range))	No. of cells to give 10,000 c.p.m. (mean ± s.d. (range))
10	Normal	1.64 ± 0.15	$49,860 \pm 4320$
		(1.38-1.84)	(41,188-57,833)
21	Progeny of males	1.70 ± 0.18	$52,495 \pm 5950$
	tolerant of PVG	(1.41-1.86)	(40,599-63,495)
		CTL assay	
		Slope	No. of cells to give
		(mean ± s.d. (range))	33.3% specific lysis
10	Normal	85.6 ± 7.4	$49,279 \pm 12,226$
		(76-94)	(45,481-65,234)
21	Progeny of males	84.3 ± 6.3	47.321 ± 10.119
	tolerant of PVG	(73-93)	(33,258-64,145)

Dose-response curves were done for each animal as described for Fig. 1, and the data analysed by simple linear regression. The relative response to PVG alloantigens was computed for each animal as the number of cells required to incorporate 10,000 c.p.m. of ³H-thymidine or give 33.3% specific lysis of PVG of concanavalin A (Con-A) blasts labelled with ⁵¹Cr. Normal and experimental groups are not different statistically by Student's t-test. The correlation coefficient was >0.95 for all regression lines. P > 0.2 for the normal and experimental groups in both the MLC and CTL assays

compatible to B. Because of the generally accepted results of this type of experiment, I believe it is unlikely that 'antigen' is being transferred genetically, and thus this does not explain why tolerant males should pass on their tolerance to their progeny.

Of course, the point of Gorczynski and Steele's work is that genetically transmitted acquired characteristics in mice is not the usual form of inheritance, and that such an event is truly exceptional. It is hoped that the experiments of Gorczynski and Steele^{1,2} can be repeated in the same strains of mice which they used, preferably derived from the same stock.

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- Gorczynski, R. M. & Steele, E. J. Proc. natn. Acad. Sci. U.S.A. 77, 2871-2875 (1980).
 Gorczynski, R. M. & Steele, E. J. Nature 289, 678-681 (1981).
 Billingham, R. E., Brent, L. & Medawar, P. B. Phil. Trans. R. Soc. B239, 357-414 (1956).
 Smith, R. N. & Howard, J. C. J. Immun. 125, 2289-2294 (1980).
 Gorczynski, R. M., Macrae, S. & Till, J. E. Scand. J. Immun. 7, 453-465 (1978).
 Wilson, D. B., Silvers, W. K. & Nowell, P. C. J. exp. Med. 126, 655-665 (1967).
 Dorsch, S. & Roser, B. J. exp. Med. 145, 1144-1157 (1977).
 Smith, R. N. & Powell, A. E. J. exp. Med. 146, 899-904 (1977).
 Butcher, G. W. & Howard, J. C. Nature 266, 362-364 (1977).
 Antezak, D. F., Brown, D. & Howard, J. C. Cell Immun. 43, 304-316 (1979).

Two major genes, linked to HLA and Gm, control susceptibility to Graves' disease

Hisamitsu Uno*, Takehiko Sasazuki*, Hajime Tamai† & Hideo Matsumoto‡

- * Department of Human Genetics, Medical Research Institute,
- Tokyo Medical and Dental University, Tokyo 113, Japan
- † Department of Psychosomatic Medicine, Faculty of Medicine,
- Kyushu University, Fukuoka 812, Japan
- ‡ Department of Legal Medicine, Osaka Medical School,
- Takatsuki 569, Japan

Graves' disease is a multifactorial disease in which immunogenetic as well as environmental factors have important roles. Recently, cumulative evidence has shown that genes controlling immune responses are linked to the MHC (major histocompatibility complex)1 and/or immunoglobulin allotype genes2,3. To identify the genes governing susceptibility to Graves' disease, we have studied 30 Japanese families where more than two first degree relatives were affected with the disease. From genetic analysis of HLA (human MHC) and immunoglobulin allotype of all family members, we present here evidence for the existence of two genes controlling the susceptibility to Graves' disease, one closely linked to HLA-DR, the other linked to the gene coding for the Gm allotype.

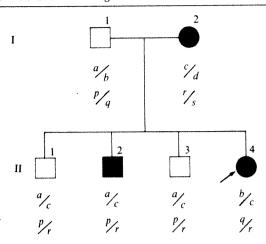


Fig. 1 A family affected with Graves' disease. ● ■, Graves' disease. a: HLA-A26, Bw54, Cw1; b: HLA-A2, Bw54, -; c: HLA-A11, Bw39, -; d: HLA-A2, -; p: Gm(1, 13, 15, 16, 17); q: Gm(1, 17, 21); r: Gm(1, 3, 5, 13, 23); s: Gm(1, 13, 15, 16, 17).

The families comprised 189 members, 71 (37.6%) of whom were affected with Graves' disease (60 female). The diagnosis of Graves' disease was based on the history and signs of hyperthyroidism with diffuse goitre, increased ¹³¹I uptake, raised serum thyroid hormone levels, the presence of thyroid autoantibodies, a positive stimulation test to thyrotropin releasing hormone (TRH test) and triiodothyronine suppression test, and histological examination if necessary. All these examinations except the triiodothyronine suppression test were also applied to healthy family members. They were typed for HLA-A, -B, -C and -DR antigens and immunoglobulin allotypes, Gm and Km.

We analysed the segregation of Graves' disease and HLA and Gm haplotypes in 15 selected families. We identified the disease-associated haplotypes of HLA and Gm by determining the haplotypes shared between two affected siblings selected if necessary by random number. We found that all affected siblings except one female shared the disease-associated haplotypes of both HLA and Gm. However, siblings who shared the diseaseassociated haplotypes did not necessarily suffer from the disease (Fig. 1). Statistical significance of this genetic association between the development of Graves' disease and particular HLA and Gm haplotypes in each family was analysed by the sib-pair method⁴ using 62 siblings in the families. Siblings under 10 yr old were excluded from analysis because Graves' disease is very rare below this age (Table 1). A significant association between the development of Graves' disease and particular HLA and Gm haplotypes in each family was observed ($P < 2.0 \times$ 10⁻³). The association was strong and significant in female siblings ($P < 2.0 \times 10^{-3}$), but not significant in male siblings, partly due to the small numbers of male patients in these families. The strong association between the development of Graves' disease and HLA and Gm haplotypes indicated that there might be two genes linked to HLA and Gm, respectively, which had a major influence on the development of Graves' disease. The one affected female sibling who did not share the disease-associated haplotype of HLA with other affected siblings could be explained by a recombination between HLA and an HLA-linked susceptibility gene for Graves' disease. Fourteen healthy siblings who had the disease-associated haplotypes were aged from 12 to 49 yr (mean age 27.6 yr). Because the age of onset of Graves' disease varies widely, they may possibly develop the disease later in life.

Furthermore, in a study of 30 probands in these families, the antigen frequency of HLA-DR5 was significantly increased to 30.0%, compared with 5.0% in control group ($Pc < 3.0 \times 10^{-3}$). The association between Graves' disease and HLA-DR5 indicated that an HLA-linked disease susceptibility gene was in strong linkage disequilibrium with HLA-DR5 (Table 2). There was, on the other hand, no significant association between

Table 1 Association between Graves' disease and HLA and Gm haplotypes in 15 families

		haple of HLA and	associated otypes I Gm allotype Negative†	P
Total sib	Affected	17	1	$< 2.0 \times 10^{-3}$
(n = 62)	Unaffected	15	14	~ a.o ~ 10
Female sib	Affected	14	1	$< 2.0 \times 10^{-3}$
(n = 50)	Unaffected	8	12	~2.0 \ 10
Male sib	Affected	3	0	NS
(n = 12)	Unaffected	7	2	149

One affected sibling used to determine disease-associated haplotypes in each family was excluded from this analysis. The P value was calculated by Fisher's exact method. NS, not significant.

* Positive for disease-associated HLA and Gm haplotypes.

Graves' disease and any particular immunoglobulin allotype in these probands, indicating the absence of significant linkage disequilibrium between Gm and a Gm-linked disease susceptibility gene.

The HLA-D region is assumed to be comparable to the I-region of the murine H-2 complex, in which immune response genes (Ir genes) and immune suppression genes (Is genes) are located^{5,6}. Recently, it has become more evident that HLA-linked Ir genes or Is genes exist in man⁷⁻⁹. Analysis of a human population has also revealed a statistical association of Gm allotypes with immune responsiveness to specific antigens^{10,11} and with several autoimmune diseases^{12,13}.

In the murine system, it has been demonstrated that idiotypic determinants are linked to heavy-chain constant region allotypic markers^{14,15}, and the idiotypic determinants of antigen-specific receptors on lymphocyte membranes are likely to have

Table 2 Association between Graves' disease and HLA

HLA- antigens	Graves' disease $(n = 30)$	Control group (n = 80)	χ^2	Pc	RR
DR5	9(30.0%)	4(5.0%)	13.08	<3.0×10 ⁻³	8.14
DRw8	11(36.7%)	13(16.3%)	5.82	NS	3.07

DR5 and DRw8 were determined using 11 and 5 sera, respectively, from the 8th International Histocompatibility Workshop¹⁸. Pc: P multiplied by the number of HLA-DR antigens studied. RR, relative rick

fundamental roles in immune regulation¹⁶. The immuno-regulatory function of idiotypes was revealed in deliberate immunization experiments¹⁷. It was therefore assumed that two genes, HLA-linked and Gm-linked, would govern the specific immune response to certain antigen(s) relevant to the development of Graves' disease. The elucidation of the antigen(s), and environmental factors involved in the pathogenesis of Graves' disease will, in turn, help to clarify the immunogenetic factors controlling the susceptibility to Graves' disease.

This is the first description of a strong association between a human disease and not only HLA but also immunoglobulin allotype in a familial analysis.

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- 1. McDevitt, H. O. & Benacerraf, B. Adv. Immun. 11, 31-37 (1969).
- Biozzi, B. in Genetic Control of Immune Responsiveness (eds McDevitt, H. O. & Landy, M.) 174–178 (Academic, New York, 1972).

[†] Negative for either or both disease-associated HLA haplotype and Gm haplotype.

- 3. Smith, S. M., Ness, D. B., Talcott, J. A. & Grumet, F. C. Immunogenetics 4, 221-232
- Penrose, L. S. Ann. Eugen. 6, 133-138 (1935)
- Benacerraf, B. & McDevitt, H. O. Science 175, 273-279 (1972).

 Benacerraf, B. & Devitt, H. O. Science 175, 273-279 (1972).

 Benacerraf, B. & Dorf, M. in The Role of Products of the Histocompatibility Gene Complex in Immune Responses (eds Katz, D. H. & Benacerraf, B.) 225-248 (Academic, New York,
- Greenberg, L. J., Bradley, P. W., Chopyk, R.-L. & Lalouel, J.-M. Immunogenetics 11, 145-167 (1980)
- Sasazuki, T., Kohno, Y., Iwamoto, I., Tanimura, M. & Naito, S. Nature 272, 359-361
- Sasazuki, T. et al. J. exp. Med. 152, 297s-313s (1980)
- Nakao, Y. et al. New Engl. J. Med. 304, 407-409 (1981)
- Whittingham, S. et al. Clin. exp. Immun. 40, 8-15 (1980).
- Nakao, Y. et al. Clin. exp. Immun. 42, 20-26 (1980).
 Farid, N. R., Newton, R. M., Noel, E. P. & Marshall, W. H. J. Immunogenet. 4, 429-432 (1977)
- 14. Schwartz, M., Lifshitz, R., Givol, D., Mozes, E. & Haimovich, J. J. Immun. 121, 421-426
- Pawlak, L. L., Mushinski, E. B., Nisonoff, A. & Potter, M. J. exp. Med. 137, 22-31 (1973).
- Jerne, N. K. Annls Immun. Inst. Pasteur, Paris 125, 373-389 (1974)
 Eichman, K. Adv. Immun. 26, 195-254 (1978).
- Mickey, M. R. & Terasaki, P. I. in *Histocompatibility Testing 1980* (ed. Terasaki, P. I.) 21-136 (UCLA Tissue Typing Laboratory, California, 1980).

Association between HLA-DR antigens and helper cell activity in the control of dental caries

Thomas Lehner, Jonathan R. Lamb. Kenneth L. Welsh & Richard J. Batchelor

Department of Oral Immunology and Microbiology, Guy's Hospital Medical and Dental Schools, London SE1 9RT, UK Department of Tissue Immunology, Royal Postgraduate Medical School, The Hammersmith Hospital, London W12 0H5, UK

Dental caries affects the majority of people in developed countries but about 3% of them seem to be resistant. An immunological basis for this resistance has been suggested by the findings that relatively caries-free subjects have increased serum antibody titres1 and that their T lymphocytes have a greater potential to proliferate on stimulation with streptococcal antigens2. Helper cells or their factors3 are required for formation of antibody to the streptococcal antigen4. In this study specific helper factor activity was released by 1-10 ng of the purified streptococcal antigen I/II (SAI/II) from lymphocytes of cariesresistant subjects, compared with 1,000 ng of SAI/II required to release similar helper factor activity from lymphocytes of cariesprone subjects. The low-dose helper activity of lymphocytes was associated predominantly with the HLA-DRw6,1,2,3 crossreactive groups, whereas the high-dose helper activity was associated with the HLA-DR4-related gene product.

The most important microorganism to be identified as a causative agent of dental earies is Streptococcus mutans⁵ purified protein antigen (SAI/II) isolated from this organism^{6,7} induced protection against caries on immunization of rhesus monkeys⁸. SAI/II stimulates monkey peripheral blood lymphocytes to generate T helper cells after in vitro incubation for 4 days in a modified Marbrook-Diener system⁴. Further incubation of the helper cells with an optimum dose of SAI/II for 24 h releases antigen-specific helper factor (HF) which stimulates a cooperative antibody response by normal mouse spleen cells to dinitrophenylated SAI/II (DNP-SAI/II). We have now modified this entirely in vitro method of generating both helper cells and factors, by assuming that in vivo natural immunization in man and artificial immunization in rhesus monkeys should lead to generation of helper cells. We have then used 1-10,000 ng of SAI/II to stimulate the relase of HF from the putative helper cells.

We selected a population of 24 young subjects (mean age 23 yr), 12 of whom showed a low DMFS index (decayed, missing, filled tooth surfaces) of 0-3 (mean \pm s.e., 1.0 ± 0.4), and the remaining 12 showing a high DMFS index of 5-35 (18.8 ± 3.0). Lymphocytes from the caries-resistant subjects released HF on addition of 1-10 ng SAI/II, whereas lymphocytes from

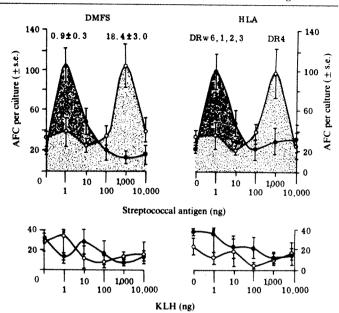


Fig. 1 The effect of dose of streptococcal antigen I/II (SAI/II) on the T-cell helper function in caries-resistant and caries-prone subjects. Human peripheral blood lymphocytes were separated by Ficoll–Isopaque density gradient $^{18}.5 \times 10^6$ viable cells per ml were cultured with 1, 10, 100, 1,000 and 10,000 ng of SAI/II $^{6.7}$ or keyhole limpet haemocyanin (KLH) for 24 h in Marbrook flasks⁴ The cell-free supernatant (HF) was collected, Millipore filtered and 1 μl of the HF was assayed in Marbrook-Diener culture flasks with 10⁷ unprimed B10.BR spleen cells and 0.1 μg ml⁻¹ DNP-haptenated SAI/II or TNP-KLH⁴. The anti-DNP antibody-forming cells (AFC) were assayed on day 4 in triplicate using the modified Cunningham assay¹⁹. The results are expressed as the number of AFC per culture ± s.e.m. and are given for the low DMFS (decayed, missing and filled surfaces; n = 12) and high DMFS group (n = 12) on the left, and DRw6,1,2,3 (n = 14) and DR4 group (n = 10) on the right. Lymphocytes responding to 1-10 ng SAI/II (DMFS 1.0 ± 0.4; HLA-DRw6,1,2,3) are shaded and those responding to 1,000 ng SAI/II (DMFS 18.8 ± 3.0; HLA-DR4) are stippled.

caries-prone subjects required 1,000 ng SAI/II for a similar release of HF, assessed by the antibody-forming cell assay (Fig. 1). Indeed, 11/12 (92%) of the caries-resistant, compared with 3/12 (25%) caries-prone subjects, released a significant amount of HF from lymphocytes stimulated with 1-10 ng SAI/II (P =0.0027 by Fisher exact test). In contrast, 8/12 (68%) of the caries-prone subjects and 0/12 caries-resistant subjects released a significant amount of HF from lymphocytes stimulated with 1,000 ng SAI/II (P = 0.0013). Significant HF was taken as the mean + 3 s.d. (79) antibody-forming cells resulting from the HF activity released by lymphocytes in the absence of SAI/II in 24 subjects.

The striking difference in the dose response to the SA between the disease-resistant and prone subjects was then analysed in relation to the HLA-A,B,C and DR(w) antigens' There were no obvious differences with the HLA-A.B and C antigens but a significant relationship was observed with the HLA-DR antigens (Fig. 1). Stimulation of lymphocytes with 1-10 ng SAI/II released significant amounts of HF activity in 7/9 subjects with DRw6, as compared with 1/10 with DR4 (P = 0.0097). Furthermore, a smaller group of five DR1,2,3typed lymphocytes also showed a significantly greater number of lymphocytes responding to the lower dose of SAI/II than the DR4-typed lymphocytes (P = 0.0039). It seems, therefore, that the cross-reacting DRw6,1,2,3 antigens¹⁰ are associated wih helper cells responding to low doses of SAI/II (12/14) whereas the DR4 antigens are not (1/10; P = 0.00074). However, lymphocytes with DR4 responded to 1,000 ng SAI/II in 6/10 (60%) subjects, compared with 3/14 (14%) subjects with lymphocytes bearing DRw6,1,2,3 antigens (P = 0.0566). Note that DR4 seems to be functionally dominant, because in all 10

Table 1 Reproducibility of the dose responses of antibody-forming cells of lymphocytes

			ng of streptococcal antigen I/II					
Subject	DRw	No. of times tested	0	1	10	100	1,000	10,000
1 2 3 4	6 6 4 4	4 3 5 3	21.8 (2.8) 15.3 (2.9) 22 (3.3) 15.3 (2.9)	127.2 (7.2) 84.7 (30.4) 34 (5.6) 15 (4)	15.8 (5.6) 93 (15.1) 40 (20) 45 (15)	31.3 (14.4) 14.3 (9.8) 34.3 (1.3) 43 (12)	16.8 (7.1) 15.7 (8.7) 136.5 (12.6) 133.3 (20.3)	17.5 (5.9) 12.3 (4.7) 47.7 (3.9) 60 (21.6)

Results are the mean (±s.e.) of antibody-forming cells (AFC). The AFC of human peripheral blood lymphocytes were determined to five concentrations of streptococcal antigen I/II, as described in Fig. 1 legend. This was performed on three, four or five occasions on lymphocytes from the same four donors.

subjects in whom DR4 was associated with DRw6,1,2,3 the lymphocytes responded to the high dose of SAI/II. The reproducibility of the effect of increasing doses of SAI/II on lymphocytes was tested on three to five different occasions in four subjects (Table 1). The maximum number of antibodyforming cells resulted from 1-10 ng SA for DRw6 or 1,000 ng for DR4 on each of the 15 occasions tested; the variations between different tests in the same subjects were small, as shown by the standard errors of the means.

A parallel study of HF activity of in vivo immunized, cariesresistant monkeys with SAI/II revealed that lymphocytes from these monkeys responded to 1-10 ng of SAI/II but not to keyhole limpet haemocyanin (KLH; Fig. 2). However, cariesprone monkeys which were not immunized showed no response to any of the antigen concentrations. The results of naturally acquired immunity in man and artificially acquired immunity in monkeys suggests that protection against dental caries in both primates is associated with T helper cells capable of responding to 1-10 ng SAI/II; this might be controlled in man by the cross-reactive DRw6,1,2,3 gene products. However, the cariesprone subjects have T cells which require a 1,000 times higher dose of SAI/II to respond with a HF activity comparable to cells of the caries-resistant subjects, and DR4 may be one of the gene products controlling this response. As such a high dose is unlikely to be reached in biological conditions, the end result might be similar to that found in monkeys which failed to respond to any of the antigen doses.

The specificity of the HF to DNP-SAI/II and characterization of HF have been described for rhesus monkeys elsewhere 4.11. Corresponding specificity studies have been carried out on human lymphocytes. Culturing human or monkey lymphocytes with 1-10,000 ng KLH and using trinitrophenyl (TNP)-KLH in the cooperative culture had no effect on the number of antibodyforming cells (Figs 1, 2). To exclude further the possibility that the SAI/II might stimulate a nonspecific helper factor, the factor was added to the cooperative culture with TNP-KLH. This had no effect on the number of antibody-forming cells (22.5 \pm 7.4, n = 4), as compared with the same four factors added to the cooperative culture with DNP-SAI/II, which increased the antibody-forming cells to 115.0 (\pm 2.2).

Immunoadsorption of HF on Sepharose 4 beads coated with SAI/II bound the HF activity but did not do so when the beads were coated with KLH (in preparation). Furthermore, as with monkey HF11, human HF was bound by antisera to anti-HF, but not anti-suppressor factor antisera. The factor also had DR region-encoded determinants, as insolubilized monoclonal anti-DR framework antibody (DA2) bound HF. Moreover, a monoclonal antiserum to DRw6,1,2 (Genox 353) absorbed out all the helper activity of DRw6 lymphocytes, without any effect on the helper activity from DR4 lymphocytes (in preparation). The factor binds IgM but not IgG or IgA antisera, as has been found in mouse and monkey helper factors 11-13. The significance of IgM has not been adequately explained, but it has been interpreted as a cross-reaction with some region of the μ chain. It seems, therefore, that HF prepared from human lymphocytes has, in addition to an antigen-binding site, a 'constant' region and determinants encoded by the HLA-D region, as has been found in HF prepared from mouse lymphocytes¹⁴

The finding that T helper cells respond to either low or high doses of antigen in man has no direct parallel in animal experiments. T-cell involvement has been observed in low responder mice, for in addition to the decreased antibody formation there is a decrease in delayed hypersensitivity and in vitro T-cell proliferative response¹⁵. Furthermore, immunization with an antigen under Ir gene control showed that in high responder mice the number of antigen-specific T cells increases, whereas no change occurs in the low responders¹⁶. The association of low-dose T helper cell responses in man with the DRw6,1,2,3 cross-reactive antigens and high-dose helper responses with DR4-related antigen might be an expression of , gene control. However, in vitro secondary responses to KLH revealed that human T-cell monocyte interactions are governed by HLA-D region antigens¹⁷. It is therefore possible that the functional high- and low-dose responses to SAI/II which are associated

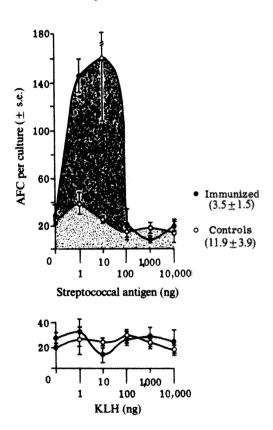


Fig. 2 The effect of streptococcal antigen I/II (SAI/II) T-cell helper function in immunized and control monkeys. Rhesus monkey peripheral blood lymphocytes were separated and cultured with 1, 10, 100, 1,000 and 10,000 ng of SAI/II or KLH. HF was prepared and the AFC were assayed and expressed as described for human lymphocytes in Fig. 1 legend. The results are given for six monkeys immunized with SAI/II and with a scare of 1.8 (±3.9). Lymphocytes responding to 1-10 ng of SAI/II (DMFS of 3.5) are shaded and there are no lymphocytes responding to 1,000 ng of SAI/II among the control monkeys.

with the HLA-D region may be related to monocyte antigenbinding cells and not necessarily to T cells. This possibility will be examined, although preliminary results with radiolabelled SAI/II suggest that selective binding of small doses of SA to HLA-DRw6,1,2,3 cells and high doses to HLA-DR4 cells are related to T cells. Whichever set of cells is involved, it seems that the aims of immunization should be to induce with very small doses of protein antigen T helper cells which cooperate with B cells in the formation of high-avidity antibodies, required in protection against the microorganisms.

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Received 1 April; accepted 16 June 1981.

- Challacombe, S. J. & Lehner, T. J. dent. Res. 55, C139-C148 (1976).
- 2. Lehner, T., Challacombe, S. J., Wilton, J. M. & Ivanyi, L. Archs oral Biol. 21, 749-753
- Feldman, M. J. evn. Med. 136, 737 (1972)
- Fetuman, N. J. & K., Kontiainen, S. & Lehner, T. J. Immun. 124, 2384-2389 (1980). Fitzgerald, R. J. & Keyes, P. H. J. Am. med. Ass. 61, 23-33 (1960). Russell, M. W. & Lehner, T. Archs oral Biol. 23, 7-15 (1978).

- 7. Russell, M. W., Bergmeier, L., Zanders, E. D. & Lehner, T. Infect. Immunity 28, 486-493
- Lehner, T., Russell, M. W. & Caldwell, J. Lancet i, 995-996 (1980).
 Welsh, K. I. & Batchelor, J. R. in Handbook of Experimental Immunology 3rd edn (ed. Weir,
- D. M.) 35.1-35.20 (Blackwell, Oxford, 1978). 10. Bodmer, J. G. Br. med. Bull. 34, 233-240 (1978).
- Zanders, E. D., Lamb, J. R., Kontiainen, S. & Lehner, T. Immunology 41, 587-596 (1980).
 Feldman, M. & Basten, A. Nature 237, 13 (1972).
 Taniguchi & Tada J. Immun. 113, 1757 (1974).
 Kontiainen, S. & Feldman, M. Thymus 1, 59-64 (1979).

- Benacceraf, B. & McDevitt, H. O. Science 175, 272-277 (1972). Hämmerling, G. I. & McDevitt, H. O. Transplantn Proc. 5, 179-182 (1973)
- Rodey, G. E., Luehrman, L. K. & Thomas, D. W. J. Immun. 123, 2250-2254 (1979).
 Wilson, B. J. & Kocvara, H. J. Immun. Meth. 9, 67-68 (1975).
- 19. Cunningham, A. & Szenberg, A. Immunology 14, 599-600 (1968)

Cross-reactivity between Thy-1 and a component of intermediate filaments demonstrated using a monoclonal antibody

R. Dulbecco*, M. Unger, M. Bologna†, H. Battifora†, P. Syka & S. Okada

The Salk Institute, 10010 N. Torrey Pines Rd, La Jolla, California 92037, USA and *Department of Pathology and Medicine, University of California at San Diego, La Jolla, California 92037, USA

Monoclonal antibodies1 are powerful tools for identifying antigenic molecules because they recognize a single antigenic site. In contrast, conventional immune sera contain mixtures of antibody molecules with a large variety of specificities. Some of these specificities may be cross-reactive with antigenic sites present on unrelated molecules, thus tending to decrease the overall recognition specificity. Such a cross-reactivity should be observed with some monoclonal antibodies, in which it might be expected to be both strong and specific (that is, directed against a restricted number of targets2). We report here such a crossreactivity in a monoclonal antibody that recognizes equally well both Thy-1 (ref. 3) and a component of intermediate filaments, probably vimentin^{4,5}.

The hybridoma producing the monoclonal antibody in question was isolated by Lake and Clark⁶. This antibody is an anti-Thy-1.1 mouse IgM designated T11A9e, which binds to Thy-1 at the surfaces of various cells, for example, the fusiform derivatives of the mammary cells RAMA7,8 and RANI9 or the





Fig. 1 Immunofluorescence of T11A9e serum with F2408 cells. a, A glass cover slip on which cells had grown was washed in phosphate-buffered saline (PBS) containing 0.01% Na azide and was then covered with antiserum diluted 1/50 in the PBS-azide saline. After 30 min, it was washed three times with PBS-azide, then overlayered with a 1/50 solution of fluorescein-labelled goat anti-mouse immunoglobulin (Antibodies, Inc.) for 30 min, washed again three times and mounted. Similar staining, of slightly reduced intensity, was obtained if the cells were fixed in 3% formaldehyde for 20 min, b. The cover slip was fixed in 3% formaldehyde for 20 min, rinsed in PBS-azide containing 0.01% thymerosal, dipped in cold methanol and then acetone (30 s each), hydrated in PBSazide and then stained as in a, except that the three washings after each antibody application were for 10 min, 2 h and 10 min. Similar results were obtained if formaldehyde fixation was omitted. The disappearance of the Thy-1 surface dots was observed for all antisera. Photographed using a Zeiss microscope with epi-illumination. a, $\times 1,300$; b, $\times 900$.

F2408 rat fibroblasts 10 (Fig. 1a). In the present experiments, the antibody used was either in the form of serum obtained from mice in which the T11A9e hybridoma had given rise to a tumour after subcutaneous injection, ascitic fluid after intraperitoneal cell injection, or supernatant of the hybridoma cultures. The IgM in the supernatant was partially purified and concentrated about 30 times by retention on an Amicon XM300 filter, followed by sizing in a Biogel A5M column. All these preparations had identical specificities.

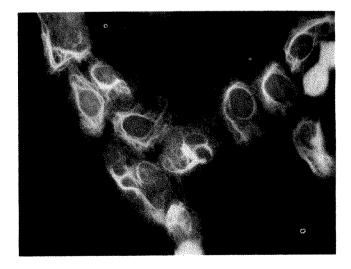


Fig. 2 Immunofluorescence of F2408 cells treated with colchicine. Cover-slip cultures were grown for 18 h in the presence of colchicine, 20 µg ml⁻¹, then stained with T11A9e serum as described for Fig. 1b. ×700.

[†] Permanent addresses: Department of Pathology, University of L'Aquila Medical School, L'Aquila, Italy (M.B.) and Department of Surgical Pathology, Northwestern University Medical School, Chicago, Illinois 60611, USA (H.B.).

Immunofluorescence experiments showed that T11A9e stains, in addition to surface Thy-1 in unfixed cells, a system of filaments in methanol-acetone-treated cells. Stained filaments were seen in the fusiform but not in the epithelial derivatives of mammary cells, and in the F2408 fibroblasts (Fig. 1b). They were also stained in cells that do not contain a Thy-1.1 antigen on their surfaces, such as BALB/c macrophages or BALB/c 3T3 fibroblasts, which have Thy-1.2. In all cells the pattern resembled that of intermediate filaments stained by antibodies to vimentin. In fact, in double immunofluorescence experiments, an identical network was stained in F2408 cells using either T11A9e or a rabbit antiserum to vimentin (Fig. 2). In cells pretreated with colchicine (20 µg ml⁻¹) for 12 h, the filaments stained by T11A9e showed the behaviour reported for intermediate filaments4 (Fig. 3). To exclude the possibility that the ability to stain these filaments was due to an accidental contamination of the hybridoma culture with hybridoma cells producing a different antibody, the T11A9e hybridoma was recloned. This recloned hybridoma produced an antibody with the same properties.

To determine whether the same antibody molecules stained Thy-1 and intermediate filaments, a T11A9e antiserum was absorbed with cells of two Thy-1.1-positive thymoma cell culture lines, or their Thy-1 negative derivatives¹¹. The ability to stain the filaments was absorbed by the Thy-1-positive cells but not by their negative variants; it was not absorbed by Thy-1.2 cells, whether positive or negative for Thy-1 (Fig. 4).

To test whether T11A9e antibody recognizes intermediate filaments through its Fc segment, the T11A9e antibody was preincubated for 30 min at room temperature with various concentrations of Thy-1.1 purified from rat brain (provided by I. Trowbridge). The mixture was then used to stain either Thy-1 at the surface of unfixed F2408 cells, or intermediate filaments in acetone-treated F2408 cells. Thy-1 at a concentration of 30 μg ml $^{-1}$ completely inhibited both types of staining. The Thy-1 concentrations at which the proportion of surface-stained cells or the intensity of filament staining (as judged from visual observation) was reduced to 50% were similar, $\sim 1~\mu g$ ml $^{-1}$. Therefore both target molecules are recognized by the same antibody site.

In addition to T11A9e, two out of 13 monoclonal anti-Thy-1 antibodies also stain intermediate filaments; these are T12G11c

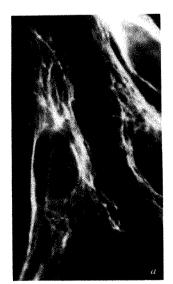




Fig. 3 Double immunofluorescence of F2408 cells with T11A9e and anti-vimentin serum. Immunofluorescence was carried out as described for Fig. 1b. a, The first antibody was a rabbit anti-vimentin serum and the second antibody was rhodamine-conjugated goat anti-rabbit immunoglobulin. b, The first antibody was T11A9e serum and the second, fluorescein-conjugated goat antimouse immunoglobulin. Photographs were taken using the appropriate filters for detection of fluorescence by rhodamine (a) or fluorescein (b). \times 1,400.

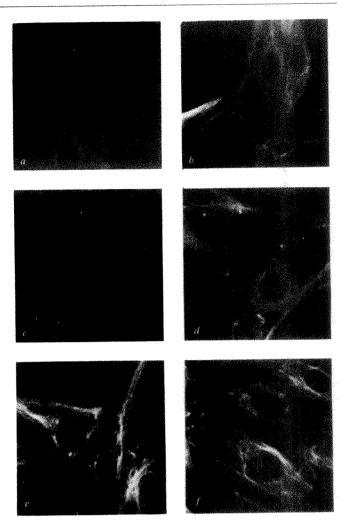


Fig. 4 Absorption of T11A9e serum with Thy-1-positive cells and their negative mutants. $100~\mu l$ of serum diluted 1/100 in PBS were absorbed in each case with $5\times10^8-5\times10^9$ cells of various mouse thymoma lines 12 (provided by R. Hyman). After 1 h at room temperature with frequent shaking the mixtures were centrifuged at 2,000 r.p.m. for 10 min. The supernatants were used in immunofluorescence experiments with F2408 cells as described for Fig. 1b. Absorbing cells: a, BW5147.3 · Thy-1.1 · ; b, BW5147.3 · Thy-1.1 · ; c, BW5147 · Thy-1.1 · ; d, BW 5147 · Thy-1.1 · ; e, S49 · Thy 1.2 · ; f, S49 · Thy 1.2 · a. The fields were photographed and printed using identical exposures. $\times 800$.

(IgM) and T32B11e (IgG) (both from P. Lake and E. Clark). The others give only a faint uniform fluorescence, probably due to surface Thy-1. Of the positive antibodies, T12G11c may be from the same lymphocyte clone as T11A9e, but T32B11e is likely to be different. Two conventional anti-Thy-1 rabbit antisera, obtained using purified Thy-1 from rat brain or from mouse thymocytes, did not stain the filaments.

To identify the protein to which the T11A9e antibody binds in the intermediate filaments, cytoskeleton proteins of F2408 cells were isolated as previously described¹², labelled with ¹²⁵I then immunoprecipitated with T11A9e antiserum, using a second antibody. Separation by polyacrylamide gel electrophoresis and autoradiography did not reveal any band of precipitated material. This antibody also fails to precipitate surface Thy-1. These failures may reflect a low affinity of the antibody. In spite of the lack of immunoprecipitation it is likely that T11A9e antibodies bind to vimentin because the T11A9e immunofluorescence of intermediate filaments is suppressed by anti-vimentin antiserum.

Because serum from mice inoculated with another isolate of the same hybridoma (T11A9a, no longer available) had a strong in vitro anti-doming effect on mammary cells¹³, we examined the possible relationship of this effect with our results. We found that the anti-doming effect was not removed by absorption to Thy-1.1-positive cells and was therefore unrelated.

We do not know whether the cross-reactivity we have observed is due to the proteins or possibly to some common carbohydrate. If the former is correct, it might reveal either a relatedness of the two proteins or a chance similarity of antigenic sites. In the latter case, our observation would be an example of the cross-reactivity of monoclonal antibodies predictable on theoretical grounds². The frequency of cross-reacting antibodies is unknown. When mice and rats were immunized with Thy-1, antibodies showing the cross-reaction we described occurred in 3 out of 13 monoclonal antibodies examined, but may not represent the true proportion because two of the antibodies may have a common origin. No general statement about the frequency of cross-reactivities can be made on the basis of these results or of past experience with immune sera. This remains to be established in further experiments using monoclonal anti-

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- Williams, A. F., Galfre, G. & Milstein, C. Cell 12, 663-673 (1977).
- Milstein, C. & Lennox, E. in Curr. Topics dev. Biol. 14, 1-32 (1980). Williams, A. F. in Contemporary Topics in Molecular Immunology (eds Porter, R. R. & Ada, G. L.) 83-114 (Plenum, New York, 1977)
- 4. Franke, W. W., Schmied, E., Osborn, M. & Weber, K. Proc. natn. Acad. Sci. U.S.A. 75, 5034-5039 (1978).
- Hynes; O. & Destree, A. T. Cell 13, 151-163 (1978).

- Tyles, O. & Desiree, A. I. Cea 15, 151-165 (1978).
 Lake, P. & Clark, E. A. Eur. J. Immun. 9, 875-886 (1979).
 Bennett, D. C., Peachey, L. A., Durbin, H. & Rudland, P. S. Cell 15, 283-298 (1978).
 Rudland, P. S., Bennett, D. C., Ritter, M. A., Newman, R. A. & Warburton, M. J. in Control Mechanisms in Animal Cells (eds Jemenez de Asua, L. et al.) 341-365 (Raven, New Mechanisms in Animal Cells). York, 1980).
- Dulbecco, R. et al. Proc. natn. Acad. Sci. U.S.A. 78, 2345-2349 (1981).
- Freeman, A. E., Igel, H. J. & Price, P. J. In Vitro 11, 107-116 (1975). Trowbridge, I. S., Hyman, R. & Mazauskas, C. Cell 14, 21-32 (1978).
- Franke, W. W., Schmid, E., Osborn, M. & Weber, K. J. Cell Biol. 81, 570-580 (1979). Duibecco, R., Bologna, M. & Unger, M. Proc. natn. Acad. Sci. U.S.A. 76, 1848-1852

Enzymatic synthesis of deshexapeptide insulin

Q. P. Cao, D. F. Cui & Y. S. Zhang

Shanghai Institute of Biochemistry, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, China

The semisynthesis of deshexapeptide insulin (DHI) from desoctapeptide insulin (DOI) reported earlier1 showed that DHI was biologically active. However, the product was not quite homogeneous, mainly due to the esterification side reaction and the following saponification². Recently, the catalytic action of trypsin was successfully used in the preparation of human insulin from porcine insulin3. It was also used in the preparation of insulin analogues with substitutions of B24 or B25 phenylalanine by leucine^{4,5}. Here, we report the synthesis of DHI from DOI and glycylphenylalanine through the catalytic action of trypsin. The product, purified by gel filtration and DEAE-Sephadex ion-exchange chromatography, showed insulin activity of 7.5 IU mg-1 in vivo and could be obtained in crystalline form.

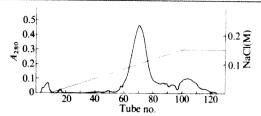


Fig. 1 Isolation of DHIOBu' from DOI by ion-exchange chromatography on a DEAE-Sephadex A-25 column (1×25 cm). 50 mg of sample were applied to the column, eluted using 50 ml of the initial 0.05 M Tris buffer, pH 7.6, with 40% isopropanol and then eluted through a salt gradient (from 200 ml of initial buffer containing 0.15 M NaCl to 200 ml of initial buffer alone). The eluent was collected in ~4-ml fractions. Active fractions were pooled, desalted by dialysis and lyophilized. The main peak is DHIOBu and the last peak, DOI.

The conditions for the enzymatic synthesis of DHI were different in certain aspects from those reported by Inouye et al.3. The alkali-labile methylsulphonylethoxycarbonyl group (Msc) was used⁶ instead of the t-butyloxycarbonyl group (Boc). The t-butyl ester (OBu') was not affected by the removal of Msc⁶, and DHIOBu' had one less negative charge than DOI; thus, they could be easily separated from each other by ion-exchange chromatography.

120 mg of (Msc)₂DOI and Gly-Phe-OBu' in excess (molar ratio = 1:60) were suspended in 0.16 ml of dimethyl sulphoxide (DMSO) and 1.08 ml of dimethylformamide (DMF) were added to ensure complete solubility. The solution was warmed to 37 °C and 0.96 ml 0.5 M Tris buffer, pH 8, added dropwise and the pH maintained at 7.8-8.0 by continual addition of triethylamine. Crystalline ovine trypsin was added and the solution incubated at 37 °C for 20 h. The total amount of enzyme used (12 mg) was added in three portions: at the start and after 2 and 6 h of incubation. At the end of the incubation, 40 ml of water were added and the pH adjusted to 4.2 using 1 M HCl. After cooling, the precipitate was collected by centrifugation, washed in water (pH 4.2) and lyophilized; 95.5 mg of dry powder were obtained. This powder was suspended in 4.78 ml of a solvent mixture, dioxane-methanol-water (3:0.5:3.5) and dissolved by adding a small amount of 2 M NaOH. The solution was cooled to 0 °C and 2 M NaOH at 0 °C added until the total amount was 0.68 ml. The deblocking reaction was carried out at 0 °C for 2 min and immediately stopped by the addition of acetic acid. The solution was then applied to a Sephadex G-50 column and eluted with 1 M acetic acid to remove high-molecular weight polymers. DHIOBu' and DOI were separated by DEAE-Sephadex chromatography (see Fig. 1). The purified DHIOBu'

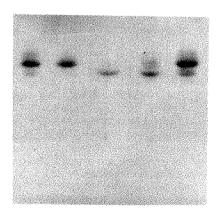


Fig. 2 Polyacrylamide gel electrophoresis of, from left to right: crude DHIOBu', purified DHIOBu', purified DHI, DOI and crude DHIOBu'; the lower end is the anode.

was treated with trifluoroacetic acid (TFA, 2.5 mg ml⁻¹) at 0 °C for 1 h. TFA was then removed by evacuation and the residue washed twice with ethyl acetate. 10 mg of the product were further purified by DEAE-Sephadex A-25 chromatography to remove any unreacted DHIOBu' and 7 mg of purified DHI were obtained. The purified DHI was dissolved in 0.01 M HCl (1.5 mg ml⁻¹) and dialysed against citrate buffer containing 25% acetone (2% citric acid, 50 ml; 6.5% zinc acetate, 1 ml; acetone, 25 ml; adjusted to pH 5.7-5.8 with NaOH and diluted to 100 ml). DHI crystals appeared after standing overnight.

The biological activity of purified DHI was determined in a mouse convulsion assay (according to British Pharmacopoeia) as 7.5 IU mg⁻¹. DHIOBu' also possessed insulin activity, thus it was easily detected and collected by DEAE-Sephadex chromatography. However, its activity is about four times lower than that of DHI. The activity of DOI is less than 1% compared with insulin.

After DEAE-Sephadex chromatography (Fig. 1), the DHIOBu' peak yielded 29.8 mg and the DOI peak, 4.9 mg; a ratio of 86:14. Polyacrylamide gel electrophoresis7 showed that

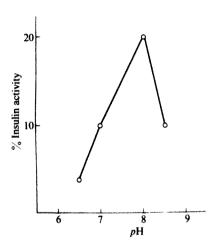


Fig. 3 The optimum pH for the enzymatic synthesis of DHI from (Boc)2 DOI and Gly-Phe-OBut.

most of the DOI was converted into DHIOBu' (Fig. 2). DOI moved faster towards the anode than DHIOBu'. When OBu' was removed, DHI occupied the same position as that of DOI. Purified DHI gave one major band after electrophoresis. Its amino acid composition was determined as: His, 2.4 (2); Arg, 0.9 (1); Asx, 3.0 (3); Glx, 6.3 (7); Thr, 1.1 (1); Ser, 2.7 (3); Gly, 3.8 (4); Ala, 1.1 (1); Val, 3.4 (4); Ile, 1.7 (2); Leu, 5.1 (6); Tyr, 2.6 (3); Phe, $1.9 (2) (Cys)_2$ not determined.

In enzymatic synthesis, co-solvent must be used—we used Tris buffer containing 50-60% DMF with a small amount of DMSO to increase the solubility. In tryptic hydrolysis, the optimal pH was found to be around 8 (Fig. 3). Inouye et al.3 used a pH of 6.5 for their synthesis. We have compared the enzymatic synthesis of DHI from (Boc)2DOI and Gly-Phe-OBu' in solutions of different pH, and found that the optimum pH was 8. The yield of the enzymatic reaction of (Msc)₂DOI and Gly-Phe-OBu' was 74%, calculated from the amount of DHIOBu' and DOI obtained by DEAE-Sephadex chromatography and the small portion of DOI polymers in gel filtration. This result could be attributed to several factors: (1) An increase in the equilibrium constant of synthesis due to the presence of co-solvent, which decreases the equilibrium constant of proton transfer from the carboxyl to the amino group of the reactants, as reported by Homandberg et al.8. (2) The high concentration of reactants and the large excess of Gly-Phe-OBu' drive the reaction towards synthesis according to the law of mass action. (3) The pH (8) was optimal for the catalytic action of trypsin. (4) Both reactants are very soluble at this pH.

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- Shanghai Insulin Research Group. Scientia sin. 16, 61-70 (1973).
 Gattner, H.-G. & Schmitt, E. W. Z. physiol. Chem. 358, 105-113 (1977).
 Inouye, K. et al. J. Am. chem. Soc. 101, 751-752 (1979).
 Gattner, H.-G. et al. Z. physiol. Chem. 361, 1135-1128 (1980).
 Tager, H. et al. Proc. natn. Acad. Sci. U.S.A. 77, 318: 3185 (1980).
 Geiger, R. et al. Chem. Ber. 108, 2758-2763 (1975).
 Cao, Q. P. et al. Scientia sin. 23, 1309-1315 (1980).
- Homandberg, G. A. et al. Biochemistry 17, 5220-5227 (1978).

Comparative antiviral efficiency of leukocyte and bacterially produced human α -interferon in rhesus monkeys

Huub Schellekens*, Arie de Reus*, Reinder Bolhuis†, Michael Fountoulakis‡, Catherine Schein‡, Josef Ecsödi‡, Shigekazu Nagata‡ & Charles Weissmann‡

- * Primate Center TNO, PO Box 5815, 2280 HV Rijswijk, The Netherlands
- † Rotterdam Radiotherapeutic Institute, 3075 EA Rotterdam, The Netherlands
- ‡ Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland

Interferons are being tested as antiviral and antitumour agents in man1-5, but because of their limited availability, clinical trials have remained inconclusive. Most of the interferon (IFN) preparations tested in man have been derived from human peripheral blood, buffy-coat leukocytes (HuIFN- α)⁶ induced by Sendai virus or from human diploid fibroblastoid cells (HuIFNβ) superinduced with poly(rI)-poly(rC)7. As production of interferon from leukocytes is restricted by the availability of blood donors, and production from cultured cells is a laborious and relatively costly process, the use of recombinant DNA technology may provide the most economical approach to the large-scale production of pure species of human interferon. The testing of such interferons is in its infancy. With the availability of bacterially produced HuIFN-a2 we have been able to compare its activity against vaccinia virus infection of the rhesus monkey with that of HuIFN-α from leukocyte buffy coats. Here we provide evidence that the two human α -interferons have comparable antiviral activity but that the bacterially produced form has fewer side effects, at least in the rhesus monkey.

There have been reports of the isolation of cDNAs encoding different HuIFN-a species, and of the production of polypeptides with the antigenic and biological properties in vitro of interferon from Escherichia coli harbouring appropriate hybrid plasmids⁸⁻¹⁰. An important question is whether interferons produced by E. coli have the same efficacy in vivo as natural interferons. The polypeptides produced in E. coli may differ from those produced in leukocytes due to postsynthetic modifications such as proteolytic cleavage, acylation or glycosylation. Recent evidence suggests that, contrary to previous indications, most if not all HuIFN-α species from leukocytes or lymphoblastoid cells are not glycosylated11, therefore, at least in this regard, we expect interferons made in E. coli to be no different. In addition, HuIFN-α from buffy coats is a mixture of several distinct species11,12 which are encoded by different genes¹³. The different IFN- α species differ substantially in their amino acid sequences (refs 10, 14, 15 and K. Henco, J. Fujisawa and J. Schmid, unpublished results) and in some of their biological activities, determined in vitro 14. In view of these considerations, the effect in vivo of a single species of α -interferon produced in E. coli may differ from that of a preparation of natural α -interferon. One report has described the protection of squirrel monkeys against a lethal dose of encephalomyocarditis by both natural HuIFN- α and a HuIFN- α from E. $coli^{10}$; in this experiment only one very high dosage of the interferons was tested, thus no conclusions could be drawn regarding their relative effectiveness.

There have been reports of rhesus monkeys infected intradermally with vaccinia virus to test the antiviral activity of human interferon¹⁶⁻¹⁸. The parameters influencing the activities of human interferon in vivo were extensively tested in this model system. The pharmacokinetics and the sensitivity of the rhesus monkey to the antiviral activity and side effects of human interferons were similar to those of man. Suboptimal doses of interferon can also be tested in this model.

We have compared the antiviral effects of different doses of HuIFN- α from buffy coats with HuIFN- α from E. coli in our rhesus monkey vaccinia model and studied the possible side effects of these products. The natural HuIFN- α , prepared as previously described⁶, had a specific activity of $10^{6.2}$ U per mg. HuIFN- α_2 was expressed in E. coli from an IFN- α_2 cDNA from which the signal sequence 14 had been removed and replaced by a methionine codon (M. Mishina, W. Boll and A. Hall, unpublished results). Purification of interferon from E. coli extracts involved acid precipitation, chromatography on agarose-linked Cibacrone blue and reversed-phase HPLC; the final product had a specific activity of 10^{7.6} U per mg protein and showed a single major interferon band and one minor band on SDSpolyacrylamide gel electrophoresis.

Three doses of each interferon (50,000, 150,000 and 500,000 U per kg body weight) were administered to groups of three monkeys each, by daily intramuscular injections, starting the day before challenge with vaccinia and continuing for 7 days after challenge. Three hours after the second interferon treatment the levels of circulating interferon were similar for both types, at all dosage levels (Table 1). All three dosages of natural and bacterial interferon had significant antiviral activity and the degree of protection was dose dependent (Table 1). The highest doses used completely abolished vaccinia-specific lesions. The in vivo antiviral activities of the two preparations were similar to their activities in vitro. The side effects of the two interferons. however, differed significantly (Table 2). Natural α -interferon from buffy coats, at a dose of 500,000 U per kg, caused leukopenia and fever, whereas the interferon from E. coli did not. Liver and kidney function tests showed no adverse effects of either type of interferon. Subsequently, doses of 10⁷ U E. coli

Table 1 Comparison of the efficacy of natural HuIFN-α (buffy coat) and HuIFN- α_2 (E. coli) in vaccinia-infected rhesus monkeys

Dose	Circulating	Circulating interferon		Lesion score (day 7)		
(U per kg body weight)	HuIFN-α (buffy coat)	HuIFN-α ₂ (E. coli)	HuIFN-α (buffy coat)	HuIFN-α ₂ (E. coli)		
500,000	480 (±0)	380 (±92)	$0.7*(\pm0.8)$	1.2* (±1.0)		
150,000	$80 (\pm 35)$	$120(\pm 43)$	$1.5*(\pm0.5)$	1.5* (±0.5		
50,000	40 (±17)	73 (±42)	2.2* (±0.8)	2.4* (±1.2		
Untreated cor	ntrols	<20	4.0 (0.0)			

Rhesus monkeys (Macaca mulatta; bred at the Primate Centre, The Netherlands) weighing 1.5-2 kg were used. Natural HuIFN- α was induced in buffy coats using Sendai virus and purified to the stage PIF-1 (see ref. 6); the specific activity was 10^{6.2} U per mg protein. HuIFN-α₂ (E. coli) was prepared from E. coli trans formed by the plasmid Z-pBR(Δ Ampi)/IFN- α_2 (Δ sig)-M2lb (M. Mishina, W. Boll and A. Hall, unpublished results) and had a specific activity of $10^{7.6}$ U per mg protein. The monkeys were injected intramuscularly with the doses indicated from day -1 until day +7. Each treated group consisted of three monkeys. Four untreated monkeys served as controls. On day 0, the animals were sedated with ketamine hydrochloride, blood was taken and all monkeys were vaccinated with vaccinia virus (Elstree strain, 10^8 plaque-forming units per ml) using a Sterneedle device. At day +7, the skin lesions were scored according to an arbitrary scale16 from 0 to 4 based on the appearance of papules and pustules, and severity. Results are the mean ±s.d. Interferon activity was assayed using cytopathic inhibition effect test on WISH cells.

Significant protection at the P < 0.01 level when compared with untreated controls in the Mann-Whitney U-test. A score of 0.5-1.0 is equivalent to the response found after injection of 0.9% NaCl (refs 16, 17).

Table 2 Comparison of the side effects of HuIFN- α (buffy coat) and HuIFN- α_2

	Leukocyte c	Temperature °C	
	Day -2	Day 0, 3 h after interferon	(Day 0, 3 h after interferon)
Natural HuIFN-α (buffy coat) (500,000 U per kg body weight)	14.1 (±3.6)	7.2* (±1.3)	38.1† (±1,5)
Bacterial HuIFN-α ₂ (500,000 U per kg body weight) Untreated controls	12.6 (±2.7) 15.7 (±4.8)	15.0 (±3.1) 14.0 (±4.1)	36.3 (±0.6) 36.7 (±0.4)

For experimental details see Table 1 legend. On day -2 and day 0 (3 h after interferon administration), the animals were sedated using ketamine hydrochloride and blood was taken for routine blood chemistry and haematology. At day 0, the body temperature was measured rectally and the monkeys were vaccinated (see Table 1 legend). Values are mean leukocyte counts and temperature ±s.d. Significant reduction (P < 0.05) when compared with day -2 in the Mann-Whitney U-test.

Significant temperature rise at a P = 0.053 level when compared with untreated controls and P < 0.05 when compared with the animals treated with HuIFN-α2 (E. coli).

IFN α_2 per kg were administered to three monkeys, again without giving rise to fever, leukopenia, anaphylactic reactions or other evidence of toxicity. The difference in toxic effects between the two interferon preparations when administered to rhesus monkeys could be due to the higher degree of purity of the bacterial product. However, recent work by Scott et al. 19 shows that even highly purified leukocyte interferon (NK2-IF), prepared using antibody affinity chromatography, shows the same toxic effect as the much less pure PIF preparation. Perhaps the toxic effects are due to one or more of the α -interferon species other than IFN- α_2 , present in the preparation derived from buffy coats. Alternatively, as Genentech's α -interferon (type A, which is equivalent to our α_2) purified from E. coli apparently still causes fever and leukopenia in humans (see Nature 291, 105–106; 1981), it could be that the rhesus monkey is not sensitive to the pyrogenic activity of pure α -interferon, and the response we found with the natural interferon was due to impurities in that preparation.

Our study illustrates the use of the vaccinia-infected rhesus monkey as a model for comparing the effects of different interferon preparations. We show that an interferon species produced by recombinant DNA techniques has the same antiviral efficacy in vivo as a natural interferon preparation. If the lack of side effects demonstrated in the rhesus monkey proves to be the same for humans, it may be possible to increase the dosage by one order of magnitude or more over that now used.

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- Stewart, W. E. II The Interferon System (Springer, New York, 1979). Scott, G. M. & Tyrell, D. A. J. Br. med. J. 280, 1558–1562 (1980). Krim, M. Blood 55, 875–884 (1980). Sikora, K. Br. med. J. 281, 855–858 (1980).

- Priestman, T. J. Lancet ii, 113-118 (1980). Cantell, K. & Hirvonen, S. J. gen. Virol. 39, 541-543 (1978).
- Billiau, A. et al. Antimicrob. Ag. Chemother. 16, 49-55 (1979). Nagata, S. et al. Nature 284, 316-320 (1980).
- Masucci, M. G. et al. Science 209, 1431-1435 (1980) Goeddel, D. et al. Nature 287, 411-417 (1980).
- Allen, G. & Fantes, K. H. Nature 287, 408-411 (1980). Rubinstein, M. et al. Proc. natn. Acad. Sci. U.S.A. 76, 640-644 (1979)
- 13. Nagata, S., Brack C., Henco, K., Schamböck, A. & Weissmann, C. J. Interferon Res. 1,
- 14. Streuli, M., Nagata, S. & Weissmann, C. Science 209, 1343-1347 (1980).
- Mantei, N. et al. Gene 10, 1-10 (1980).
- Weimar, W., Stitz, L., Billiau, A., Cantell, K. & Schellekens, H. J. gen. Virol. 48. 25-30
- Schellekens, H., Weimar, W., Cantell, K. & Stitz, L. Nature 278, 742 (1979) Schellekens, H. & Weimar, W. thesis, Erasmus Univ., Rotterdam (1980).
- 19. Scott, G. M. et al. Br. med. J. 282, 1345-1348 (1981).

Interaction of the antihypertensive drug felodipine with calmodulin

Stig-Lennart Boström*, Bengt Ljung*, Sven Mårdh†, Sture Forsen‡ & Eva Thulin‡

* AB Hässle Research Laboratories, S-431 83 Mölndal, Sweden and Department of Physiology, University of Göteborg, Sweden † Institute of Medical and Physiological Chemistry, Biomedical Center, Box 575, Uppsala University, S-751 23 Uppsala, Sweden ‡ Physical Chemistry 2, Chemical Center, POB 740, S-220 07 Lund, Sweden

Despite the marked differences in their chemical structure, members of a heterogeneous group of pharmacological agents are thought specifically to block Ca2+ influx through calcium channels and thus mediate negative inotropic cardiac effects and vasodilatation. Electrophysiological studies of the myocardium have shown that the slow inward Ca2+ current is blocked1, but the cellular mechanism of these agents in vascular smooth muscle is largely unknown. Felodipine [4-(2,3-dichlorophenyl)-1,4-dihydropyridine-2,6-dimethyl 3,5-dicarboxylic 3-ethylester and 5-methylester)] is a new antihypertensive agent which seems specifically to dilate precapillary resistance vessels in vivo. It is a structural analogue of nifedipine and SKF 242602, both of which have been classified as 'calcium antagonists', implying that their vascular as well as myocardial actions are due to a blockade of Ca2+ influx. However, the findings reported here point rather to an interaction between felodipine and calcium-binding proteins such as calmodulin.

The effects of felodipine were studied in the isolated rat portal vein. The smooth muscle of this vessel shows many similarities to that of the haemodynamically important small resistance vessels, including its pronounced dependence on extracellular $[Ca^{2+}]$ (refs 3-5). Figure 1a shows the spontaneous phasic electrical and mechanical activity of the rat portal vein in the absence of inhibitors. The addition of felodipine (30 nM) produced a gradual decrease of integrated electrical membrane discharge and reduced the amplitude and duration of the contractions (Fig. 1c, d), whereas tetrodotoxin, at a dose level capable of blocking sodium channels, did not affect this spontaneous activity (Fig. 1b).

Figure 2 shows the dose response of the portal vein to cumulative doses of CaCl₂ in a K⁺-rich solution. The presence of felodipine at low concentration (0.1 pM) in a Ca²⁺-free solution for 1 h markedly depressed the contractile responses when Ca²⁺ was reintroduced (Fig. 2).

Similar experiments investigated transmembrane Ca^{2+} influx in K^+ -depolarized muscle. The Ca^{2+} -depleted portal veins were

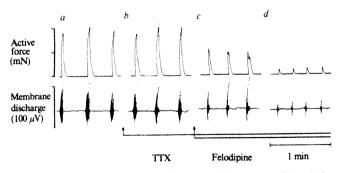


Fig. 1 Effects of felodipine (30 nM) on extracellularly recorded electrical membrane discharge and contractile force of the spontaneously active smooth muscle of the rat portal vein. Vessel mounted for recording of activity in longitudinal media layer²⁰. Intermittent bursts of electrical membrane discharge and associated phasic contracting are seen in the control (a). Administration of tetrodotoxin (TTX, 1 µM; b) in a concentration which would abolish neurogenic vasoconstrictor responses²¹, reflecting blockade of Na* channels, did not affect the activity. However, addition of felodipine (30 nM) led to a gradual inhibition of membrane discharge and of mechanical force illustrated 20 min (c) and 40 min (d) after administration.

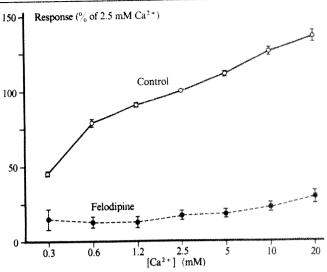


Fig. 2 Effects of felodipine (0.1 pM) on isometric force development in response to graded concentrations of Ca²⁺ in isolated rat portal vein lying in high-K⁺ (128 mM) Tris-buffered Krebs solution. Ca²⁺ added to solution after 1 h incubation in nominally Ca²⁺-free Krebs ('Control') and after a subsequent 1 h in Ca²⁺-free solution containing the drug. All responses expressed as a percentage of the initial response to 2.5 mM Ca²⁺. Values obtained in the presence of felodipine were corrected for the decline observed in control experiments where no drug was added (mean±s.e., n = 8). Note the pronounced felodipine blockade of Ca²⁺ responses in the depolarized vascular smooth muscle.

'pulse-labelled' with 45Ca²+ in 1.2 mM CaCl₂ for 2 min, sufficient time for a contracture to develop. The tissues were then washed in a Ca²+-free lanthanum buffer for 1 h to displace extracellularly bound Ca²+. As Fig. 3 shows, the 45Ca²+ uptake was identical after 1 h incubation with or without 1 nM felodipine. However, after treatment with 2 mM LaCl₃ for 1 h, the cellular 45Ca²+ uptake was <10% of control (Fig. 3). In agreement with these results, Church and Zsoter were unable to detect any alteration in 45Ca²+ uptake on treatment with nifedipine, verapamil or diltiazem in concentrations producing a clear-cut inhibition of smooth muscle activity. It thus seems that felodipine and some other calcium antagonists interfere with intracellular Ca²+ utilization rather than with transmembrane Ca²+ influx during activation.

To explore possible interactions between felodipine and myofibrillar proteins, 14 C-felodipine was incubated for 1 h with a Ca^{2+} -sensitive crude actomyosin preparation from porcine aorta⁸; samples were then subjected to SDS-polyacrylamide gel electrophoresis. One gel was stained for protein with Coomassie brilliant blue and in the other the distribution of radioactivity was measured in 5-mm sections of the gel. Molecular weight (M_r) estimations were made with standard proteins. Radioactivity was detected only at the dye front and in the 15,000–20,000 M_r region.

Calmodulin $(M_r 16,700)$ occurs in the cytoplasm and is bound to a membrane fraction⁹ and to other proteins¹⁰; its interaction with a drug could therefore affect smooth muscle activity¹¹⁻¹³. When ¹⁴C-felodipine was incubated with calmodulin and subjected to electrophoresis, the radioactivity in the gel was found to coincide with the site of protein staining.

On binding calcium, calmodulin is converted from an inactive to an active form which may influence various cellular processes $^{14.15}$, possibly also membrane activation 16 . Apparently, calmodulin can bind a maximum of 4 mol Ca^{2+} per mol protein when assayed at high ionic strength $^{14.15}$. Potentiometric titration of smooth muscle calmodulin with $CaCl_2$ [in 20 mM imidazole buffer (pH 7.0, 22 ± 1 °C) with 100 mM KCl] resulted in an equivalence point corresponding to an uptake of 4 mol Ca^{2+} per mol protein, as determined using the Gran-plot technique 17 . However, in a concentration of 4 mol felodipine per mol of protein, the binding was reduced to ~ 2 mel Ca^{2+} .

In NMR studies, ¹¹³Cd has a spin I = 1/2 magnetic nucleus and the similarity of the ionic radii of Cd (0.0097 nm) and Ca²⁺

(0.0099 nm) makes Cd2+ a good substitute for Ca2+ in Ca2+ binding protein18. The addition of felodipine to (Cd)4-calmodulin produced dramatic changes in the 113 Cd-NMR spectrum (Fig. 4), which can be taken to indicate that the binding of felodipine to calmodulin causes the conformation of the latter to change in such a way that at least one of the Ca2+-binding sites is altered. This interaction is apparent at a molar ratio of felodipine to calmodulin of only 0.5: 1. The ¹¹³Cd-NMR results obtained also show that 1-2 mol felodipine are bound per mol calmodulin with a binding constant of $10^5 - 10^6 \,\text{mol}^{-1}$.

Trifluoroperazine, which is known to interact with calmodulin did not inhibit smooth muscle in the rat portal vein (results not shown). Furthermore, their different 113Cd-NMR spectra 18 indicate that the conformational change of calmodulin induced by trifluoroperazine is qualitatively different from that caused by felodipine.

Overall, these results indicate that felodipine may exert its vasodilator effects by interacting with a Ca2+-binding protein rather than by inhibiting Ca2+ influx. Church and Zsoter7 have recently shown that nifedipine and other so-called Ca24 antagonists do not impair transmembrane Ca2+ transport in either vascular or cardiac tissue. Furthermore, it is debatable whether Ca2+ antagonists do indeed act by selective inhibition of slow Ca²⁺ channels in the myocardium¹⁹. We suggest that felodipine and possibly other Ca2+ antagonists exert at least some of their effects by an action on calmodulin or other Ca²⁺-binding proteins.

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45Ca2+ uptake (c.p.m. per mg dry weight)

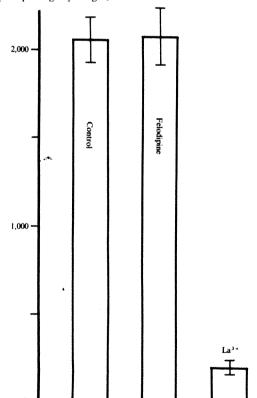


Fig. 3 Effect of felodipine and lanthanum (La3+) on 45Ca2+ influx in the rat portal vein. After an initial period of 1 h in Tris-buffered Krebs solution, the muscles were kept for 1 h in a Ca²⁺-free solution. The tissues were then transferred to a high-K⁺ (128 mM), Ca²⁺-free Tris-buffered solution for 10 min. The muscles were then pulse-labelled⁶ with ⁴⁵Ca²⁺ for 2 min using 45Ca²⁺ in 1.2 mM CaCl₂ in the high-K⁺ buffer. During such a 2-min CaCl₂ exposure a contracture developed in the control specimen. After the initial period experimental muscles were exposed to felodipine (1 nM) or La3+ (2 mM). After the 2-min labelling period, the muscles were washed for 1 h in nominally Ca2+-free Tris-Krebs solution including 2 mM LaCl2. The muscles were then blotted lightly, dried overnight at 100 °C, weighed, dissolved and analysed for 45Ca.

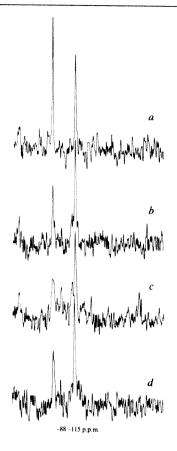


Fig. 4 The effects of felodipine on the 113Cd-NMR spectrum of a 1 mM solution of (Cd)₄-calmodulin (bovine testes) at pH 8.4 (Tris-perchlorate buffer). In the absence of felodipine (a) the two 113Cd-NMR signals are observed at -88 and -115 p.p.m., respectively, relative to 0.05 m Cd(ClO₄)₂; higher fields have negative shifts. The molar ratio of felodipine to calmodulin is: b, 0.6: 1; c, 0.9: 1, d, 2.0: 1. The felodipine was added as a 10 mM solution in ethanol-cremaphor RH410 (9:1). Apart from a small change in chemical shift of signal at -88 p.p.m. (~2 p.p.m. towards higher fields, the effects of the felodipine solvent corrected for), a broadening of both 113Cd-NMR signals is observed. The broadening which is most marked on the signal at -88 p.p.m. has its maximum at a molar ratio of felodipine to calmodulin of ~1:1. Further addition of felodipine causes the signals to become narrower again. The observed effect of felodipine on the ¹¹³Cd-NMR spectra of (CD)₄-calmodulin is similar to the effect observed on the addition of 1 mol of trifluoperazine per mol calmodulin. The 113Cd-NMR spectra were obtained at 55.54 MHz on a 6.0 T FT NMR spectrometer. The sample volume was 2.5 ml and the sample was placed in a solenoid perpendicular to the axis of the superconducting magnet. Each spectrum is the result of 25,000 transients obtained during 4 h. The sample temperature was 23 ± 1 °C.

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- Fleckenstein, A. A. Rev. Pharmac, Tox. 17, 149-166 (1977).
- Taira, N., Himori, N. & Iami, Y. Clin. exp. pharmac. Physiol. 3, 567-574 (1976). Sigurdsson, S. B., Uvelius, B. & Johansson, B. Acta physiol. scand. 95, 263-269 (1975). Sutter, M. C., Hallbäck, M., Jones, J. V. & Folkow, B. Acta physiol. scand. 99, 166-172
- Sutter, M. C. & Ljung, B. Acta physiol. scand. 99, 484-495 (1977). Kroeger, E. A., Marshall, J. M. & Bianchi, C. P. J. Pharmac. exp. Ther. 193, 309-316
 - Church, J. & Zsoter, T. T. Can. J. Physiol. Pharmac. 58, 254-264 (1980).

- Courch, J. & Zsolet, T. I. Can. J. Physiol. Pharmac. 58, 234-204 (1960).
 Sobieszek, A. & Bremel, R. D. Eur. J. Biochem. 55, 49-60 (1975).
 Kakiuchi, S. et al. Adv. Cyclic Nucleotide Res. 9, 253-269 (1978).
 Grand, R. J. A. & Perry, S. V. Biochem. J. 183, 285-295 (1979).
 Dabrowsky, R., Aromatorio, D., Sherry, J. M. F. & Hartshorne, D. J. Biochem biophys. Res. Commun. 78, 1263-1272 (1977).
- Hathaway, D. R. & Adelstein, R. S. Proc. nam. Acad. Sci. U.S.A. 76, 1653–1657 (1979).
 Walsh, M. P., Vallet, B., Autric, F. & Demaille, J. G. J. biol. Chem. 254, 12136–12144
- Cheung, W. Y. Science 207, 19-27 (1980).
 Wang, J. H. & Waisman, D. M. Curr. Topics cell. Regulation 15, 47-107 (1979).
- Thorens, S. J. Muscle Res. Cell Motility 1, 455-456 (1980).
 Dyrssen, D., Jagner, D. & Wengelin, F. Computer Calculation of Ionic Equilibria and Titration Procedures, 204-238 (Almqvist & Wiskell, Stockholm; Wiley, New York; 1968). 18. Forsén, S., Thulin, E. Drakenberg, T., Krebs, J. & Seamon, K. FEBs Lett. 117, 189-194
- Vaughan Williams, E. M. Anti-Arrhythmic Action and the Puzzle of Perhexiline, 32-33
- (Academic, London, 1980).
- Ljung, B. & Stage, D. Acta physiol. scand. 80, 131-141 (1970).
 Ljung, B. & Stage, D. Acta physiol. scand. 94, 112-127 (1975).

TERS ARISING

Disruption of meteoritic iron parent bodies

THE discovery by Clarke et al.1 of preterrestrial shock polymorphism (& structure) and shock-induced diamond in Allan Hills A77283 has implications for the process by which the parent body of this meteorite disrupted.

The internal metallographic structures of meteoritic iron seem to require well insulated parent bodies of 10-100 km size^{2,3}, whereas iron meteorites typically fall to Earth as bodies of 10-100 cm with occasional crater-forming masses of perhaps up to 100 m size. However, of the 70 or so IA irons (excluding A77283) only 3 show sufficient signs of mechanical damage to produce the ε structure⁴. Of these three Cañon Diablo is well known to contain diamond, but is a crater-forming mass and the indications are that its shock effects arose during Earth impact. Cranbourne and Magura are showers, not associated with known craters, and are badly corroded. Magura has been reported to contain diamond4.

In the absence of ablative heat effects it is difficult to say whether the shock effects in Cranbourne and Magura are pre-terrestrial or not, but the Allan Hills A77283 observations now open up the possibility that they may be.

Thus, the disruption of the IAB parent body seems to have been effected with major damage (ε) to only two or three of the resulting fragments. By contrast, most of the 130 or so members of the IIIAB group show ε structures or shock heating effects.

It is therefore easy to accept a collision process for the disruption of the IIIAB parent body but the marked absence of shock polymorphism in the IAB irons is puzzling. One possibility is that the distribution of non-metal phases (silicates, sulphides) was different in the two parent bodies and allowed damage to be more concentrated in the non-metal portion of the IAB parent. The new evidence on A77283 and the possible pre-terrestrial character of shock effects in Cranbourne and/or Magura now indicate that collision was involved in the disruption of the IAB parent body. The nature of the assumed non-metal portion of the IAB parent which bore the brunt of collision damage remains unresolved but the position of the shocked diamondiferous ureilites might be reconsidered in this context.

H. J. AXON

Metallurgy Department, University/UMIST, Manchester M1 7HS, UK

1. Clarke, R. S. Jr. Appleman, D. E. & Ross, D. R. Nature 291.

- 2. Wood, J. A. in Asteroids (ed. Gehrels, T.) 849-891 (University of Arizona Press, 1979).

 3. Goldstein, J. I. & Axon, H. J. Naturwissenschaften 60,
- 313-321 (1973).
- 4. Buchwald, V. F. Handbook of Iron Meteorites (University of California Press, 1976).

Fish versus zooplankton predation in lakes

FISH predation has traditionally been viewed as one of the most important factors regulating zooplankton community structure in freshwater lakes 1,2 Recently Lane³, approaching the problem from a different point of view, has conducted comparative studies of vertebrate and invertebrate predation in a temperature freshwater lake numerous species of zooplanktivorous fish and concluded that piscine predators "probably have little effect on the myriad of interactions among most zooplankton species". Lane's comparative approach for studying predation in aquatic systems is commendable. However, we question her conclusion that fish were unimportant in affecting the zooplankton community which she studied because the following methodological problems of her work would result in a gross underestimate of the relative impact of fishes.

(1) Fish biomasses were poorly estimated. Estimates of absolute densities of fish cannot be made with gill nets unless ancillary studies are conducted (that is, mark and recapture). Additionally, gill nets are not available in mesh sizes which will catch larval and small juvenile fish. Consequently, Lane's estimates of fish density ignored juveniles of the 70 fish species in the lake. Most juvenile fish are planktivorous4, and although small, are more abundant than large fish; their relative consumption rates are also greater⁵. Therefore, they may significantly affect total piscine predation rates. Only predation by one fish, Osmerus mordax, was considered in her study. Consumption by other species was ignored because they were seldom caught in gill nets or were considered "inshore species". However, gill nets are highly selective for some species⁶, and absence from these nets does not indicate absence from a system. Inshore species may also have access to pelagic plankton by undertaking diel migrations to the pelagic zone^{7,8} or by locating plankton concentrated near the littoral by currents9. We have observed both types of behaviour in a zooplanktivorous fish, Menidia audens (W.W. and H.L., unpublished results).

(2) Prey consumption rates by fish were severely underestimated. Samples to estimate gut fullness and evacuation rates were taken at only three periods during 24th cycles: significantly, none was taken during daylight periods. Consequently, feeding peaks may well have been missed. For example, our work with Menidia indicates that dawn and dusk feeding peaks would be largely missed with Lane's sampling regime and would result in an underestimate of consumption rates by at least 100%. Lane's data suggest that at least some feeding by Osmerus occurs during daylight, as on one of three sample dates, fish were 60% full at sunset. She also assumed a linear instead of an exponential model of gut evacuation and that feeding and gut evacuation are mutually exclusive events. These assumptions, coupled with infrequent sampling, can also lead to severe underestimates of consumption rates10

(3) Additionally, there are apparent difficulties in estimating invertebrate predation rates. Lane's previously published estimates of zooplankton predation rates 11,12 differ by more than one order of magnitude. Such differences could lead to considerable errors in interpreting the relative importance of vertebrate and invertebrate predation. We also question the statement that "Cyclops . . . perhaps selects prey more for their availability than for any other factor",3 because prey morphology, size and behaviour contribute significantly to prey selection 13-15.

Lane's work has shown that invertebrate predators must exert an important evolutionary pressure on zooplankton community structure. Additionally, she has emphasized that the effects of fish on he often the zooplankton may indirect 14,16. However, given methodological difficulties listed above, we are unconvinced that fish have little effect, whether direct or indirect, on zooplankton communities.

WAYNE WURTSBAUGH

Department of Wildlife and Fisheries, University of California, Davis, California 95616, USA

> HIRAM LI JUDITH LI

Oregon Cooperative Fishery Research Unit. Department of Fisheries and Wildlife. Oregon State University, Corvallis, Oregon 97331, USA

- 1. Hall, D. J., Threlkeld, S. T., Burns, C. W. & Crowley, P. H. A. Rev. Ecol. Syst. 7, 177-208 (1976).

 O'Brien, W. J. Am. Scient. 67, 572-581 (1979).

 Lane, P. A. Nature 280, 391-393 (1979).
- Nikolsky, G. V. The Ecology of Fishes (Academic, New York, 1963).
- 5. Brett, J. R. in Fish Physiology Vol. 8 (eds Hoar, W. S. et al.) 599-667 (Academic, New York, 1979).

- 6. Lagler, K. F. in Fish Production in Fresh Waters (ed. Bagnal, T.) 7-47 (Blackwell, New York, 1978)
- D. J. et al. J. Fish. Res. Bd Can. 36, 1029-1039
- Bohl, E. Oecologia 44, 368-375 (1980)
- George, D. D. & Edwards, R. W. J. appl. Ecol. 13, 667-
- 10. Elliott, J. M. & Persson, L. J. Anim. Evol. 47, 977-991
- 11. Lane, P. A., Klug, M. J., & Loudon, L. Trans. Am. Microsc. Soc. 95, 143-155 (1976).
- Lane, P. A. Verh. int. Verein. theor. angew. Limnol. 20, 480-485 (1978).
- 13. Li, J. L. & Li, H. W. Limnol. Oceanegr. 24, 613-626
- Kerfoot, W. C. Limnal. Oceanogr. 22, 346–325 (1977).
 Gilbert, J. J. & Williamson, E. E. Occologia 37, 13–22
- 16. Northcote, T. G., Walters, C. J. & Hume, J. M. B. Verh, int. Verein, theor. angew. Limnol. 20, 2003-2012 (1978).

LANE REPLIES—Wurtsbaugh et al. state that I concluded fish to be unimportant with regard to zooplankton community structure¹. I did not draw this conclusion. They confuse two questions: (1) Which predators, vertebrate or invertebrate. exert the most predation pressure on lake zooplankton?, and (2) what is the significance (importance) of these predation pressures? My paper was concerned with providing a quantitative answer for question (1). With regard to question (2) I was careful not to make value judgements. I stated that vertebrate predators often "dramatic effects" have on lake zooplankton. I shall reply to the three methodological criticisms of Wurtsbaugh et al., then clarify the problem of significance (silver bullet obsession).

(1) Fish density: my report gave only a small part of a 4-yr collection effort of the Gull Lake fish community. In addition to gill nets, mark and recapture, a high-speed sampler, a purse seine, sonar, dip nets and hand lines were used for field sampling, and experimental cages were used for predation studies on immature fish. We did not have access to a midwater trawl which has been successfully used in the Great Lakes². Our laboratory studies showed that clipped smelt were highly susceptible to fungus infections and exhibited high mortalities. Others have found summer marking to be impractical³. Consequently, we abandoned this method of estimating fish populations. As Wurtsbaugh et al. point out, most types of fishing gear are selective4; however, sonar traces gave reasonable agreement with gill-net results.

They are correct in stating that juvenile fish "may affect significantly total piscine predation rates" for some environments and there is undoubtedly some error there. Their argument for designating juvenile fish as important predators was based on the reasoning that they are more numerous and have greater relative consumption rates than adults. When I used a similar argument in comparing vertebrate with invertebrate predators, Wurtsbaugh et al. failed to acknowledge it. The reasoning is correct for both arguments, if smaller predators are more numerous. The combined sampling

methods failed to reveal large numbers of immature fish occupying the central station. References to littoral Menidia audens, the Mississippi silverside, which inhabits shallow, warm Clear Lake, California, are irrelevant to the Gull Lake situation for almost all comparable criteria⁵⁻¹⁵. My study¹ was restricted to the pelagic zone.

Fish consumption rates: all diurnal sampling was done with four periods as Table 1 of my paper clearly shows. Use of sonar to check the absence of fish during the day and their diurnal movements were mentioned in Fig. 1 legend. In a subsequent study, smelt were collected on transects from the central station to shore. Data on their stomach contents provided no evidence of inshore feeding during the day or of a dawn feeding peak. Wurtsbaugh et al. make two useful points about linear compared with exponential models of gut evacuation and the assumption of independence of feeding and gut evacuation. Usually gut evacuation time and feeding periodicity are determined experimentally. The smelt did not cooperate in our laboratory studies thus I kept the model as simple as possible.

Invertebrate predation rates: these do differ by an order of magnitude over the large range of conditions in my experiments. Feeding rates for filter-feeding zooplankton are also as variable; density relationships are even more variable. Environmental heterogeneity and feeding periodicity also contribute to rate variations. I stated in Fig. 1 legend1 that many other invertebrate predators consume the prey species; this would underestimate the degree of invertebrate predation extant in Gull Lake^{16,17}

(2) Significance: I concluded that smelt account for $\sim 5\%$ of the total predation on Gull Lake zooplankton¹. This does not mean that invertebrate predators are 20 times more important than vertebrate predators. Important to whom?-A particular population? The daphnids? The community? Important in what way?-For calculating nutrient and energy budgets? For determining community structure and trophic networks? For quantifying stability?

In aquatic ecology there is an obsession with the silver bullet—the belief that a single factor can be found to explain ecosystem dynamics. For example, the limiting factor in eutrophication studies¹⁸,

the arguments on density-dependent versus independent population regulation, the controversy regarding whether competition or predation regulates the intertidal zone and now the pelagic zone. and the suggestions that aquatic ecosystems should be organized around the axis of body size. Some of these are parameter inputs. They do not control the ecosystem but rather set particular variables in motion. Variables included within a network possess similar rates whereas parameters are faster or slower. Wurtsbaugh et al. believe that fish are a variable—this may be true for littoral species in particular. In testing the Gull Lake data set with loop analysis, it seems that smelt act as a parameter input to Chaoborus spp. and large Daphnia pulex. Inclusion of smelt as a variable reduced the agreement of model predictions with the data. In fact, smelt are not even the predominant input-those enter at nutrient and algal levels. This is why I stated that "smelt probably have little effect on the myriad of

species". It is not possible to take one predation link out of a whole ecological network and declare that this controls the system or is the most important part of the dynamics¹⁸. The effect of any given parameter or variable on another variable is a consequence of the configuration of the whole network. In addition, indirect pathways often swamp direct ones. Smelt have reduced Chaoborus spp. populations, which in turn have eased the predation pressure on many small prey species. Thus, ignoring indirect pathways could cause substantial overestimates of smelt predation. Similar results have been noted elsewhere 19. This positive effect on small species demonstrates that ecologists should not hasten to load their ecological pistols with silver bullets for they will undoubtedly be caught in the cross-fire.

interactions among most zooplankton

PATRICIA A. LANE

Department of Population Sciences, Harvard School of Public Health, Harvard University. Boston, Massachusetts 02115, USA and Department of Biology, Dalhousie University,

Halifax, Nova Scotia,

Canada

- 1. Lane, P. A. Nature 280, 391-393 (1979).
- MacCallum, W. R. & Regier, H. A. J. Fish. Res. Bd Can. 27, 1823–1846 (1970).
- O'Connor, J. F. & Power, G. Naturaliste can. 101, 755-762 (1974).
- Hamley, J. M. J. Fish. Res. Bd Can. 32, 1943–1969 (1975).
 Cook, S. F. & Moore, R. L. Trans. Am. Fish. Soc. 99, 70-73 (1970)
- 6. Moyle, P. B., Fisher, E. W. & Li, H. W. Calif. Fish Game 60, 144-149 (1974).
- 7. Hubbs, C., Sharp, H. B. & Schneider, J. F. Trans. Am. Fish. Soc. 100, 603-610 (1971).
- Mense, J. B. Bull. Okla. Fish Res. Lab. 6, iii-32 (1967). Elston, R. & Bachen, B. Trans. Am. Fish. Soc. 105, 84-88
- 10. Burbridge, R. Trans. Am. Fish. Soc. 98, 631-640 (1969).

- 11. McKenzie, R. A. J. Fish. Res. Bd Can. 15, 1313-1327
- Ferguson, R. G. Great Lakes Res. Div. 13, 47-60 (1965). 13. Li, H. W., Moyle, P. B. & Garrett, R. L. Trans. Am. Fish. Soc. 105, 404-408 (1976).
- 14. Goldman, C. R. & Wetzel, R. G. Ecology 44, 283-294
- 15 Moss R Freshwater Biol. 1, 309-320 (1972).
- Lane, P. A. Verh. int. Verein. theor. angew. Limnol. 20, 480–485 (1979).
- Lane, P. A., Klug, M. J. & Louden, L. Trans. Am. Microsc. Soc. 95, 143-155 (1976).
- Lane, P. A. & Levins, R. Limnol. Oceanogr. 22, 454-471 (1977).
- Northcote, T. G., Walter, C. J. & Hume, J. M. B. Verh. int. Verein. theor. angew. Limnol. 20, 2003-2012 (1978).

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BOOK REVIEWS

Mechanisms of scientific revolution

G. N. Cantor

HISTORIANS of science have for a long time been interested in periods of major, rapid and partially discontinuous change in science. The most radical of these changes dates from, roughly, the first half of the seventeenth century and is generally known as the scientific revolution. Other more parochial revolutions, such as the Darwinian revolution, have also attracted much attention. In physics two main periods perhaps deserve the appellation 'revolution'; one associated with Fresnel's work on light, Carnot's and Fourier's on heat and other developments in the late eighteenth and early nineteenth centuries, and the other related to the rise of quantum and relativity theories a century later. Whether we decide to characterize either of these as a "revolution" depends on how we define the term in respect to changes in many facets of science.

Enrico Bellone's A World on Paper: Studies on the Second Scientific Revolution is concerned, as the sub-title suggests, with substantiating the claim that a revolution occurred in physics in the late eighteenth and early nineteenth centuries. The author claims that during this period physics became more mathematical, theoretical and abstracted from experience; physicists thus constructing "a world on paper". This book, first published in Italian in 1976, now appears in English translation. Stillman Drake, an authority on Galileo, has provided a somewhat eulogistic foreword. Bellone's book encompasses an important topic for current historical research, since despite the considerable amount of respectable secondary literature on this period there is comparatively little of a synthesizing nature. Unfortunately, this study is disappointing and fails to achieve its objective.

Problems arise partly because Bellone, a professor of the history of science at the University of Genoa, is anxious to demolish what he sees as two pernicious and pervasive doctrines. First, he intends to refute the view, which he appears to attribute indiscriminately to philosophers and most historians of science, that from the seventeenth to the end of the nineteenth century physics was dominated by the doctrine of mechanism. Not only does he fail to explain what he understands by this term but he also does not name his opponents, with the exception of Pierre Duhem. Yet Duhem's aim was to contrast the British tendency to construct mechanical models of physical phenomena

A World on Paper: Studies on the Second Scientific Revolution. By Enrico Bellone. Pp.220. ISBN 0-262-02147-1. (MIT Press: 1980.) \$20.25, £10.50.

with the continental methods of mathematical abstraction. The Aim and Structure of Physical Theory was published 75 years ago and no respectable historian or philosopher of science in recent years has championed any simplistic thesis concerning the total dominance of mechanism during the nineteenth century. We can conclude that Bellone has set up a straw man as his major opponent.

The second of Bellone's opponents is less of a straw man than an inappropriate one. Apparently with Kuhn, Popper, Feverabend and Lakatos in mind, he attacks those philosophers who have proposed general theories of scientific development. However, in order to controvert, for example, Kuhn's theory it is necessary to show that either it is intrinsically incoherent or it does not accord with the actual development of science. Bellone attempts a rather inadequate variant of the latter strategy by arguing in five casestudies that physicists have articulated their own views about scientific method and have not been indebted to philosophers of science, least of all to those who espoused mechanism.

By far the longest study is an attempt to show that, contrary to Duhem's characterization, Kelvin was not a mechanist. To substantiate this claim the author discusses Kelvin's work in such areas as energy conservation and the vortex atom hypothesis. However, he seems prepared to concede that Kelvin deployed mechanistic hypotheses and analogies provided they were adequately grounded on known physical laws and expressed in mathematical terms. More positively he interprets Kelvin as being centrally concerned with inductively discovered physical laws and prepared to employ mathematics only as a useful tool. These attitudes are traced to the views of John Herschel and Thomas Reid. Kelvin's empiricist philosophy is contrasted, in the second study, with that of Boltzmann who propounded a mathematical and highly abstract form of physical theorizing which set him against Mach's sensationalism and Ostwald's theory of energetics, and also led to his work on probability calculus. Of particular importance to Bellone is Boltzmann's partial rejection of mechanistic explanations.

The third study is largely concerned with Ampère who is interpreted as having rejected action-at-a-distance forces - as championed by Newton and Laplace and adopted a contact-action ether theory of electromagnetism. There follows an essay contrasting the fluid theory of electricity held by the Abbé Nollet in the 1740s with the mathematical instrumentalism propounded by Cavendish a quarter of a century later. Finally we return to the Kelvin-Boltzmann dispute over the respective roles of mathematics and experience and to Kelvin's recognition in 1900 that physics was facing two major problems: first the problem of reconciling ether theory with the Michelson-Morley experiment and, second, the inadequacy of the Maxwell-Boltzmann theorem for the equipartition of energy. These difficulties perceived at the end of the nineteenth century are interpreted by Bellone as indicating continuity with twentiethcentury physics; thus, in his opinion, no scientific revolution occurred early this century.

I hope that I have accurately conveyed the author's major claims since I found this a surprisingly difficult, even opaque, book. While admiring Bellone's courage in attempting to cover a major historical topic, I encountered several difficulties with his thesis. Some problems stem from disagreements with his historical claims. To give two examples: there is strong evidence, some of which is cited in this book, that Ampère did not subscribe to a contact-action ether, and Kelvin's philosophy cannot be simply traced back to Reid's - indeed they differed radically over the question of hypotheses and analogies. Other difficulties arise because although the book contains summaries of various writers' highly interesting views on mathematics, experimentation, theory and mechanics, the presentation of these issues lacks coherence and is weak in analysis, so that the reader is frequently assailed by: ambiguous statements and points which need both clarification and evidential support.

Yet more problems stem from the historiography which is based on what the author calls the "scientist's dictionary" (considered by Professor Drake to be Bellone's major contribution to the study of history). The dictionary of a scientist contains, it appears, the set of his currently adopted ideas. If I have interpreted Bellone correctly, then his theory of dictionaries is unhelpful if not positively misleading.

There are many significant dissimilarities between a mind and a dictionary: a dictionary, unlike a mind, contains only definitions of words, is open to public scrutiny and so on. How are we to construct a scientist's (private) dictionary from his public statements when the latter are often shaped for the audience for which they are intended? Given these and similar queries it is unfortunate that Bellone did not discuss this theory in much greater detail.

Bellone's notion of the scientist's dictionary has a further consequence for his historical claim that a revolution occurred in physics in the late eighteenth and early nineteenth centuries. Revolutions have, as Kuhn insisted, a social component which he expressed in terms of paradigm change being a change in consensus among the members of a scientific community. Even if one does not agree with the details of Kuhn's thesis it would appear difficult for the historian interested in large-scale changes in science to omit this social dimension. Yet this is precisely what Bellone does. According to

his theory, each scientist maintains his own dictionary and yet Bellone offers the historian no means of moving from individual dictionaries to an understanding of broader historical processes. Indeed, his failure to appreciate the latter leaves the reader unclear as to what constituted the "second scientific revolution".

Unfortunately, the author conveys the impression that he is divorced from the main currents of historical scholarship. An examination of the book's endnotes reveals surprisingly few references to the major secondary works on the subjects he discusses. His indiscriminate use of terms such as deduction, crucial experiment. common sense, scientific revolution, mechanism and Newtonianism indicates that he is not aware of the specific meaning conventionally attached to them. Finally, it is surely only ignorance of recent scholarship that could have led the author to construct his book as an attack on Duhem's outmoded thesis.

G. N. Cantor is a Lecturer in the Department of Philosophy at the University of Leeds.

Across the spectrum of IR astronomy

R.F. Carswell

Infrared Astronomy. International Astronomical Union Symposium No. 96. Edited by C.G. Wynn-Williams and D.P. Cruikshank. Pp.376. ISBN hbk 90-277-1277-1; ISBN pbk 90-277-1228-X. (Reidel: 1981.) Hbk Dfl.90, \$47.50; pbk Dfl.45, \$23.50.

FEW branches of astronomy have made such rapid progress in the past few years as the infrared. With the continuing development of modern detector systems, and in particular the availability of four telescopes high on Mauna Kea in Hawaii above most of the atmospheric absorption, there is every expectation that this will continue. It was therefore appropriate that a symposium on infrared astronomy should be held in Hawaii last year, drawing together contributions from many of the most active people in the field.

The papers included in the proceedings reflect the diversity of topics to which infrared astronomy is making its significant, and often unique, contribution. Articles on planetary studies, protostellar objects, molecular clouds, dust, nebular emission lines, stars in external galaxies, and active galactic nuclei show just how wide the range is. The often dull (to an outside astronomer) technical papers describing detector systems were deliberately excluded from the symposium, and the editors chose to include only reviews on each topic rather than all the inevitable specialist results normally heard at such symposia. The result is a volume which summarizes the present state of the subject well and gives an idea of the rapid advances being made without the nonspecialist reader having to wade through a mass of detail.

To choose a few at random, the three descriptions by Evans, Hyland and Thompson highlight the value of infrared studies of the highly obscured regions where star formation is occurring, and provide useful summaries for the relatively ignorant such as myself. The review of the reviews on this topic by Zuckerman helps to give the overall picture. In an area where I have more expertise, Soifer and Neugebauer give us a comprehensive survey of the properties of active galactic nuclei and quasars.

The final summary by Longair states succinctly what emerges from the proceedings in general. Infrared astronomy is proving to be useful in probing an increasingly diverse range of astrophysical questions and, with future developments, such as grating spectrometers, area detectors and infrared astronomy satellites, the trend towards "in depth" studies of particular classes of objects at all wavelengths is likely to continue. Thus, a book on infrared astronomy is likely to give way to more specialist collections. In the meantime, this volume provides a useful, up-to-date review of the entire field.

R.F. Carswell is at the Institute of Astronomy, Cambridge.

Hot history

P.T. Landsberg

The Tragicomical History of Thermodynamics 1822–1854. Studies in the History of Mathematics and Physical Sciences, 4. By C. Truesdell. Pp.372. ISBN 3-540-90403-4. (Springer-Verlag: 1981.) DM99, \$58.50.

WHAT is the meaning of the following result: "If the total heat of a homogeneous and uniformly hot substance be conceived to be divided into any number of equal parts, the effects of those parts in causing work to be performed are equal"? If this should not be quite clear, the following may help: "If the absolute temperature of any uniformly hot substance be divided into any number of equal parts, the effect of those parts in causing work to be performed are equal". These are quotes from William John Macquorn Rankine (1820-1872), a Scottish mining engineer, writer on thermodynamics, inventor of the word "adiabatic". They are his version of the second law of thermodynamics as given in his Manual of the Steam Engine which saw many editions, starting in 1859. Little wonder, then, that James Clerk Maxwell felt in 1878 that anyone who actually understood these remarks could explain thermodynamically what Tennyson had said of the great Duke:

whose eighty winters froze with one rebuke All great self-seekers trampling on the right.

If oscillations of this order in the clarity of conceptions concerning thermodynamics could take place *after* the period covered in Truesdell's book, it is perhaps not surprising that the period itself also furnishes many challenges to those who come in today to tidy up the errors of the founding fathers of the subject. This is precisely what Truesdell seeks to do (the above quotations will not be found in this book).

Where Maxwell proceeded with delicacy and humour, Truesdell is hard-hitting, hoping to raise in this way the subject from the "Dismal Swamp of Obscurity that from the first it was and that to-day in common instruction it is" (p.6). Leaving an assessment of this provocative remark to the many students and teachers of the subject, the book deals with Truesdell's rediscovery (following J. Moutier) of F. Reech (1805-1884) and has chapters or sections on Carnot, Helmholtz (The Conservation of Force being described as his weakest paper), Fourier, Clausius, Rankine, Kelvin and many others, mathematical aspects being given prominence.

The teaching of the subject can conveniently start with the introduction of specific and latent heat functions C and l defined in a usual notation by:

 $dQ = C_v dT + l_v dV = C_v dT + l_v dp.$

To these relations a third relation may be

added, which is not independent but yields a pleasant symmetry:

$$dQ = m_v dV + m_n dp.$$

This procedure has been used in a number of text books, including my own. Truesdell relates it to what he calls the "Doctrine of Latent and Specific Heats".

The book makes interesting reading and in its emphasis on the history of mathematical formulations of thermodynamics it breaks new ground. It suffers by the awkwardness of the chapter arrangements, cross references and notation, which makes it hard to read, and by the very limited references to the writings of contemporary historians of science, which sets it apart from current historical research. Not even Clausius' 1872 paper on the history of the mechanical theory of heat is cited as a source. I conclude with quotations illuminating the harsh ethos of

Of Laplace: As a physicist he preferred contorted structural hypothesis, as a mathematician he was unusually loose, even for his day [p.41].

Of Fourier: I doubt if any critical reader today would find in his book much insight into nature beyond that common in works of his predecessors and contemporaries [p.76].

"This unhappy quality is the tough and tortuous thread of the plot of the tragicomedy. It began with Laplace and was spun out by Fourier" (p.77).

P.T. Landsberg is Professor of Mathematics at the University of Southampton.

Will plant biochemistry go to the ball?

Philip Rubery

The Biochemistry of Plants: A Comprehensive Treatise. Editors-in-chief P.K. Stumpf and E.E. Conn. Vol.1 The Plant Cell (edited by N.E. Tolbert), pp.699, ISBN 0-12-675401-2. Vol.2 Metabolism and Respiration (edited by David D. Davies), pp.687, ISBN 0-12-675402-0. Vol.3 Carbohydrates: Structure and Function (edited by Jack Preiss), pp.638, ISBN 0-12-675403-9. (Academic: 1980.) \$65, £43 per volume.

A RECENT issue of Trends in Biochemical Sciences carried the headline "Plant biochemistry - a Cinderella too long". Does the publication of a comprehensive treatise on the biochemistry of plants herald the arrival of this poor relation in good society? As the editors comment in their general preface, the study of plants has had a mixed reception in the biochemical community. Thus the treatise aims not only to instruct but also to inspire - indeed, evangelize.

The first volume is largely self-contained and is organized into chapters centring on particular organelles and cellular membrane components which collectively cover many of the topics dealt with under the more system-based approach of Vols 2 and 3. Indeed, from reading all three volumes one theme that constantly recurs is the unity of plant cells and the interdepen-

Volumes 4 (Lipids: Structure and Function), 5 (Amino Acids and Derivatives) and 6 (Proteins and Nucleic Acids) in this series have now appeared. The last two volumes making up the Treatise (Vol.7 Secondary Plant Products and Vol.8 Photosynthesis) are scheduled for publication shortly. The completed series will be reviewed in Nature at a later date.

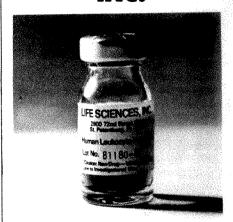
dence of organelles. For example, an overall account of the current status of carbon flow and compartmentalization in photorespiration and gluconeogenesis from fat can be synthesized from articles in Vol.1 on microbodies, on mitochondria and on chloroplasts; in Vol.2 on control of carbohydrate oxidation and on photorespiration; and in Vol.3 on integration of carbohydrate metabolism. The glyoxylate cycle is also covered in Vol.4 and a further chapter on photorespiration is promised for Vol.8.

One of the primary goals of a treatise such as this is to provide authoritative accounts of major areas which embody a large corpus of research. Many of the chapters fulfil this aim well - those on sugar nucleotide transformations (Vol.3), polysaccharide solution conformation (Vol.3), development and evolution of plastids (Vol. 1) and mitochondrial electron transport and energy coupling (Vol.2) are particularly good. The chapter on primary cell walls (Vol.1) includes a welcome critical review of the evidence for covalent linkages between macromolecules having a key role in wall structure.

A comprehensive account of plant biochemistry has other important tasks to perform. For instance, the applicability to plants of research strategies evolved in other contexts should be assessed and publicized. This is epitomized by ap Rees (Vol.3) in his consideration of the theoretical and experimental approaches to the regulation of hexose phosphate metabolism along the lines influentially described by Newsholme and Start in Regulation in Metabolism (Wiley, 1973). Turner and Turner cover similar ground in Vol.2, where Wiskich also deals rather more phenomenologically with the control of the Krebs cycle.

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demonstrably ignorant compared to their animal colleagues (the Cinderella effect?) are frequently pointed out and many chapters suggest where plant horizons could be broadened or at least examined more closely. According to Storey (Vol.2), none of the mitochondrial redox flavoproteins has been characterized as an isolated enzyme. The contrast with the wealth of spectroscopic data available for plant mitochondria exemplifies the comparatively patchy sophistication of plant biochemistry. Similarly, the important enzyme thymidylate synthase, discussed by Cossins in Vol.2, has not been characterized from plant tissue. On a wider scale. the biochemistry of many basic physiological problems remains obscure, especially when they lie in the interstices of traditional major domains. For example, we have little detailed understanding of the differences in metabolite traffic, involved in energy supply and carbon skeleton provision, between chloroplasts, cytoplasm, mitochondria and peroxisomes in leaf cells in the light and the dark. Graham's chapter (Vol.2) on the effects of light on "dark" respiration addresses some of these questions. The excellent article on translocation of sucrose and oligosaccharides, by Giaquinta in Vol.3, combines a physiological and biochemical approach to highlight key problems such as the mechanism of sucrose unloading from phloem whose further elucidation could have agronomic and economic implications. It is important that plant biochemistry should, like mammalian biochemistry, be increasingly concerned with applying the burgeoning range of new techniques to essentially cellular problems.

As to omissions, I would have liked some treatment of the cytoskeleton in plants and also perhaps a chapter explicitly devoted to transport across the membranes of organelles. The virtual exclusion of growth substances from the consideration of cell wall dynamics and of seed reserve mobilization is also regrettable, although admittedly phytohormones are not within the plan of the treatise. Nevertheless, most topics that one would hope to find adumbrated by the volume titles are covered although the "metabolic" content of Vol.2 is largely carbohydrate; lipids, amino acids, proteins and nucleic acids, and secondary products being dealt with in Vols 4,5,6 and 7 respectively. Much of the contents of these three volumes and of the treatise as a whole are likely to remain valuable sources of reference and perspective for the rest of the decade. They are not expensive in today's terms for what they provide and the semi-autonomous nature of the volumes should enable a variety of permutations to serve the interests of different groups and individuals. The treatise is timely and, like Cinderella, the subject is of some promise.

Philip Rubery is a Lecturer in Biochemistry at the University of Cambridge

Muscle: from hypertension to erection

Andrew P. Somlyo

Smooth Muscle: An Assessment of Current Knowledge. Edited by E. Bülbring et al. Pp.576. ISBN 0-7131-4348-7/0-292-77569-5. (Edward Arnold/Texas University Press: 1981.) £45, \$95.

SMOOTH muscle cells in blood vessels. bladder, uterus, gastrointestinal tract and airways support and regulate, directly or indirectly, nearly all bodily functions. Nevertheless, physiologists, until recently, have shied away from studying these cells because of their small size, relatively poor ultrastructural organization and great individuality. Our knowledge of the cell physiology of smooth muscle, with a few notable exceptions, is largely due to studies conducted within the past 15 years and, thus, a timely subject for the stated aim of this book - "an assessment of current knowledge". The book is a "family affair"; the editors and contributors are Professor Bülbring of Oxford and her former pupils who, individually and in collaboration, have contributed vigorously to this field. Nevertheless, the volume is not parochial; geographically, the authors range from Oxford to continental Europe, including Russia, and through the United States to Australia and Japan. Intellectually, the contents extend to cover work from laboratories outside "the family" and successfully reconcile previously opposing views, such as the normal occurrence of graded changes in membrane potential in tonic smooth muscles and the existence of smooth muscle regulation by mechanisms independent of the membrane potential (pharmacomechanical coupling).

The major emphasis is on electrophysiology and ion transport, areas of research in which Professor Bülbring and her pupils have been most active. Of what is known about these subjects, the contributions by Brading, Casteels, Jones, Kuriyama and Tomita leave little uncovered. Jones, perhaps having planned successfully his chapter as the basis of a graduate course on smooth muscle, presents a particularly broad view, covering not only relevant aspects of the ultrastructure, electrophysiology and the ionic permeability of normal smooth muscle, but also the membrane properties of vascular smooth muscle altered by high blood pressure. Voltage clamping of smooth muscle is assessed with admirable caution by Bolton, Tomita and Vassort. The application of this technique to tissues having such complex geometries as smooth muscle is a terrain where most researchers fear to tread, and, therefore, the identification of the ionic currents responsible for action potentials in smooth muscles has been ambiguous. The task of identifying such currents is not made easier by the large variations among different smooth muscles in which the contributions of, respectively, sodium and calcium to

the early current are quite variable. Burnstock's contribution on smooth muscle development and innervation and Gabella's extensive discussion of the ultrastructure of smooth muscle successfully relate structure to function. Gabella, incidentally, also reflects the consensus of experienced electron microscopists that, in both relaxed and contracted smooth muscle, myosin is organized in thick filaments.

Given the highly individual properties of smooth muscle cells in different organs and the variety of their functions, the subjects to be covered are limited only by space and the authors' interests. These range from the aforementioned studies of vascular smooth muscle altered by hypertension to the role of penile blood vessels in erection. It may come as a surprise to some that erection is due to the relaxation of smooth muscle of the penile arteries, the flaccid state being the result of vasoconstriction.

The flaws of the book are minor, although the mistaken identification of one of the editors, a Professor of some years standing, as an Assistant Professor is certainly an editorial "blooper". There is considerable overlap between the various chapters covering electrophysiology and ion distribution, perhaps unavoidable in a multi-author volume that is the product of a single scientific "school", and also forgiveable in a book that boasts several thousand references dealing with this subject. It is not a textbook, since some important aspects of smooth muscle, including biochemistry, muscle mechanics and energetics did not fall within this group's research interests and are, therefore, discussed little or not at all. In this sense, this book and the recently published volume in the Handbook of Physiology series (Vascular Smooth Muscle; American Physiological Society, 1981), of which, I should confess, I am an editor, complement each other well; together they provide an encyclopaedic survey of the field.

Smooth Muscle: An Assessment of Current Knowledge brings us up to date with the use of electron probe analysis and ion selective electrodes, the latter in the laboratory of one of the editors, to verify the unusually high cytoplasmic concentration and activity of chloride in smooth muscle. Perhaps some of the more speculative aspects of this book, such as the postulated binding of calcium to the inner surface of smooth muscle plasma membrane, will eventually also be tested by direct methods.

The book is recommended to those with an interest in smooth muscle and to those wishing to learn about a cell system admirable in its infinite variety.

Andrew P. Somlyo is Professor of Physiology and Pathology and Director of the Pennsylvania Muscle Institute, University of Pennsylvania.

PRODUCT REVIEW

Alkalinity analyser

THE pump-colorimeter total alkalinity analyser is an addition to the Hach range of colorimetric instruments. Alkalinity is measured by colorimetric pH determination and three ranges are available: 0–50 mg 1^{-1} (as CaCO₃); 0–100 mg 1^{-1} ; and 0–500 mg 1^{-1} .

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Carbon rod atomizer

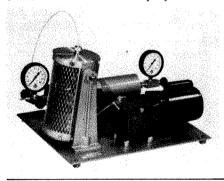
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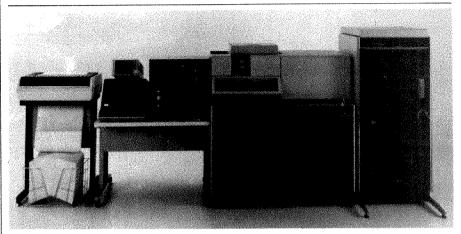
Bath circulators

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- PRODUCT REVIEW — AMERICAN CHEMICAL SOCIETY

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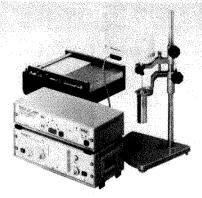
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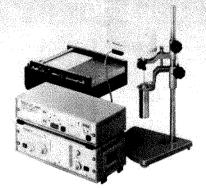
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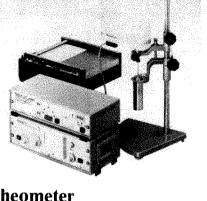
Conductivity controller

NEW literature from Beckman Instruments describes a single range conductivity controller for water purity applications requiring continuous digital conductivity indication. It has on/off control and an output for proportional control or remote recording.



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Acid digestion bombs

Teflon-lined, stainless steel bombs for dissolving or digesting analytical samples in strong acids or alkalis are described in a new publication from the Parr Instrument Co. Five models (23-125 ml) are offered for treating inorganic or organic samples with HF, HC1, H₂SO₄, or other strong acids or alkalis, at temperatures well above normal boiling points, for rapid dissolution and complete sample recovery without loss of trace elements and with no added contaminants from the container itself. Built-in safety rupture disks protect against unexpected explosion.

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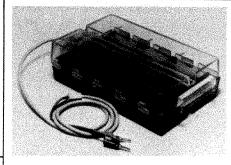
Post-column HPLC

THE URS 050 post-column reaction system has been designed by Kratos for derivatization procedures requiring the addition of a single reagent at room temperature (such as o-phthalaldehyde). The URS 050 performs all the necessary reaction steps: reagent addition, reagent/sample mixing, and reaction delay (to allow sufficient time for the derivatizing reaction to occur). Circle No. 107 on Reader Enquiry Card.

Thermocouple heads

Omega Engineering has developed new miniature aluminium heads for its line of thermocouple assemblies. Both corrosion and weather-resistant, they are designed to provide maximum protection for the electrical terminations of the thermocouple and connection to associated instruments.

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Electrophoresis chamber

THE new Semi-Micro II electrophoresis chamber from Gelman Sciences reduces buffer consumption to 200 ml per chamber. Three support bridges allow three membranes to be run simultaneously. The chamber works equally well with either Gelman's Sepraphore III or Super Sepraphore membrane, and is designed for use with Gelman's 8- or 4-place applicator. Circle No. 109 on Reader Enquiry Card.

Antisera

UNDILUTED antisera for RIA or nonisotopic immunoassay are now available from Miles Laboratories in two categories; undiluted antisera to hormones (for example, thyrotropic hormone) and undiluted antisera to drugs (digoxin, phenobarbital, gentamicin, tobramycin and amikacin).

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Scintillation cocktail

To meet the increasing demand for laboratory safety, Research Products International has developed Safety-Count, a new ready-to-use cocktail for liquid scintillation counting, designed for high efficiency counting of aqueous samples, salt-butter combinations and many other biological materials. Safety-Count uses a 100% aromatic hydrocarbon solvent. yielding a product with a flash point of 120°F TCC (tag closed cup). The cocktail is packaged in 4-l amber glass bottles covered with an impact-resistant plastic coating to reduce the risk of breakage. Circle No. 111 on Reader Enquiry Card.

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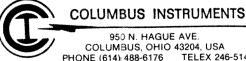
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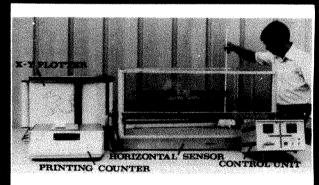


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HOCUTT, C.H. and STAUFFER, J.R. Jr. (eds). Biological Monitoring of Fish. Pp.414. ISBN 0-669-03309-X. (Lexington Books, Gower Publishing Company Ltd. Farnborough, Hampshire, England: 1981.) £20.

HÖFER, M. and HOGGETT, J.G. Transport Across Biological Membranes. Pp.184. ISBN 0-273-08480-1. (Pitman Publishing: 1981.) £15.

HOUSE, M.R. and SENIOR, J.R. (eds). The Ammonoidea. The Evolution, Classification, Mode of Life and Geological Usefulness of a Major Fossil Group. The Systemics Association Special Vol. No.18. Proceedings of an International Symposium held at the University of York. Pp.594. ISBN 0-12-356780-7. (Academic: 1981.) £36, \$86.50.

JOINER, J.N. (ed.). Foliage Plant Production. Pp.614. ISBN 0-13-322867-3. (Prentice-Hall International: 1981.) £16.20.

KILDEBERG, P. Quantative Acid-Base Physiology. System Physiology and Pathophysiology of Renal, Gastrointestinal, and Skeletal Acid-Base Metabolism. Pp.206. ISBN 87-7492-327-7. (Odense University Press: 1981.) Dan. kr. 130.

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Edn. Pp.655. ISBN 0-13-208256-X. (Prentice-Hall: 1981.) £12.95.

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LLOYD, C. Birdwatching on Estuaries, Coast and Sea. Pp.160. ISBN 0-7278-2003-6. (Severn House, London: 1981.) £7.95.

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McKINNELL, R.G. et al. (eds). Differentiation and Neoplasia. Results and Problems in Cell Differentiation. A Series of Topical Volumes in Developmental Biology, Vol.11. Pp.310. ISBN 3-540-10177-2. (Springer-Verlag: 1980.) DM129,

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RAGER, G.H. Development of the Retinotectal Projection in the Chicken. Advances in Antomy, Embryology and Cell Biology, Vol.63. Pp.92. Flexi ISBN 3-540-10121-7. (Springer-Verlag: 1980.) DM52, \$30.70.

READINGS FROM SCIENTIFIC AMERICAN. Genetics, with Introductions by Cedric I. Davern. Pp.331. Hbk ISBN 0-7167-1200-8; pbk ISBN 0-7167-1201-6. (W.H. Freeman: 1981.) Hbk \$19.95; pbk \$8.95.

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REMMERT, H. Arctic Animal Ecology. Pp.250. Flexi ISBN 3-540-10169-1. (Springer-Verlag: 1980.) DM37, \$21.90.

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SCHMITT, F.O. et al. (eds). The Organization of the Cerebral Cortex. Proceedings of a Neurosciences Research Programme Colloquium. Pp.592.ISBN 0-262-19189-X. (MIT Press: 1981.) \$50.

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WHALLEY, P. Butterfly Watching. Pp.160. ISBN 0-7278-2002-8. (Severn House, London: 1980.) £7.95.

WILSON, R. The Back Garden Wildlife Sanctuary Book. Pp.152. Flexi ISBN 0-14-046-915-X. (Penguin: 1981.) £2.95.

YOUDIM, M.B.H. et al. (eds). Essays in Neurochemistry and Neuropharmacology, Vol.5. Pp.153. ISBN 0-471-27879-3. (Wiley: 1981.) £16.50.

Applied Biological Sciences

CAVALLA, J.F. (ed.). Risk-Benefit Analysis in Drug Research. Proceedings of an International Symposium held at the University of Kent at Canterbury, England, March 1980. Pp.197. ISBN 0-85200-364-1. (M.T.P., Laneaster, England: 1981.)

COLLINS, C.H. et al. (eds). Disinfectants: Their Use and Evaluation of Effectiveness. The Society for Applied Bacteriology Technical Series, No.16. Pp.230. ISBN 0-12-181380. Academic: 1981.) £16, \$38.50.

FIECHTER, A. (ed.) Advances in Biochemical Engineering, Vol.17, Products from Various Feedstocks. Pp.172. ISBN 3-540-09955. (Springer-Verlag: 1980.)

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CONOLLY, B. Techniques in Operational Research. Vol.2, Models, Search and Randomization. Pp.338. ISBN 0-85312-240-7. (Wiley: 1981.) £21.50, \$56.75.

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BIOLOGIST/IMMUNOLOGIST

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For all these positions, a highly competitive salary is offered together with a non-contributory pension and life assurance scheme, subsidised B.U.P.A., 21 days' holiday, flexible working hours and relocation expenses where applicable.

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(9370)A

THE UNIVERSITY **OF SHEFFIELD DEPARTMENT OF PHYSICS**

Applications are invited from

men and women for an SERC POSTDOCTORAL RESEARCH ASSISTANT

to study the atomic structure of metallic alloy glasses, using neutron and X-ray diffraction techniques. The post involves joining an established group investigating both chemical and topological aspects of metallic glass structure. The neutron experiments will be made at AERE, Harwell and the ILL Grenoble. The work arises from collaboration between Dr N°Cowlam, Department of Physics, and Dr H A Davies, Department of Metallurgy and forms part of a wider programme of research on metallic glasses whose properties are currently of particular scientific and technological interest. Tenable for three years. Initial salary in the range £6,070 - £6,880 a year. Experience in neutron diffraction or in structural studies of amorphous materials an advantage.

Applications including curriculum vitae, and the names of two referees should be sent, as soon as possible to Dr N Cowlam/Dr H A Davies, c/o Department of Physics, The University, Sheffield S10 2TN, Quote Ref. R/627/G

(9345)A

A search is being conducted for an individual to fill an endowed **PROFESSORSHIP**

in the

DEPARTMENT OF TROPICAL PUBLIC HEALTH

We will consider candidates who are actively utilizing modern biological approaches to understand and combat tropical diseases, particularly those caused by parasites. Preference will be given to persons whose expertise includes the biology and biochemistry of membranes.

The person to be selected should be willing to interact with members of a multi-disciplinary department which includes molecular biologists, immunologists, cell and vector biologists, be able to establish working relationships with epidemiologists and field research workers, and be interested in teaching at the graduate and post graduate level.

Harvard University is an Equal Opportunity Employer committed to an Affirmative Action Program.

Send curriculum vitae, bibliography and three (3) references to: Dr Konrad Bloch, Chairman, Search Committee, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts, 02115.

(NW828)A

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (University of London) Keppel Street (Gower Str) London WCIE 7HT

Under a joint agreement between the Wellcome Foundation Limited and the London School of Hygiene and Tropical Medicine, a new laboratory is being formed to study the chemotherapy of protozoan diseases, particularly leishmaniasis and Chagas' Disease. The work of the laboratory will be under the direction of Dr R A Neal and located in the Department of Medical Protozoology (Professor W Peters).

A POSTDOCTORAL **FELLOW**

is now required to continue studies in progress on leishmaniasis chemothe-Applicants should have completed a PhD degree on aspects of parasitic protozao, eg biochemistry, that would be of relevance to the chemotherapy of these organisms. Experience in culture techniques would be an advantage. The appointment would be for up to three years.

The salary will be in the range £6,070 — £10,575 plus £967 London Weighting per annum.

Applications, consisting of full curriculum vitae and naming two referees, should be sent to the Senior Assistant Secretary at the School.

(9354)A

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to work in their Science and Mathematics department on all aspects of the publication process from receipt of manuscripts in-house through to publication including liaison with authors, marking up, commissioning artwork, proofreading, liaison with the departmental Production and Design Manager and generation of pub-licity material. The editor will work closely with a small team involved in the expansion of HEB's successful science and mathematics lists at primary, secondary and tertiary levels. There is considerable opportunity to become involved in the development of new projects.

The job would suit a graduate in science or mathematics with at least three years' relevant experience in publishing.

Salary will be at least £6,200, or more, commensurate with experience.

Applicants should write to Graham Taylor with full details of career to date



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Applications are invited from recent graduates in geology or an equivalent qualification.

EXPLORATION GEOLOGIST I

Applications are invited from geologists with 3-5 years experience in exploration for base metals and a strong background in geochemistry, geophysics and drill supervision.

EXPLORATION GEOLOGIST II

Applications are invited from geologists with 3-5 years experience in exploration for base metals in carbonate environments. For this position a strong background in carbonate petrology and statistics would be an asset.

SENIOR EXPLORATION GEOLOGIST

Applications are invited from geologist with 7-10 years experience in mineral exploration involving a variety of geological environments, particularly carbonates. For this position proven ability of crew supervision and conceptual thinking is essential.

Salaries and conditions of employment will be discussed at interview. Applications quoting references and including comprehensive curriculum vitae should be forwarded to: Mr Sean Egan, Administration Manager, Irish Base Metals Limited, 162 Clontarf Road, Dublin 3, Ireland. (9362)A

UNIVERSITY OF LONDON

DEPARTMENT OF BIOCHEMISTRY St. Thomas's Hospital Medical School, London, SE1 7EH

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited from Postdoctoral Biochemists to work on a project supported by the British Diabetic Association to investigate the mechanisms of sugar transport into brain synaptosome preparations. The appointment is available from 1 October 1981.

Applications, accompanied by a curriculum vitae and the names of two referees, should be sent to Professor H S Bachelard, Department of Biochemistry, St Thomas's Hospital Medical School, London SE17EH. (9365)A

READVERTISEMENT MEMORIAL UNIVERSITY OF NEWFOUNDLAND St. John's Newfoundland

St John's, Newfoundland, Canada

DEPARTMENT OF

COMPUTER SCIENCE

HEAD

Applications are invited for the position of Head, Department of Computer Science in the Faculty of Science, Memorial University of Newfoundland. Position to be filled as soon as possible.

Candidates should have demonstrated teaching and scholarly research abilities indicative of senior rank, with a fairly broad background in computer science and the capabilities of exercising academic and administrative leadership. A PhD in Computer Science or related area is required.

The Head would be responsible for developing future research programs, including the introduction of a PhD program. An MSc program is already in existence. The undergaduate program is well established and is rapidly expanding with approximately three hundred full-time majors. At present there are nine full-time faculty members with additional post to be created. In addition there are several part-time Sessional Lecturers.

Departmental Computing facilities include a VAX 11/780 currently running under UNIX Version 7 Operating System and a PDP 11/34 and 11/10 also under UNIX. A large number of terminals, graphics equipment and other facilities are also available.

Canadians or Landed Immigrants will be given first preference. Salary is negotiable.

Please send curriculum vitae and the names of three references to: Dr PJ Heald, Dean of Science, Chemistry-Physics Building, C-335, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X7, Canada. (NW823)A

FRESHWATER BIOLOGICAL ASSOCIATION BIOMETRICIAN/ STATISTICIAN

required for Windermere Laboratory to initiate research in biometry as applied to pupulation dynamics of benthos, plankton and fish, sampling freshwater organisms and chemical/physical variables and experimental studies in collaboration with biologists and chemists. Minimum of 2.1 honours degree in statistics/biometry/mathematics or science degree plus statistical training or experience. Conditions analogous to HSO/SSO in Civil Service, starting salary between £6,075 and £7,644 pa.

Full details: Secretary, FBA, The Ferry House, Ambleside, Cumbria LA22 0LP (096 62 2468). Closes 7 Sept. (9314)A

AUSTRALIA New South Wales

Department of Agriculture

Senior Livestock Research Officer

Agricultural Research Station, Trangie, N.S.W. POSITION NO AG. 81/247

Salary: A\$21,779 range A\$24.690.

Qualifications: Essential — Post-graduate degree in Animal Breeding and Genetics.

Duties: Responsible for directing and supervising the N.S.W. Department of Agriculture's Merino breeding activities. Successful applicant will have a major research programme on the inheritance of production characters in Merino sheep especially on the inheritance of wool production.

In addition will be responsible for development of breeding programmes in collaboration with Sheep and Wool Advisory Officers. Sheep breeding is part of a multi disciplinary animal production research group located at Trangie. This group has good laboratory and statistical support.

Accommodation: Single Officer accommodation or house for married applicant is available at the Research Station.

Inquiries: (Sydney (02) 217-5262 (Dr. G.E. Robards) Inspection of work location prior to interview is welcome. Applications on form 59 available from any N.S.W. Government Office are invited from persons irrespective of sex, race, marital status or physical impairment. Envelopes containing applications should be clearly marked "Confidential-Position No. Ag. 81/247 and forwarded to Personnel Resources Branch, Level 10 McKell Building, Rawson Place, Sydney. 2000, N.S.W., Australia by 11th September, 1981.

(9382)A

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(NW833)A

THE WEIZMANN INSTITUTE OF SCIENCE ISRAEL

POST-DOCTORAL POSITION AVAILABLE

For electrophysiological studies in procaryotic cells, to learn the mechanisms of sensory transduction.

Applicants should have a PhD degree. Preference will given to those having research experience in electrophysiology or in measuring membrane potential using optical techniques.

Applications or requests for more details should be addressed to: The Weizmann Institute of Science, Membrane Research Department, 76100 Rehovot — Israel. (W405)A

UNIVERSITY OF GUELPH

DEAN OF THE COLLEGE OF BIOLOGICAL SCIENCE

Nominations and applications are sought for the position of Dean of Biological Science which will become vacant on 1 July, 1982. The College of Biological Science consists of the Department of Botany and Genetics, Microbiology, Nutrition, and Zoology, and the School of Human Biology and has approximately 90 faculty members. Approximately 2,000 students are enrolled in the undergraduate programmes in the College with 150 graduate students in Masters and PhD programmes. Research projects in the College cover a wide spectrum and attract considerable external funding. Strong academic leadership qualities and distinguished scholarly reputation, together with previous teaching and administrative experience are requirements for the position.

Applications and nominations should be sent by October 31, 1981 to: Dr. H. C. Clark, Vice-President, Academic, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (NW825)A

BIOTECHNOLOGISTS

Graduates (2) and Technicians (2) are required by Cambridge Life Sciences Ltd, a new British biotechnology company based at the Cambridge Science Park, England.

The successful candidates will have had considerable experience of commercial scale biochemical production, particularly enzymes, and be familiar with the problems of scale-up from bench to pilot plant.

This is an excellent opportunity for scientists with initiative to work in this new and rapidly developing technology in a stimulating and pleasant environment.

Salaries are negotiable according to qualifications and experience.

Please write, enclosing full CV to:

Dr M. Gronow CAMBRIDGE LIFE SCIENCES LTD Science Park Milton Road Cambridge CB4 4BH

or phone 0223 835221

(9393)A

BIOPHYSICIST/ MOLECULAR GENETICIST

The Donner Laboratory, Lawrence Berkeley Laboratory, is seeking a Biophysicist/Molecular Geneticist to lead a research program designed to investigate the fundamental nature of mutational events in mammalian cells. Applicants should have a doctorate and an established record of creative research in the molecular mechanisms of mutation and altered gene expression.

Research experience in the effects of ionizing and ultraviolet radiation, and chemical mutagens and carcinogens on mammalian genomes is also desirable. Salary will depend on the level of achievement and professional maturity. Applications must be received by September 30, 1981.

Send curriculum vitae, publications list, and names and addresses of at least two references to: Dr Eleanor A. Blakely, Chairman, Biophysicist Search Committee; c/o Mr Ron Lowder, Lawrence Berkeley Laboratory, Personnel Office, One Cyclotron Road, Berkeley, CA 04720. An Affirmative action, equal opportunity employer, M/F/H.



Lawrence Berkeley Laboratory

(NW819)A

READVERTISEMENT MEMORIAL UNIVERSITY OF NEWFOUNDLAND

St John's, Newfoundland, Canada

COMPUTER SCIENCE ASSISTANT/ASSOCIATE PROFESSORSHIPS

Applications are invited for fulltime, ternure-track appointments at the rank of Assistant or Associate Professor level, to commence at the earliest possible date. Applicants should have a PhD or nearing completion of a PhD in Computer-Science. Specialization in the areas of computer graphics, microprocessors, computer systems, data base systems or programming languages would be highly desirable.

Responsibilities will include teaching at the undergraduate and graduate levels, research and some committee work.

Departmental computing facilities include VAX 11/780 currently running under UNIX Version 7 Operating System and a PDP 11/34 and 11/10 also under UNIX. A large number of terminals, graphics equipment and other facilities are also available.

Canadians or Landed Immigrants will be given first preference. Salary is negotiable.

Please send curriculum vitae and names of three references to: Prof J M Foltz, Acting Chairperson, Department of Computer Science, Memorial University of Newfoundland, St John's, Newfoundland A1C 5S7, Canada. (NW822)A

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due to expansion of its laboratories, is seeking additional PhD's familiar with:

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Please send curriculum vitae, reprints of pertinent publications, and letters of recommendation to ARMOS CORPORATION, 180 Kimball Way, South San Francisco, California 94080. (NW821)A

UNIVERSITY OF MELBOURNE FACULTY OF ENGINEERING APPROPRIATE TECHNOLOGY COORDINATOR

Applications are invited for the above-mentioned post at the level of Lecturer/Senior Lecturer (Limited Tenure). The appointment will be for three years commencing 1st December, 1981 or as soon as possible thereafter.

The Appropriate Technology Section was established to encourage and coordinate activity in the Faculty of Engineering in the technologies appropriate to the needs of developing countries, both in education and training and through research and development work. The Section is responsible for the Master's Programme in Development Technologies.

Applicants should have a degree in engineering or applied science, and have had professional experience in developing countries and a strong interest in their problems. Teaching experience is expected, and experience in some field of appropriate technology is desirable.

Salary in the ranges \$A19,821 — \$A26,037 and \$A26,593 — \$A30,995 per annum.

Further details of the position are available from the Dean of the Faculty of Engineering.

Further information regarding details of application procedure and conditions of appointment, is available from the Staff Officer, University of Melbourne, Parkville, 3052, Victoria, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 36 September 1981. (9357)A

KING'S COLLEGE LONDON DEPARTMENT OF BIOPHYSICS POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship to study the regulation of globin gene expression during the development of the frog, *Xenopus laevis*. Expertise in Molecular biology and a knowledge of vertebrate development would be beneficial. Post tenable for up to 3 years from 1 October 1981. Salary in the range £6,475 — £7,700 pa plus £967 London allowance. USS.

Apply in writing as soon as possible with a *curriculum vitae* and the names and addresses of two referees, to Dr R K Patient, Imperial Cancer Research Fund, Mill Hill, London NW7 1AD.

RESEARCH TECHNICIAN (Grade 3)

Applications are invited from suitably qualified persons interested in working on DNA gyrase and the enzymology of DNA supercoiling in bacteria and eukaryotes. Experience in the techniques of DNA biochemistry, protein chemistry or enzymology an advantage. This post, funded by the Cancer Research Campaign, commences on or around 12 October and is renewable annually for up to three years. Salary in the range £4,672 — £5,473 plus £1,027 London Allowance. 37 ½ hour week, Monday to Friday. Five weeks' annual holiday. Superannuation scheme.

Apply in writing by September 7 1981, with a curriculum vitae and the names and addresses of two referees, to Dr L M Fisher, Department of Biphysics, Kings College, 26-29, Drury Lane, London WC2B 5RL. (9377)A

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UNIVERSITY OF MELBOURNE READER/SENIOR LECTURER

(Two Positions) in the DEPARTMENT OF PHYSICS (RAAF ACADEMY)

Applications are invited for the above-mentioned positions. The Physics Department is located both within the grounds of the University and at Point Cook where the bulk of cadet teaching to BSc level is undertaken. The main areas of research are infrared, gamma-ray and neutron astrophysics, and remote sensing investigations of the lower atmosphere. Applicants should have expertise in one or other of these areas.

The appointment will be made to the Staff of the University of Melbourne and will be tenable upon University conditions initially for five years in the case of an appointment as Réader and for four years in the case of an appointment as Senior Lecturer. Either during the initial period or if confirmed by Council after the initial period, the appointment will continue until 31 December of the year immediately preceding that in which the proposed Defence Force Academy commences to operate, when the appointment with the University of Melbourne will terminate. The holder of the appointment will then have the right to transfer to the employment of the Defence Force Academy. The Department of Defence has entered into an agreement with the University of New South Wales whereby the University will establish a College of the University within the Defence Force Academy.

The Department of Defence will provide twelve months' formal notice prior to requiring a decision from a person in relation to the exercise of that right to transfer.

The Department of Defence will also arrange for removal to Canberra, in accordance with normal Commonwealth standards and procedures, for a person who transfers to the Defence Force Academy.

When the Defence Force Academy commences to operate, a person employed by the University in connection with the RAAF Academy who transfers to the Defence Force Academy will be employed by the Defence Force Academy under conditions not less favourable than those which that person enjoyed at the University.

Salary within the range: Reader \$A35,049 per annum; Senior Lecturer \$A26,593 to \$A30,995 per annum.

Salary and classification will be determined according to qualifications and experience.

The successful applicants will be expected to commence duties on 1st January, 1982.

Enquiries may be directed to Professor J A Thomas in the University.

Further information including details of application procedure and conditions of appointment are available from the Staff Officer, University of Melbourne, Parkville, 3052, Victoria, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on 30 September 1981.

(9358)A

The Australian Institute of Marine Science RESEARCH POSITION

Chemical Oceanography

The Australian Institute of Marine Science was established as a Statutory Authority of the Commonwealth Government in 1972 and in 1977 moved into a modern comprehensively equipped laboratory complex at Cape Ferguson near Townsville. The Institute is well situated for studies in tropical marine science including phenomena and the land-sea interface, on the complex environment of the Great Barrier Reef and in the adjacent ocean. The ongoing programs of the Institute are augmented by active collaboration and interaction with visiting investigators from research organizations within Australia and overseas.

APPLICATIONS are invited from researchers with interests and experience in chemical and related aspects of Oceanography to take part in comprehensive investigations of the environment and living communities of the Great Barrier Reef. Within the overall program, an understanding is sought of the physical, chemical and biological interactions taking place within reefal systems and between those systems and adjacent waters.

QUALIFICATIONS: The appointee must possess PhD level qualification and preferably experience demonstrably applicable to the research program outlined.

SALARY: The salary offered shall be commensurate with the qualifications and experience of the successful candidate.

Applications including a complete C.V. and the names of at least three (3) but not more than five (5) referees should be forwarded to: The Secretary, Australian Institute of Marine Science, P.M.B. No. 3, M.S.O. Townsville, Q. 4810, Australia.

Closing date for applications is 30 September 1981.

(9351)A

UNIVERSITY OF STRATHCLYDE Applications are invited for a POSTDOCTORAL RESEARCH FELLOW and for TWO RESEARCH ASSISTANTS IN CARDIOVASCULAR

CARDIOVASCULAR
PHARMACOLOGY
in the
DEPARTMENT OF
PHYSIOLOGY AND
PHARMACOLOGY
details as follows

 Postdoctoral Research Fellow, financed by the British Heart Foundation for work on early post-infarction arrhythmias. Period two years. Salary Range IA £6,880 per annum.

2. Research Assistant, financed by the Scottish Home and Health Department to work on the cardiovascular effects of prolonged exposure to lead. Period two years. Salary Range IB £5,285 per annum. Registration for a higher degree possible.

3. Research Assisant, financed by the Medical Research Council to work on the release of vasoactive agents in shock. Period two and a half years. Salary Range IA up to £6,880 per annum. Registration for a higher degree possible.

USS benefit in each case.

Applications (quoting R15/81) including a brief curriculum vitae and the names of two referees should be sent to Professor J R Parratt, Royal College, University of Strathclyde, Glasgow G1 1XW as soon as possible, stating clearly the particular post in which interested. (9356)A

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The Transatlantic Agency (N2) 33 Great James Street, London WC1.

Enclose SAE please.

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY CLINICAL STUDIES

RESEARCH ASSISTANT PHYSIOLOGY/ BIOMECHANICS

Applications are invited from recent graduates with an interest in physiology and biomechanics to participate in an investigation into the mechanism of dilation of the sheep cervix at birth. The project is financed by the Wellcome Trust.

Initial salary will be either £5,285 or £5,675 per annum.

Applications, together with the names of three referees, should be received not later than 4th September, 1981, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/866/N. (9363)A

UNIVERSITY OF OXFORD University Laboratory of Physiology RESEARCH ASSISTANT GRADE IB

Salary £5,285 — £7,290

Applications are invited for the post of Research Assistant, supported by a grant form the National Institute of Health, Washington, USA, for research on the mechanism of action of antidiuretic hormone. Candidates should have an honours degree in Physiology, Biology or Biochemistry; experience in transport physiology or cell biology would be an advantage. The post is tenable until April 30th, 1984.

Applications stating age, qualifications and experience, and giving the names and addresses of two academic referees should be sent to: The Administrator, University Laboratory of Physiology, Oxford OXI 3PT. (9390)A

POSTDOCTORAL Fellows Research Associates and Technicians, Molecular Genetics and Neurobiology, University of Colorado. Applications are invited from individuals with PhD or MD to pursue studies on the molecular genetics of the mammalian brain. Emphasis is placed on use of recombinant DNA technology to study the structure and regulation of genes expressed postnatally. Background in nucleic acid chemistry is essential Technicians with experience in cell fraction and DNA-RNA hybridization are also needed. All positions have potential of leading to long term appointments. Beginning salary range \$18,000 - \$32,000. Travel expenses for relocation and meetings. Send CV, names and phone numbers of 3-5 references, and copies of recent publications to: William E Hahn, Professor, University of Colorado, School of Medicine, B-111, Denver, Colo. 80262. (NW818)A

KING'S COLLEGE LONDON (University of London) DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH ASSISTANT

to join a research group investigating the selective intracellular catabolism of abnormal proteins.

The position, funded by SRC, will be tenable for two years and the project has as its objective the purification and characterisation of a size-discrimination protease from rabbit reticulocytes.

Applicants should have experience in enzyme purification and protein chemistry, an interest in the mechanism of protein turnover would also be of advantage. Salary in the range £6,070 to £6,880 plus £967 London Allowance.

Applications, including curriculum vitae and names and addresses of two academic referees to Dr A R Hipkiss, Department of Biochemistry, King's College London, Strand, London WC2R 2LS as soon as possible.

(9359)A

UNIVERSITY OF DUNDEE DEPARTMENT OF BIOCHEMISTRY

Applications are invited from holders of good Honours degrees in Biochemistry or a related discipline for the post of

RESEARCH ASSISTANT

for a period of three years to work on a project to define the molecular mechanism in which plasma lipoproteins take part in lutropin-dependent progesterone synthesis by the corpus luteum. Salary according to age and experience in the range £5,285 — £6,070. Further particulars available from the Personnel Office, The University, Dundee DD1 4HN where applications should be returned by 17th Sep-

tember, 1981. Please quote Ref EST/50/81/J. (9344)A

UNIVERSITY OF MELBOURNE Australia CHAIR OF AGRICULTURE (ANIMAL SCIENCE)

Applications are invited for appointment to the Chair of Agriculture (Animal Science). Applicants should have had extensive research experience in some field of animal production and a demonstrated interest in and knowledge of agriculture.

Salary: \$A41,509 per annum.

Further information is available from the Registrar of the University or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H OPF. All correspondence (marked 'Confidential') should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia

Applications close on 31 October 1981. (9372)A

UNIVERSITY OF MELBOURNE Australia CHAIR OF AGRICULTURE (PLANT SCIENCE)

Applications are invited for appointment to the Chair of Agriculture (Plant Science) in the School of Agriculture and Forestry.

Applicants should have had extensive research experience in some field of plant science related to plant production and a demonstrated interest in, and knowledge of agriculture.

Salary: \$A41,509 per annum.

Further information may be obtained from the Registrar of the University or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. All correspondence (marked 'Confidential') should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia.

Applications close on 31 October 1981. (9373)A

RESEARCH FELLOW

Position will be available August 1, 1981. The work involves studies of molecular biology, including cloning of insulin receptor. Salary is \$15,000 (negotiable).

Please send résumé c/o
Dr K Itakura,
Molecular Genetics
City of Hope Res. Inst.
Duarte, Calif. 91010 USA
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(NW817)A

THE MATHILDA AND TERENCE KENNEDY INSTITUTE OF RHEUMATOLOGY

A position is available for a graduate

RESEARCH ASSISTANT

to work in the Biochemistry Division (Head: Dr Helen Muir). The research project will involve studies on the mechanism of biosynthesis and degradation of cartilage proteoglycans and collagen in relation to arthritis. The salary will be based on Range IB of the University of London scales for Research and Analogous staff.

Applications, including curriculum vitae and the names of two referees, should be sent to the General Secretary, The Mathilda and Terence Kennedy Institute of Rheumatology, Bute Gardens, Hammersmith W6 7DW. (9346)A

UNIVERSITY OF YORK

DEPARTMENT OF CHEMISTRY
Applications are invited from suitably
qualified persons for the post of

POST-DOCTORAL RESEARCH ASSISTANT

to work with Dr Guy Dodson on the ligation of haemoglobin crystallised from polyethylene glycol. The project is financed by the Medical Research Council and the appointment is available for three years from 1 October 1981.

The salary scale is £6,070 to £10,575 (Research 1A).

Closing date for applications — 11 September 1981.

Six copies of applications, including full curriculum vitae and naming three referees, should be sent to the Registrar, University of York, Heslington, York YO1 5DD, from whom further particulars are available. Please quote reference number 7097. (9347)A

ONTARIO CANCER TREATMENT & RESEARCH FOUNDATION LONDON CLINIC

London, Ontario, Canada

Applications are invited for the position of

CAREER SCIENTIST

from applicants with a minimum of 2-3 years post-doctoral research experience in the field of

CELL GENETICS

This position is to join a recently formed multidisciplinary research group studying the biology of cancer metastasis. The research objectives of the group are to gain an understanding of how the tumor microenvironment may modulate the metastatic potential of tumor cell populations.

The successful candidate will have proven expertise in a relevant field of research and the ability to develop an interactive research program with other members of the group. Salary will be commensurate with experience.

Applications to Dr K Moore, The Ontario Cancer Foundation, London Clinic, London, Ontario N6A 4G5. (NW829)A

UNIVERSITY COLLEGE GALWAY

DEPARTMENT OF PHYSICS
POST DOCTORAL
RESEARCH ASSISTANT

Applications are invited to work on laser transmission through laboratory generated fog and aerosol media. The work will also involve computer processing of data. Tenable for 2 years from 1 October 1981.

Applications with statement of research experience and the names of two referees should be sent as soon as possible to Dr S G Jennings, Department of Physics, University College Galway, Galway, Ireland. (Tel: Galway 7611 ext 704).

Salary within range IR£7,590 — IR£8,495 pa, depending on age and experience. (9361)A

FACULTY POSITIONS BIOCHEMISTRY

Tenure-track positions at a level to be determined by qualifications are open in the Biochemistry Department, School of Medicine at the University of North Carolina at Chapel Hill, We seek to establish expertise in any of the following research areas: cell surfaces, cell surface interactions and/or membrane biosynthesis; culture, genetics and/or differentiation of eukaryote cells; use of recombinant DNA methods in eukaryote molecular genetics; neurochemistry. Excellence in teaching and a desire to teach is expected.

Send curriculum vitae to: Dr Mary Ellen Jones, Chair, Biochemistry Department, University of North Carolina, Chapel Hill, NC 27514. We are keenly interested in attracting outstanding women and/or minority scientists to this department. Equal Opportunity/Affirmative Action Employer. (NW826)A

INGENE is a young and rapidly growing company creating industrial and biomedical products through genetic engineering technologies. Our laboratory facilities support projects utilizing recombinant DNA approaches, molecular genetics, cell culture, DNA synthesis and sequencing, and immunology. We are recruiting personnel in the fields of:

GENETIC
ENGINEERING/RECOMBINANT
DNA TECHNOLOGY
MOLECULAR
GENETICS/MICROBIOLOGY
BACTERIA/YEAST
IMMUNOLOGY/HYBRIDOMA
TECHNOLOGY
PROTEIN
BIOCHEMISTRY/ENZYMOLOGY
PLANT CELL GROWTH
AND
GENETICS/SEED SCIENCE

Opportunities exist for qualified PhD candidates with postdoctoral experience and an interest in participating in a stimulating, scholarly research program. We also are seeking technical personnel and postdoctoral fellows for work on diversified genetic engineering projects.

INGENE has attractive laboratory facilities in coastal Santa Monica, California, one of the most desirable commercial and residential sections in the Los Angeles area.

Please send a résumé and list of three references to: INGENE 1701 Colorado Avenue Santa Monica, CA 90404.

INGENE is an Equal Opportunity Employer and welcomes applications from qualified women and minorities.

(NW816)A

UNIVERSITY OF OXFORD NUFFIELD LABORATORY OF OPHTHALMOLOGY RESEARCH ASSISTANT

required for 3 years for a study of post-translational modification of proteins in cataract with special reference to diabetes and uraemia. Starting salary £5,285 (Scale 1B).

Further particulars may be obtained from Dr J J Harding, at the above address, to whom applications with full curriculum vitae and names of two referees should be sent as soon as possible. (9360)A

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY TRANSLOCATION IN FUNGI

Applications are invited from those with experience in fungal or higher plant physiology for the post of Post-doctoral Senior Research Assistant (SERC supported) to work on long-distance translocation in Serpulla lacrimans with Professor D H Jennings. The post is tenable for up to three years from 1st October, 1981, at an initial salary within the range £6,070 — £6,880 per annum.

Applications, together with the names of 3 referees should be received not later than 7 September, 1981 by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref: RV/867/N. (9364)A

"POSITION Open" Postodoctoral — Utah in surface Raman spectroscopy. Experience in SERS and total internal reflection optics; interests in biophysical-bioengineering-biochemical applications of Raman spectroscopy desirable. Send vita, letter of interest, publication list, reprints, and three letters of reference to J D Andrade, Bioengineering Department, University of Utah, Salt Lake City, Utah 84112 USA.

(NW830)A

MANCHESTER AREA
HEALTH AUTHORITY
(TEACHING)
SOUTH DISTRICT
Christie Hospital and Holt
Radium Institute

BASIC GRADE BIOCHEMIST

to join a research group studying mechanisms of response to hormone therapy in breast cancer.

Previous experience in radioreceptor analysis, radioimmunoassay or a related field is important.

The starting salary will be within the range £5,346 — £6,162 (increase pending).

For informal enquiries telephone Dr Diana Barnes, 061-445 8123 ext 437.

Application forms from Sector Administrator, Christie Hospital, Wilmslow Road, Withington, Manchester M20 9BX extension 520. Closing date: 9th September, 1981.

(9352)A

SOUTH GLAMORGAN HEALTH AUTHORITY (T) UNIVERSITY HOSPITAL OF WALES

REGIONAL CYTOGENETICS SERVICE B/G CYTOGENETICISTS

Salary: £4,839 — £6,363 per annum

The above vacancy has arisen in this department which provides a comprehensive cytogenetics service. The person appointed will be required to work in all sections of the service.

Applicants should have a good honours degree in genetics or in one of the other Biological Sciences. Experience in tissue culture techniques and diagnostic cytogenetics will be an advantage, but not essential.

Further information can be obtained from: Peter J. Gregory, Department of Child Health, University Hospital of Wales, Heath Park, Cardiff. Tel: (0222) 755944 ext 2287.

Application forms available from: Personnel Department, University Hospital of Wales, Heath Park, Cardiff. Please enclose S.A.E.

Closing Date: 10th September 1981.

(9386)A

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

DEPARTMENT OF HAEMATOLOGY

TECHNICIAN

required to work with a team carrying out clinical bone marrow transplantation. Duties include tissue typing, mixed lymphocyte culturing and carrying out various immunological assays. Experience in any of these fields or in haematological techniques desirable. This is a post for someone with initiative and interested in research projects. The post is funded by the Leukaemia Research Fund and is tenable for two years.

Salary up to £5,284 plus London Allowance (under review), initial placing dependent on age and qualifications.

Application forms and further particulars are available from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 7/233.

(9353)A

UNIVERSITY OF OXFORD NUFFIELD DEPARTMENT OF

ORTHOPAEDIC SURGERY

MRC BONE RESEARCH LABORATORY

Applications are invited for two posts to work on the isolation and characterization of factors that induce the differentiation of bone cells.

(1) POSTDOCTORAL RESEARCH ASSISTANTSHIP

Starting salary (Range 1A) — £6,070 — £7,290 pa for biochemists with experience in protein isolation + cell culture techniques

(2) MEDICAL LABORATORY SCIENTIFIC OFFICER

Salary range — £4,851 — £5,334 for technical assistance with above project

The posts are supported by MRC for a three year period. Applications with full curriculum vitae and names and addresses of three referees to Dr J T Triffitt MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX3 7LD, as soon as possible. (9367)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF ZOOLOGY POSTDOCTORAL BIOCHEMIST

Applications are invited for a postdoctoral research fellowship on a cancer research campaign supported project on cell surface glycoproteins in chronic lymphocyte leukemia supervised by Dr A H Maddy. Previous experience in membrane or protein biochemistry would be an advantage but candidates of other backgrounds would also be considered.

The post is for three years starting from October 1st 1981, or as soon as possible thereafter, with a research range 1A salary scale and superannuation.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Dr A H Maddy, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, from whom further details may be obtained. Please quote reference No. 5047. (9380)A

RESEARCH ASSISTANT

Immunologist/biochemist required to work as part of a team investigating immune reactions against liver cell membrane antigens in relation to autoimmune liver disease. An interest in characterisation of monoclonal antibody specificities and experience in enzyme-linked immunoassays and related techniques will be an advantage. The post is funded for 2 years in the first instance at a salary of up to £6,475 plus £967 London Weighting Allowance subject to the usual conditions.

Further information may be obtained from Dr I G McFarlane, Liver Unit, King's College Hospital (telephone: 01-274 6222 ext 2747).

Applications giving full curriculum vitae plus names and addresses of two referees should be sent to the Secretary, King's College Hospital Medical School, Denmark Hill, London SE5 8RX. Closing date 30th September 1981. (9355)A

UNIVERSITY OF FLORIDA DEPARTMENT OF MEDICINE University of Florida seeks

Scientist for appointment as
ASSISTANT PROFESSOR
OF MEDICINE

to join Medical Oncology Research team primarily involved in immunology and experimental Hematology. Candidates must have a doctoral degree (PhD or equivalent), at least 2 years experience, and demonstrated research productivity in human cellular immunology. Responsibility will include teaching at all levels. Salary \$23,000—negotiable.

Application recruiting deadline 12-31-81, anticipated starting date April 1, 1982. Contact Roy S Weiner, MD, Division of Oncology, Box J-277, J Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610. An Equal Employment Opportunity/Affirmative Action Employer. (NW820)A

UNIVERSITY OF LEICESTER DEPARTMENTS OF PHYSIOLOGY

AND MEDICINE

GRADE 3 TECHNICIAN

A Grade 3 Technician is required to assist in laboratory studies into electrolyte transport by body cells in relation to blood pressure. The post is financed by the Medical Research Council and is tenable in the first instance for one year and is renewable for a further two years. The project will be carried out jointly in the two departments under the supervision of Professor J D Swales, Dr H Thurston and Professor R Whittam.

Previous laboratory experiencen in a biological sciences department is desirable. Salary scale £4,672 to £5,473.

Applications including a curriculum vitae and the names of two referees should be sent to Professor J D Swales, Department of Medicine, Clinical Sciences Building, Leicester Royal Infirmary, Infirmary Square, Leicester LE2 7LX by 28th August 1981. (9369)A

UNIVERSITY OF WARWICK RESEARCH ASSOCIATE IN VIROLOGY

Applications are invited from biochemists, microbiologists and other suitably qualified candidates to work in the Department of Biological Sciences on an ARC funded project concerned with the epidemiology of rotaviruses. Candidates should have either an upper second class honours degree plus at least one years postgraduate experience or an MSc. The appointment on the salary Range IB scale: £5,285 pa — £7,700 pa is for three years.

Applications (no forms) should include a curriculum vitae and the names of two referees to the Academic Registrar, University of Warwick, Coventry CV4 7AL and quote Ref. No: 2/2A/81/0. Closing date for receipt of applications 4th September, 1981. (9387)A

FELLOWSHIPS

UNIVERSITY OF GLASGOW DEPARTMENT OF BOTANY POST-DOCTORAL RESEARCH FELLOWSHIP

A post is available for a molecular biologist or virologist to work on the replication of a plant rhabdovirus. This will involve the cloning in *Escherichia coli* of various viral RNAs. Previous experience of recombinant DNA techniques would be an advantage. Familiarity with plant viruses is not a prerequisite.

The post will be available from 1st October, 1981 for three years. Starting salary will be within range 1A of the scales for Research and Analogous staff (£6,070 - £6,880).

Applications giving the names of three referees should be sent to Dr J J Milner, Department of Botany, The University, Glasgow G12 8QQ by 18th September, 1981.

In reply please quote Ref. No. 4847. (9384)E

continued on page xxviii

UNIVERSITY OF CAPE TOWN Chief Technical Officer

Applications are invited for the above post which falls vacant on 1st April 1982 in the Department of Inorganic Chemistry. The successful applicant will take charge of the general workshop in the School of Chemical Sciences. Experience in metalwork, woodwork, instrument-making and the maintenance of scientific equipment and appropriate technical qualifications will be a recommendation for the post.

Appointment, according to qualifications and experience, will be made on the salary scale R13,905 \times 495 - R14,400 \times 735 - R15,135 \times 735 - R15,870 per annum.

Applications, (quoting ref. number AC/8) including the names and addresses of two referees, should be addressed to the Head, Department of Inorganic Chemistry, University of Cape Town, Rondebosch, 7700, South Africa not later than 30 September 1981.

The University's policy is not to discriminate in the appointment of staff on the grounds of sex, race or religion. Further information on the implementation of this policy is obtainable from the Registrar. (W404)A

THE OPEN UNIVERSITY **EARTH SCIENCES**

NERC RESEARCH **ASSISTANTSHIPS**

Troodos Deep Bore

As part of an international deep drilling project involving scientists from Canada, Cyprus, Denmark, Iceland, UK, USA and W. Germany, a grant has been awarded to Professor I. G. Gass from The Open University and Professor F. J. Vine from The University of East Anglia to study the Troodos ophiolite (Cyprus) by drilling.

Funds have been made available to employ two Research Assistants to work on geophysical aspects with Professor Vine (UEA) and petrological/geochemical aspects with Professor Gass (OU); these posts are available from 1st January 1982 for three years. This advertisement is for both posts. Candidates for the Open University Petrology-Geochemistry post should hold a good first degree in geology with a specialization and interest in the petrology and geochemistry of basicultrabasic igneous rocks. Candidates for the geophysics post should have undergraduate experience in geophysics and will be expected to investigate the physical properties of bore hole specimens at varying temperature and pressures.

Both candidates will be involved in field studies and be present during some if not all of the drilling programme. There may be an opportunity to study for a higher degree. Salary will be within the Research and Analogous 1B scale: £5,285 - £7,700 p.a. but not exceeding £6,880 p.a. at the highest point.

Further particulars and application forms are available from the Assistant Secretary (Science), (4223/2), The Open University, Walton Hall, Milton Keynes MK76AA or telephone Milton Keynes (0908) 653481; there is a 24 hour answering service on 653868.

Closing date for applications: 31st October.

(9371)A

UNIVERSITY OF LEICESTER | DEPARTMENT OF ZOOLOGY

RESEARCH **ASSISTANTSHIP**

Applications are invited for the post Research Assistant at postdoctoral or graduate level to join a group working on the arrangement and expression of DNA sequences in the chromosomes of amphibians. Applicants should have some experience with recombinant DNA and related technologies. The appointment will be for one year in the first instance, commencing 1st October, 1981, but is likely to be renewable for a further 2 or 3 years. Salary scale £5,285 to £7,700.

Applications, with the names of 2 academic referees, to Professor H C Macgregor, Zoology Department, University of Leicester, Leicester LE1 7RH. Enquiries by telephone welcome (0533-554455 ext 115)



UNIVERSITY OF WARWICK

RESEARCH **ASSISTANTSHIPS**

HIGH PERFORMANCE CERAMIC ALLOYS

Applications are invited for postgraduate and postdoctoral research assistantships concerned with the development of new structural ceramic alloys within a group in the Department of Physics, led by Dr M H Lewis, supported by the Wolfson Foundation. The research has evolved from a collaborative programme with Lucas Industries in studying microstructural develop-ment, deformation and fracture mechanisms in ceramics based on the β — Si₃N₄ crystal structure.

Applicants should preferably have experience in electron-microscopy and associated microanalytical techniques or in ceramic fabrication and mechanical testing. Appointments are for 2 years from October, 1981 or as soon as possible thereafter. Starting salary either up to £6,475 pa on the Range IB scale: £5,285 — £7,700 pa or £7,700 pa on the Range IA scale: £6,070 — £10,575 pa.

Applications (no forms) should include a curriculum vitae and the names of two referees to the Academic Registrar, University of Warwick, Coventry CV4 7AL. Please quote Ref. No: 2/A/81/0. Closing date for receipt of applications 4th September, 1981.

THE INDUSTRIAL LIAISON PROGRAM OF THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY PRESENTS A COLLOQUIUM ENTITLED

"Microcapsules and Microcarriers in Biotechnology"

October 15, 1981 Date:

Professor Robert S. Langer, Jr., MIT Chairman:

Professors Marcus Karel, Alexander Klibanov, Co-Chairmen:

William Thilly, MIT Professor E. Katchalski-Katzir Other Speakers:

Weizmann Institute of Science Professor D. Papahadjopoulos University of California, San Francisco Professor T. Chang, McGill University

J. Salk, M.D., Salk Institute

MIT Kresge Auditorium, Cambridge, MA 02139 Location:

Maria Clara V. Suva Martin Contact: Industrial Liaison Program Tel: (617) 253-2691. Telex: 921473

Attendance Fee: Industrial Liaison Program (ILP) Members - free

Non-ILP Members - \$500 Immobilized Enzymes

Enzyme Stabilization

Controlled Drug Delivery Systems Liposomes — Drug Targeting Microcapsules

Diffusion Control in Food Systems Microcarriers for Mammalian Cell Culture Microcarriers in Vaccine Production

Abstract:

Topics:

Major advances have been witnessed in the past few years in the area of biotechnology, which can be defined as the application of engineering principles to biological processes. In this coloquium, the use of novel concept — microcapsules and microcarriers — in biotechnology will be discussed. These particles possess significant potential in both basic and applied research. The applications in several important areas will also be discussed including their use as (i) supports for cells which make useful products, (ii) matrices for enzymes to be used as bioproducers or bioconverters, and (iii) the central components in drug and food delivery systems (NWX793)C delivery systems.

Frontiers in Liposome Research: **Targeted Chemotherapy** November 5-7, 1981

A meeting sponsored by the Northern California Cancer Program and the School of Medicine, UC — San Francisco and organized by Dr. D. Papahadjopoulos. Will bring together a group of investigators with diverse backgrounds to critically evaluate the use of liposomes for targeted chemotherapy. Recent advances in the field will be presented as a background for discussions on the prospects for future progress and clinical trials. The meeting will be limited to 75 participants.

For additional information contact Dr. S. Szoka, School of Pharmacy, University of California at San Francisco, San Francisco, CA. 94143 USA. (NW832)C

GRANTS and SCHOLARSHIPS

IMPERIAL CANCER RESEARCH FUND Research Studentship (Bursary)

Applications are invited from recent honour graduates for a Bursary in the Transcription Laboratory to study the molecular biology of the DNA tumour virus polyoma.

The award, for full time studies leading to a higher degree (PhD) will be tenable for three years with a non-pensionable grant of £4,140 a year (subject to tax) and in some cases additional allowances. Candidates should have a first or upper second class honours degree in one of the biological sciences if they are UK graduates, but non-U.K. residents are not necessarily excluded. A strong background in molecular biology will be important and some training in recombinant DNA techniques would be particularly desirable.

For further information telephone Dr R. Kamen (01-242 0200 ext 277). Application forms are available from The Personnel Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields London WC2. Tel. as above ext 305. Completed application forms should reach the Fund no later than 1st September 1981.

THE OPEN UNIVERSITY FACULTY OF SCIENCE

J

RESEARCH FELLOWSHIP

(Brain Research Group) (Temporary)

Applications are invited for a temporary post as Research Fellow to join the Brain Research Group in the Biology Discipline at the Open University.

The successful candidate will be expected to pursue their own line of research within the interests of the Group and also to collaborate on existing projects, which include the neurochemical correlates of developmental plasticity and early learning, the role of glycoproteins in synaptogenesis and synaptic function and the development of transmitter systems.

Candidates should hold a PhD degree in biochemistry/cell biochemistry/cell biology. Experience in neurochemistry and immunology will be an advantage.

The post was first advertised in December 1980 but an appointment was not made. Applicants who replied to the original advertisement will be automatically re-considered and need not apply again.

The post is available immediately for a period of two years, with a strong possibility of renewal for a further year. Salary will be on the Research and Analogous 1A scale £6,070 - £10,575 p.a. An appointment will be made within the range £6,070 - £8,515 p.a. on the 1A scale.

Further particulars and application forms are available from the Assistant Secretary Science (4152/1), The Open University, Walton Hall, Milton Keynes MK7 6AA or telephone Milton Keynes (0908) 653481: there is a 24 hour answering service on 653868.

Closing date for applications: 7th September. (9374)E

UNIVERSITY OF ABERDEEN DEPARTMENT OF CHEMISTRY DOCTORAL

POST-DOCTORAL RESEARCH FELLOWSHIP

Applications are invited for an SRC funded Postdoctoral Research Fellowship concerned with the decomposition of alkoxy radicals in relation to fundamental aspects of unimolecular reactions and atmospheric chemistry using photolytic techniques. The post is tenable for one year working with Dr L Batt.

Salary within Range IA, £6,070 — £7,290 per annum, with appropriate placing.

Further details from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by 11 September 1981. (9378)E

UNIVERSITY OF GLASGOW INSTITUTE OF NEUROLOGICAL SCIENCES POST DOCTORAL FELLOWSHIPS (Immunology)

Two vacancies exist for Immunologists with post doctoral experience, to study auto-immune diseases of the nervous system. Candidates should have general immunological experience; an interest in tissue culture an advantage. The positions are for three years in the first instance.

Applicants should send Curriculum Vitae together with the names of three referees to: Dr P O Behan, Dept of Neurology, Institute of Neurological Sciences, Southern General Hospital, 1345 Govan Road, Glasgow G51 4TF. (9381)E



DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOSYNTHESIS

A Fellowship is available from 1st October, 1981, renewable for a second year, for research on the biosynthesis of the polyether ionophore antibiotic monensin. The project will involve the application of C-13, H-2 and O-18 labelled precursors. Experience in organic chemistry and/or mechanistic enzymology is required.

Salary on 1A Scale within range £6,070 to £6,880 plus superannuation. Starting point dependent on age and experience.

Applications, with the names of two referees, should be sent as soon as possible to Dr J A Robinson, Department of Chemistry, University of Southampton, Highfield, Southampton SO9 5NH. Please quote ref: N. (9368)E

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF MICROBIOLOGY RESEARCH FELLOWSHIP

Applications invited for a Post Doctoral Research Fellow IA for a project to produce monoclonal antibodies to *Treponema pallidum*. Experience in cell culture and/or immunology would be advantageous.

The post is tenable from 1 October 1981 for 18 months.

Informal enquiries to Dr C W Penn, Department of Microbiology, (021 472 1301 ext 3673).

Salary on Research Fellow IA scale £6,070 — £10,575 plus superannuation. Maximum starting salary

Further particulars from Assistant Registrar (Sci & Eng), University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees should be sent by Friday 11th September 1981. Please quote reference NM5. (9383)E



UNIVERSITY OF DUBLIN
Trinity College

POST DOCTORAL RESEARCH FELLOWSHIP MICROBIAL ECOLOGY

Applications are invited for the above post from microbiologists or botanists, to work on microbial aspects of immobilization of fertilizer nitrogen, nitrogen fixation and mobilization of insoluble N in peat under willow coppice grown for biomass fuel.

The appointment will be tenable for a period of two years, commencing on 1st October, 1981.

Salary scale: IR£5,897 — IR£6,129 per annum.

Candidates should submit *curricu-lum vitae* together with the names of two referees to:

Dr Paul Dowding, Department of Botany, Trinity College, Dublin 2

to arrive not later than 20th September, 1981. (9376)E

UNIVERSITY OF ABERDEEN DEPARTMENT OF ZOOLOGY

POST-DOCTORAL RESEARCH FELLOWSHIP IMMUNOLOGY/ ENDOCRINOLOGY

Re-Advertisement

Applications are invited for an SRC supported post-doctoral research Fellowship studying Autoimmune Destruction of Fish Gonads. The post is tenable for three years, working with Dr I G Priede and Dr L M Laird to examine this phenomenon first demonstrated on salmonids.

Salary within Range IA, £6,070 — £7,290 per annum, with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by 11 September 1981. (9375)E

STUDENTSHIPS

THE POLYTECHNIC: WOLVERHAMPTON The South Staffordshire Medical Centre

Applications are invited for a

RESEARCH STUDENTSHIP

financed by the Rotha Abraham Bequest, to undertake a research project on "MICROPROCESSOR CONTROLLED REGULATION OF BLOOD GLUCOSE IN THE NEW-BORN"

Applicants should possess, or expect to possess, at least a second class honours degree in an appropriate discipline, eg biochemistry, clinical chemistry, analytical chemistry, or equivalent.

The studentship is tenable for up to two years and it is expected that the successful candidate will register for a higher degree.

Further particulars and application forms from; The Faculty of Science Office. The Polytechnic, Wulfruna Street, Wolverhampton WV11LY. (Tel; (0902) 27371). (9350) F

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY POSTDOCTORAL RESEARCH FELLOW

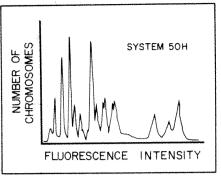
Applications are invited for a postdoctoral research fellowship in the above department for work involving the cloning of developmentally regulated MRNA sequences in Xenopus. The appointment available from 1st October 1981 will be for three years on an SERC funded research grant. Salary starting at point 3 (£6,880) on the 1A range for research and analogous staff. Candidate with directly relevant experience would be preferred, but inquiries are also invited from people who have recently completed PhD work in related fields and would like to acquire methods of nucleic acid and cloning technology. Informal inquiries may be made to Dr P Ford (031) 667 1081 ext 2873/8.

Applications should be made in writing, quoting at least two referees, to Dr P J Ford, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. Please quote reference 5042. (9379)E

No other commercially available cell sorter and analyzer approaches the power, precision and versatility of the Ortho Systems 30/50. The instrument is offered in two basic configurations: System 30-a high resolution, 21 parameter analytical flow cytometer, and System 50-which adds cell sorting capabilities to the System 30 flow cytometer. Both feature dual lasers as standard equipment for increased analytical power.

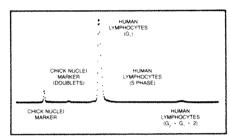
System Components

To appreciate the far-reaching capabilities of these Ortho instruments, consider that the System 50 combines: two lasers-a .8 milliwatt helium-neon for ultra high precision scatter measurements and a 5 watt argon type for fluorescence measurements (optional argon-ion, and krypton-ion lasers with U.V. capability are available); four detectors-two of the photo-multiplier type for right angle scatter, and fluorescent emission detection (S-20 response, 185 nm-850 nm), and two solid state sensors for axial light loss and narrow angle forward scatter: a sophisticated optical system incorporating fiber optics: a multiplexed multichannel analyzer, with C.R.T. display: the signal processor unit: and an advanced cell sorter module. Additionally, the Model 2150 computerized 8 parameter data handler/sorter controller is available as an option* The 2150 System features



2. Histogram of CHO chromosomes stained for D.N.A. Ethidium Bromide/Chromomycin Av

Cytofluorograph System 50H Laser-250mw @ 457nm. Red Fluorescence



2. Histogram of human lymphocytes utilizing a chick nuclei marker for standardization and stained for D.N.A. with Propidium Iodide. C.V. = 1.65% (Human Lymphocytes)

simultaneous data acquisition, and real time processing while sorting is in progress.

System Capabilities

Together, these modules allow the System 50 to provide and analyze morphological information—a significant first for a flow cytometric instrument. With four detectors and the ability to display pulse height, pulse area or pulse width analysis for each—a total of 21 distinct measurement parameters can be studied

The System 50 also features: two bidimensional regions of interest, dual histogram multi-channel analyzer with cytogram mode, super-sil quartz optics with which the cells are analyzed and easy sample entry.

In order to provide customers with complete support. Ortho maintains an Applications Laboratory that is available for confirmation and consultation services. Additionally, an international network of service technicians is ready to aid customers should calibration or repairs ever be required.

Remarkable Results

The histograms and cytograms displayed were produced by the System 50. Evaluate them. And consider the difficulty of deriving this data by any other means. The capabilities of this unique Ortho instrument will speak for themselves.

State-of-the-art cell sorting and analysis from Ortho The Cytofluorograf Systems 30/50



ilson has something new and better in pumps. It's our new Model 302 microprocessor-controlled pump. Model 302 offers this unique combination of capabilities for HPLC and preparative chromatography: (a)

microprocessor control

(b) effective flow rate range of 0.005 to 100 ml/minute with pressures to 6,000 psi (c) both flow and dispense modes (d) four easily interchangeable heads for analytical, high pressure, preparative, and recycling preparative LC.

There's so much you can do. Interface with a computer and automate your LC procedures. Prepare gradient programs. Dispense repetitively, either fixed or varied volume aliquots. Control flow rates with high accuracy.

Make this pump the heart of your LC system. Your inquiry will not only bring details on this remarkable pump but also on other fine Gilson instruments for LC and HPLC. Contact:





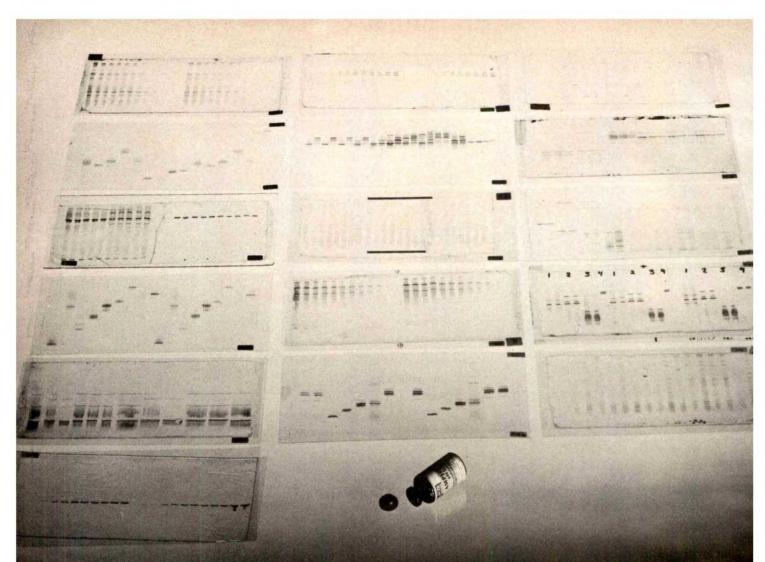
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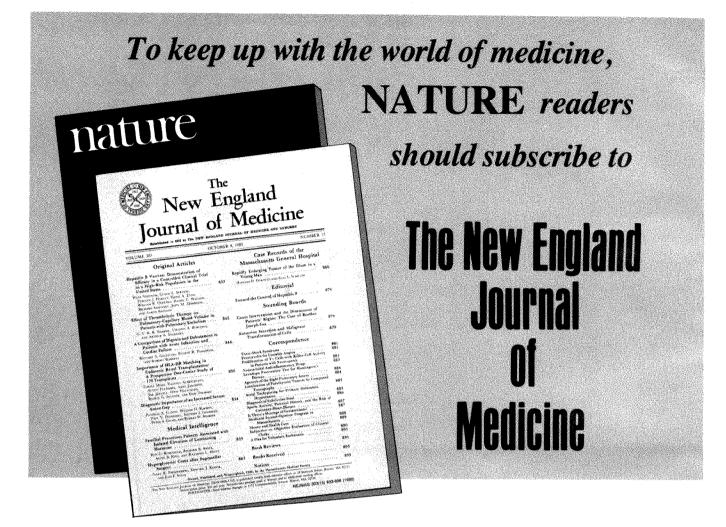
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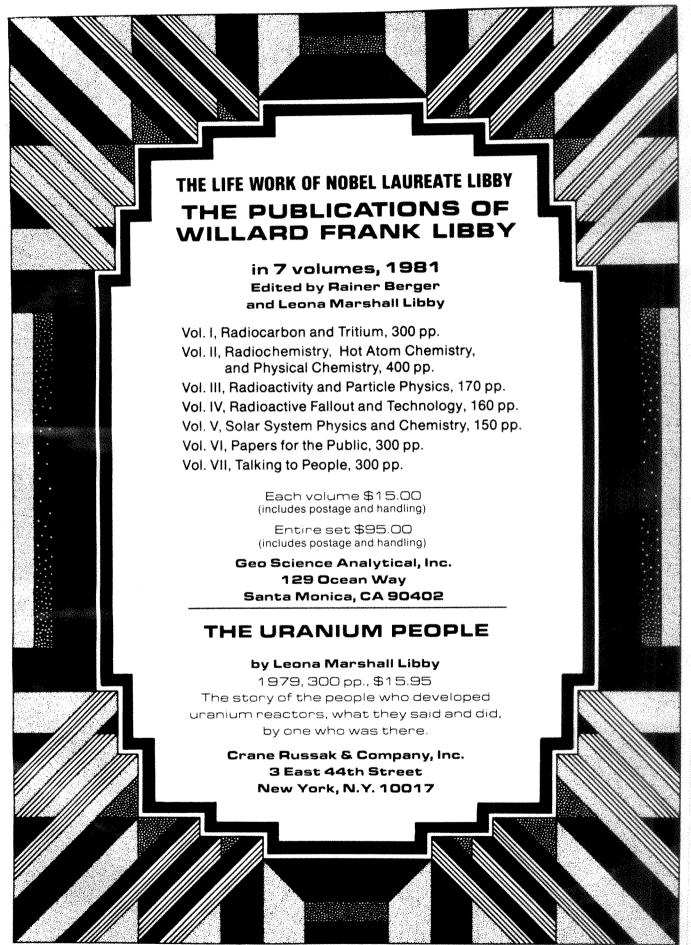
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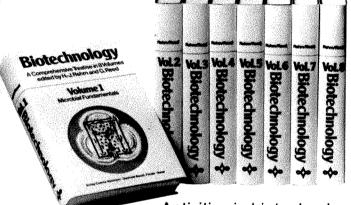
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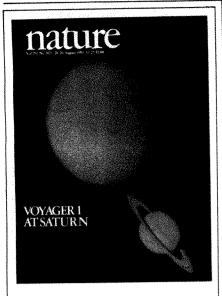
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nature

27 August 1981

More trouble for the hapless shuttle

Ironically, the shuttle device for putting objects into orbits about the Earth is being threatened now by commercial as well as technical problems (see page 789). After a decade of planning, much of it imaginative and some of it over-imaginative, it now appears that the shuttle may not be able to hold its own commercially with other means of launching satellites. The European rocket Ariane appears, in the short run at least, to be able to offer launching facilities more cheaply than comparable rocket systems in the United States, and there is at least a possibility that the shuttle itself will not be much cheaper (see page 789). In any case, the shuttle is so far behind schedule, and demands from the Pentagon on its services so clamant, that it may be some time before the device is able to perform the general service for which it was designed - making sure that the world is well supplied with telecommunications satellites (on which it is now dependent), assisting with the exploration of the Solar System and the regions beyond and giving all of us the sense that space, as we have learned to call it, is part of our domain. The irony is, of course, that the shuttle, intended as a way of cheapening the launching of satellites, should appear to be threatened by an upstart competitor from Europe. Especially in France, people will be recalling the legend of David and Goliath. The snag is that, in the end, we shall all be losers.

A device such as the shuttle (intended to have its second trial flight next month) is not merely desirable but essential. When the National Aeronautics and Space Administration decided, at the end of the Apollo programme, that sending a man to Mars would be a less useful application of the skills that it had acquired than the building of a reusable launching system, it took the right decision. Better to go step by step than to hunt for yet another worldwide television hook-up. But the shuttle, as it has been developed, is something of a bastard. The original conception of a launching device that could be used over and over again has been adulterated by arranging that the largest items of equipment, the launching rockets, should fall into the sea and be lost. Some time in the coming decade, after the system as it stands has proved itself, attention may be turned again to the recovery of these items. In the meantime, there is a serious danger that the shuttle will limp along, working principally for the military, and that the usefulness of Earth satellites will be denied.

In all the circumstances, it is necessary that the space administration, or perhaps even the US Congress, should stand back from the problems that now afflict the shuttle and ask where, in the end, it is meant to go. The issues that must be settled are not simple, but neither are they beyond comprehension.

First, there is a case for asking whether the present concept of the shuttle, as embodied in the spacecraft called "Columbia", is the most appropriate, given what has been learned in the past ten years; if it is not, the space administration (or Congress) should be prepared to write off the huge expenditures of the past decade, and to start again. Such a question could be settled only after a careful technical enquiry, but this is the time at which to make it. Second, there should be a more sober appraisal than there has been so far of the utility of being able to put satellites into orbits — a futuristic appraisal of the market. For the past several years, telecommunications authorities have been assuming that there will always be some way of launching their relay stations, and that the cost will be a small part of the cost of providing them with signals; in present circumstances, in which the benefits of terrestrial switching circuits are only just becoming clear, is that assumption still valid? And, since it would be not merely a black mark for the shuttle project but an international tragedy that the Large Space Telescope should not be launched, or not launched more or less on time, how should such projects be organized? Finally, there are questions about the future, and of several kinds. Do the difficulties that have afflicted the shuttle in

the past few years imply that it has been discovered empirically — the hard way — that even the United States is no longer capable of everything? What in the medium term, say by the end of the century, is the utility of the Earth orbit? Science and the Pentagon apart, telecommunications will have to bear the burden. And what of the more distant future? Science fiction, which has a habit of becoming fact, would suggest that the time will come when people will think it intolerable that they should be prevented by the lack of facilities from going where they choose. Such conceits, always infuriating for those concerned with next year's budget, are nevertheless historically (so far) undeniable.

Agony in London

Whom the gods would destroy, they first make seem ineffectual. This seems to be the underlying trouble at the University of London, which has just been through the traumatic process of dividing the public grant for the present academic year (which began financially on 1 August) among its several schools and specialized institutions. But the university, which is a microcosm of the British university system as a whole, is not entirely to blame. Both the university and the University Grants Committee earlier in the year were faced with an impossible task that of saving large amounts of money so quickly that they could not properly take account of academic and educational considerations. Solomon himself would have been hard pressed to make an equitable division of the diminished budget. Yet both the university and the grants committee have responded with delay. The grants committee let its dependants known only on the eve of the academic year what they would have to spend in the three succeeding years. The University of London, in which financial decisions are delegated to a body called the Court, has delayed a further six weeks and has announced financial allocations (Nature, 20 August) which are inconsistent both with the principles laid down by the grants committee and with the interim recommendations of the committee (under Sir Peter Swinnerton-Dyer) which the university had set up to suggest how its organization might be adapted to much smaller budgets.

What has happened, and is about to happen, at the University of London is important because it is among the most important of the universities in the United Kingdom. It has more students than any other British university, and is comparable in size with the Berkeley campus of the University of California. The university is the largest single source of physicians in the United Kingdom, but also has specialized institutions for training architects, veterinarians, pharmacists and even agriculturalists. Constitutionally, however, the university is not an integrated whole but an agglomeration of titularly autonomous institutions so loosely held together that they are a federation on the Canadian pattern, not that of the United States. The separate institutions include a dozen medical schools (soon to be fewer), internationally known centres of teaching and scholarship such as the London School of Economics and Imperial College, a host of graduate institutions (not only medical) whose importance nationally and internationally is beyond dispute and no fewer than eight more general colleges, scattered over twenty-five miles, which differ in size, the pattern of courses offered to undergraduates and their reputations in research. The question now is whether last week's decision by the Court will have

irreparably damaged the character of this remarkable, if anomalous, institution.

On at least three counts, the Court's decisions cannot fail to cast a long shadow over the university. First, the Court seems meekly to have accepted the grants committee's edict that the proportion of students (graduates and undergraduates) following science courses should be reduced by 11.3 per cent by the academic year beginning in 1983. This is a sharper contraction than the intended reduction of the British university student population as a whole and much sharper than the planned contraction of science teaching — a mere 2.2. per cent throughout the United Kingdom. Taking the wording of the grants committee's letter literally, however, the Court of the university has decided that teaching in the physical sciences should be maintained, and has gone on to plan for a reduction of 28 per cent in the numbers of students following courses in biology. The grants committee's recommendation that special consideration should be given to biology courses of "potential economic importance" (another name for biotechnology?) seems not everywhere to have been heeded. The result, by 1983, is likely to be that most departments of "soft" biology in the university will have shrunk to such a size that they are no longer viable. In the meantime, most of them will no doubt whistle hard to keep their spirits up and will admit more students in the next few days than prudence would dictate.

The second change of pattern now decreed that will prejudice the educational work of the university as a whole is the Court's decree that undergraduate courses in which two principal subjects are combined should bear the brunt of the contraction. To be fair, the Court has not dreamed up this conclusion of its own accord, but instead has asked the grants committee for "guidance". Having been told of what was plainly an afterthought by the committee, the court has done its arithmetic accordingly. At no point has it been considered whether these decisions are educationally desirable. Yet there are several schools within the university where combined courses of this kind, involving both general and particular studies, are much valued by employers, not to mention students. In any case, when it is plain that one of the faults of the British university system is that it offers too specialized an education to most undergraduates, by what right does the grants committee recommend that the efforts made in London to put that balance right should be negated? The Court says in its letter to its pensioners that "the far-reaching implications of this [decision] will have to be examined within the university". The snag is that its financial decisions for the coming year will have preempted sensible discussion.

Third, the allocations of funds from the university budget of £157 million for the coming year must put the survival of many of the smaller schools of the university in jeopardy. The difficulty is that institutions whose costs consist largely of salaries for academic and ancillary staffs - often three-quarters of the total - cannot hope to live within the shorter commons now decreed by getting rid of staff, for contracts of employment often require that notice of dismissal should be given before the beginning of an academic year. So how will such places manage? Especially in those schools where the numbers of students from overseas has fallen sharply, the outlook must be grim. Yet the Court of the university promises nothing in the way of help. Instead it hopes that the "cost of contraction" can be met "from some special source" and mentions the £20 million the Universty Grants Committee set aside last year to meet the cost of academic redundancy. On present form, the true cost will be far greater.

For other than financial reasons, this is an awkward time for the university. The Vice-Chancellor, Lord Annan, is about to be succeeded (on 1 September) by Professor Randolph Quirk. The senate of the university, responsible for academic policy, has just been reconstituted and will not be meeting until November. The new management has a daunting task ahead of it. It will also have an awkward constitutional problem to tackle. The Court of the university is charged with making final decisions of the distribution of funds but is barred from making decisions with academic implications without consulting the academic senate. The past few weeks have shown is that, at a time of rapid

contraction, the notion that academic and financial considerations can be cleanly separated is a fiction. But so it must be even in good times. The isolation of the Court's proceedings from the rest of the university, which has made a nonsense of the committee on academic organization, should not continue.

The university must recognize that the looseness of its constitution is increasingly an encumbrance. In spite of the committees which occupy a substantial part of many academics' time, the educational policy of the university is an amalgam of the separate decisions of individual schools. That it could or should be otherwise at places such as the London School of Economics or at Imperial College is unthinkable, but in the university as a whole, the result is inevitably duplication and incoherence. If it had the time, a stronger university might be able to create a pattern that makes more sense. But even in the short run, a stronger university could help deal with the most urgent problem — the future of academic staff. For it would be thoroughly anomalous if some parts of the university found themselves firing, or retiring prematurely, academics who might be usefully employed elsewhere within the university. And it is inequitable that the people who will be put out of work should be those who happen to be employed at schools which have been hard hit, either by the disappearance of overseas students or by the decisions forced on the Court by the arithmetic of penury — and the last-minute guidance from the grants committee.

Year of the Medfly

Californians (many of whom are ethnically Chinese) may remember 1981 as the "year of the Medfly". With a little luck, they may also come to think of 1981 as a year in which the innocence of those who pretend that nature is never malevolent was made to fade away.

The Medfly is an aphid, and its name is arrived at by a shortening of "Mediterranean". It will probably never be possible to tell how the fly reached California or the importance, in the infestation of the past few weeks, of the supposed infertility of the supposedly sterile males released by the state government into the wild. With the winter (such as it is) soon to come, Californians will with reason be hoping that most, perhaps all, of this exotic species will be killed off, and that that will be an end of the affair. Most fruit farmers will be worse off, some may even have been made bankrupt, but life will go on, on Sunset Boulevard and elsewhere.

Unfortunately, the truth is different. Aphids are pests and the stringent quarantine regulations in the United States that prohibit the unchecked importation of citrus fruit make the United States especially vulnerable to unfamiliar pests such as the Medfly. One concomitant of policies based on quarantine is that the consequences of a breakdown must be draconian. In Britain, for example, where vaccination against foot-and-mouth disease is not practised, infected animals must be slaughtered; and potential harbingers of rabies must be kept in isolation for six months. In California, the recognition of the first Medfly should have been followed by a vigorous attempt at its eradication.

Governor Jerry Brown was, by all yardsticks, slow to come to this conclusion. Everybody will sympathize, although not everybody will condone. In many ways, it is admirable that California should have taken the lead, over many years, in the protection of its own delectable environment. Governor Brown himself, one of the torch-bearers, has benefited politically as a result. Much of what has been done in California to reduce the concentration of nitric and nitrous oxides in the atmosphere has cost a great deal of money, but somehow the state (and Mr Brown) has scraped along. The trouble with the Medfly is that it has challenged the governor in a way that could not fail to be politically embarrasing. Nobody who wants to stay in Sacramento can, with an easy conscience, order out the spray guns. So the Medfly has prospered. It would be sad if Governor Brown's fortunes, as a result, went into decline.

Biotechnology's first casualty — so far

DNA Science left out on a limb

Washington

E.F. Hutton, one of Wall Street's leading investment houses, is having second thoughts about its involvement in recombinant DNA research. Earlier this month the company backed out of a deal it had set up which would have used money from over 40 institutional investors to support academic research in both US and foreign laboratories. Now it is talking of an alternative, less ambitious scheme exploiting recent changes in tax legislation to sell genetic engineering research as an attractive tax shelter.

The initial plan, first announced in February of this year, was to channel investment funds to scientists through a company set up by Hutton called DNA Science. The centrepiece of this scheme was an agreement with the Weizmann Institute of Science in Israel for collaboration on research projects. Weizmann would have provided the molecular biologists and DNA Science the chemistry through Nobel laureate Dr Christian B. Anfinsen, appointed chief scientist of an Israeli subsidiary called Taglit.

According to Hutton, the deal fell apart when the investment company began to have doubts about the legal propriety of mixing "passive" and "active" investors, some merely seeking a financial return, others demanding a greater involvement. The potential conflict between these goals surfaced when one proposed investor, Johnson and Johnson, began negotiating for exclusive marketing rights to any products which resulted from DNA Science-financed research.

Failure to reach agreement before a target date stipulated in the publicly-distributed prospectus, by which \$40 million was to have been raised, meant that Hutton was required to return the money it had already collected from outside investors. But the debate over the appropriate form of involvement by investors also reflects growing concerns that the potential commercial benefits of genetic engineering may have been oversold.

In addition to dropping the Weizmann deal, which would have focused largely on interferon, other proposals now being reassessed include an arrangement with the Battelle Memorial Institute in Columbus, Ohio, for research into projects such as the use of microorganisms to degrade toxic wastes, and collaboration with Dr John Baxter of the University of California

in San Francisco.

Dr Harsanyi, vice-president of DNA Science, says the company will now concentrate on putting together individual projects or "packages of projects" for separate funding by groups of investors, rather than trying to raise the initial capital in one lump. This means shifting the focus from long-range research to projects with more clearly-defined commercial goals.

Exploiting the tax haven incentives offered by research and development financing that were recently increased by the federal government will exclude some of the original investors, such as pension funds, which already enjoy significant tax concessions.

Ironically Hutton was one of the first major financial houses to promote the long-range commercial potential of recombinant DNA research. Nelson M. Schneider, a pharmaceutical analyst with the company who is credited as the principal architect of DNA Science and is now one of its vice-presidents, reported to a congressional committee in June that, if present trends continue, by 1985 private companies could be putting as much into biomedical research as are the National Institutes of Health (NIH).

Under the scheme unveiled in February, which had been suggested as a model for university/industry collaboration in supporting recombinant DNA research,

DNA Science would have set up a number of small "daughter companies" near university campuses in which university staff could apply themselves to commercially-oriented research while maintaining their campus links.

This was to have been the arrangement with the Weizmann Institute under an agreement reached with the institute's commercial arm, the Yeda Research and Development Corporation. Dr Anfinsen said last week that he had resigned from his post as head of the laboratory of chemical biology at the NIH's National Institute of Arthritis, Metabolism and Digestive Diseases to take up the Israeli position with DNA Science. He said that he was waiting to see what happened at the Weizmann Institute, which might continue working in the same direction on its own, before deciding what to do next.

DNA Science officials are confident that much of their work in finding both investors and research projects needing support can be salvaged. Dr Harsanyi says that several university groups have approached Hutton, interested in exploiting their research, but reluctant to become involved in the commercial management of their results. DNA Science is offering to take on this management responsibility, which could include finding potential customers for products.

David Dickson

CERN declares peace with the locals

The 26-km circumference large electron-positron ring (LEP) proposed for construction at CERN near Geneva seems to be steering itself successfully among the several political shoals that surround the project. The lastest development is that CERN and the most active environmental opposition group, Agena, have issued a mollifying joint statement.

Agena, which includes a local mayor among its six members, was successful in halting work on a reconnaissance gallery by means of a legal technicality. It has also been active in canvassing support against LEP within the Jura region of France, so any degree of rapprochement between Agena and CERN is significant.

In the statement, Agena says it is concerned about LEP polluting water and the air, and about LEP's possible effect of reducing employment at CERN (to help pay for the project). Agena asks that CERN should adopt a "different approach" and give local people time to express their opinions on the project.

For its part, CERN promises to consult all relevant associations and trade unions in "an atmosphere of cooperation"; says that it "understands" Agena's fears; that anyone is free to speak to CERN and its director-general, Professor Herwig Schopper, and that no work will be done until an environmental impact statement is complete, and the French and Swiss authorities have been given time to react through the normal national channels providing planning permission.

This latter move is an important step for CERN — although Schopper proposed it at the June meeting of CERN Council, well before the joint statement with Agena. Legally, CERN is an international organization which officially needs to deal only with the foreign ministries of member and host states. But construction of LEP, reaching deep into French and Swiss territory far from the original CERN site, has occasioned new rules.

The principal step is the production of an environmental impact statement for the French Ministry of Research and Technology, the Ministry of Environment and the Ministry of External Relations in October. Then, under French law, a commission of inquiry will be set up which will assess local opinion and invite witnesses (including, presumably, Agena itself). The commission will report, and finally a decision will be taken either by the local préfet of the département or the Conseil d'Etat. This process could take until March 1982. Swiss law is less exacting, but the French process is likely to be longer.

Meanwhile, the French research ministry

is taking steps to unblock the restraint on the LEP reconnaissance gallery, which depends on whether the gallery is a temporary or a permanent construction. If it is temporary, the law under which permission was originally granted is appropriate; if permanent — as Agena successfully argued — it is not. The ministry is attempting on the one hand to argue that the gallery is, after all, temporary and on the other to get approval through a different law. Robert Walgate

Particle physics

Director resigns

Washington

Dr George Vineyard, director of the Brookhaven National Laboratory on Long Island, New York State, announced last week that he is stepping down in order to return to full-time research. His resignation has been accepted "with regret" by the board of trustees of Associated Universities Inc., the consortium of nine universities which operates the Brookhaven Laboratory for the US Department of Energy. In a statement issued after the board's meeting last Monday, Dr Vineyard said that after nine years as director of Brookhaven, where he has been since 1954, "this appeared to be a good time to make the move".

His resignation has, however, inevitably been linked to growing speculation that the Reagan Administration is contemplating cutting funds for further development work on Brookhaven's planned 400 × 400 GeV intersecting storage accelerator (ISABELLE) which is facing delays and cost over-runs.

Uncertainty over the fate of ISABELLE has been growing ever since the laboratory encountered serious difficulties in developing the superconducting magnets, 1,100 of which will be required to complete the accelerator's two-mile circumference ring (*Nature* 286, 435; 1980).

Brookhaven now says that the problems with the magnets seem to have been solved with a new design, but the delays have inevitably resulted in cost over-runs. The bill for ISABELLE is now put at \$500 million, compared with an original estimate of \$420 million, and completion is now expected in 1988 rather than 1986.

Given the intense competition from the new proton-antiproton collider under construction at CERN in Geneva, which is now likely to beat ISABELLE to one of its principal goals, the discovery of weak vector bosons, those responsible for ISABELLE face a daunting task in maintaining political support for their machine.

Dr Vineyard denies that his resignation has anything to do with the problems with ISABELLE, pointing out that the accelerator has "turned the corner by overcoming technical difficulties with the superconducting magnets". He says that other research at the laboratory is in a healthy state, and that the new national synchroton light source is about to go into operation.

The Reagan Administration has, however, been making lukewarm noises about continued support. A meeting between the High Energy Physics Advisory Panel and the National Science Foundation next month is likely to discuss options. One is a scaled-down design, using superconducting magnets which have already been constructed at the Fermi National Laboratory but produce a slightly lower field that would mean a lower luminosity. Another is to scrap ISABELLE altogether. The Administration is awaiting the outcome of this meeting before deciding whether to go on as planned, or adopt one of these alternatives.

David Dickson

Incoherent scatter radar

Auroral visions

A new high-power radar facility, designed to study the Earth's upper atmosphere and magnetosphere at auroral latitudes, was inaugurated by the King of Sweden on 26 August. The £13 million radar's operation will be supervized by the European Incoherent Scatter Association (EISCAT) whose headquarters are in Kiruna, Sweden. The director of the association is Tor Hagfors.

The member countries of EISCAT are West Germany, France, the United Kingdom (each contributing 25 per cent to the capital and running costs), Sweden, Norway (both 10 per cent) and Finland (5 per cent). Half the working time of the facility will be spent collecting data for common use. For the remainder, each country will have control of the facility for a time proportional to its financial contribution.

The radar was originally scheduled to start operation in 1978 but was delayed due to a faulty klystron — a vital transmitter component which turned out to be more difficult to develop than expected. The delay must have proved particularly frustrating to those itching to make their reputations with the new facility, but EISCAT's twenty or so staff have spent the time making astronomical observations with the receivers and developing computer software.

The facility consists of two radar systems, one operating at VHF (224 MHz) which is expected to be ready by next year, and a tristatic UHF (933 MHz) system with a transmitter/receiver at Tromso, Norway, and receivers at Kiruna and Sodankyla, Finland. The UHF system has already produced its first crop of data. The combined systems will be able to monitor the atmosphere from 50 to 3,000 km.

The incoherent scatter technique, which EISCAT's radar utilizes, exploits the fact

Deadline missed

Legislation to bring Britain into line with the European Commission's directive on the notification of new chemicals will not now be enacted before the 18 September deadline. The Health and Safety Executive acknowledged this week that too many problems still remain to be ironed out. And in any case, Parliament will not reconvene until October.

The executive is not, however, worried that the commission will take proceedings against the United Kingdom — the United Kingdom is not the only laggard. One source of confusion is that the European directive requires the compliance not merely of governments but of other corporate bodies, including commercial companies, which may find that after 18 September they are bound by the directive.

The commission is planning to publish in the near future an inventory of all chemicals at present manufactured and sold in Europe. Companies will have nine months from the date of publication to tell the commission of substances not on the list. After that, new substances will have to be tested (toxicologically and otherwise) in accordance with the directive. Until then, however, the British and other European parliaments will have a breathing space in which to find time to comply.

Judy Redfearn

that radio waves can be scattered off individual electrons and off density variations caused by plasma waves in the ionosphere. This region of the atmosphere, which is ionized by solar and cosmic radiation, extends upwards from 50 km or so. The spectrum of backscattered radio pulses can be analysed to reveal a surprisingly large number of atmospheric parameters such as electron and ion densities, ion composition, electric fields, neutral and ionized wind velocities and temperatures.

Several incoherent scatter facilities have been constructed in the past, the most powerful being at Arecibo in Puerto Rico. Only one has operated at auroral latitudes, that at Chatanika in Alaska, which is itself to be moved to Greenland to allow more effective collaboration with EISCAT. The auroral zone is particularly important to those interested in solar-terrestrial relations because it is here that those relations are most manifest. For example, charged particles ejected from the Sun during solar disturbances can be trapped in the Earth's magnetosphere and may eventually spiral down the Earth's magnetic field lines to precipitate into the auroral upper atmosphere, causing heating and intense electric currents. EISCAT's sensitive radar should allow a detailed picture of such events to be developed, particularly when used in conjunction with other ground, rocket and satellite based Philip Campbell experiments.

Soviet academics

Three deprived

Three Soviet Jewish refusnik scientists have recently been deprived of their higher degrees, apparently as a result of their wish to emigrate to Israel. They are Aleksandr Paritskii, a Candidate of Technical Sciences, formerly employed at the Khar'kov Institute of Metrology on the design of high-precision ultrasonic ranging devices; Valerii Soifer, from Moscow, a Candidate of Biology, specializing in plant mutagenesis; and Mikhail Fuks-Rabinovich of Moscow, a meteorologist.

Paritskii and Soifer, who applied to go to Israel in 1976 and 1977 respectively, were refused permission on the grounds of the "secrecy" of their former employment. The reason that Fuks-Rabinovich did not receive his visa is unknown.

Higher degrees — those of "Candidate" (roughly equivalent to a PhD) and "Doctor" (which normally requires a minimum of ten years further research) — are not, in the Soviet Union, conferred by individual universities, but are instead awarded by a special all-union body, the Higher Attestation Commission (Vysshaya Attestatsionnaya Komissiya; VAK).

Originally VAK came under the Ministry of Higher and Specialized Secondary Education, but was transferred in 1974 to the control of the Council of Ministers by a resolution of the Central Committee of the Communist Party and the Council of Ministers of the Soviet Union. This involved what the chairman of VAK, Professor V.G. Kirillov-Ugryumov, described as "qualitative changes". In particular, he said, the new regulations required postulants for a higher degree to 'combine a profound professional knowledge with a mastery of Marxist-Leninist theory and with the convictions of an active builder of communist society".

Jews who wish to emigrate to Israel clearly do not show such "convictions", but so far the Soviet establishment has been content to expel them from professional employment. The new regulations came into force on 1 January 1976, by which time Paritskii, Soifer and Fuks-Rabinovich had presumably all obtained their Candidates' degrees. Until now there had been no suggestion that the regulation should be made retroactive.

From a practical point of view, the loss of their Candidates' degrees should make no difference to the three scientists. Indeed, the change could even benefit them, since several refusnik scientists have been unable to keep menial jobs which they took to try and support themselves, finding themselves sacked because they were "over-qualified", some facing charges of "parasitism" (being without visible means of support). However, from the point of view of Soviet academic life at large, their deprivation sets what could be a dangerous precedent.

Space economics

Ariane gets an edge

Washington

The fledgling European space industry has scored its first success in the American market. Southern Pacific Communications (SPC), a San Francisco company, has selected the European Space Agency's Ariane rocket for launching its first telecommunications satellites in preference to the Delta launcher of the National Aeronautics and Space Administration (NASA).

Two satellites will be launched for SPC in 1984, which will rent out transponders on the satellites to cable television companies. The deal is a boost for Arianespace, the French-based company which will take over responsibility for the commercial operations of Ariane when the test flights have been completed.

SPC is the first US company to make a firm agreement to use Ariane, although according to Arianespace officials reservations have been made by Western Union, RCA and Satellite Television Corporation and preliminary negotiations have started with GTE. Intelsat, the international telecommunications organization, has signed up for Ariane launches for three of its next series of satellites, due to be placed in orbit next year.

The major attraction seems to be the price. Arianespace is offering extremely favourable terms for a launch, including a requirement that only 20 per cent of the launch price—thought to be about \$40 million for the SPC satellites—need be paid before the launch, with the remainder spread over the following five years.

Officials with the Grumman Corporation, which is acting as the marketing agent for Arianespace, are also making the most of continuing delays that are expected in the initial schedule for launches by NASA's space shuttle. An advertisement placed in the US press shortly after the successful third launch of Ariane from French Guiana in late June lists among the advantages of conventional launchers "well known technology and firm dates for operational customers".

The deal with SPC reflects the growing problems that NASA faces in putting the space shuttle into operational service. Already budget restrictions and various technical difficulties have forced the agency to cut the number of flights planned in the first five years of operation from 48 to 34. Priority in scheduling these early flights is being given to the Department of Defense, requiring several commercial companies either to delay their launches or seek more conventional launches.

Intelsat, which had initially planned to launch its next series of satellites from the shuttle, chose Ariane rather than the Atlas Centaur launcher primarily because of cost. Now SPC, which is eager to tap the rapidly burgeoning field of cable television — and has already sold all the transponders on its first satellite — has selected Ariane also on

cost grounds.

NASA, not surprisingly, is not too happy with these developments. Although accepting what it sees as fair competition from the European space industry, some officials in the agency feel that the generous financing terms being offered represent a government-backed subsidy which they are unable to provide.

But the agency has enough problems of its own to worry about in getting the shuttle into full operation. There is now talk of projected cost over-runs in the shuttle programme next year of up to \$500 million, which could have a significant impact on several of NASA's activities

One way of absorbing some of these costs, if the Administration does not come up with additional funds, would be to make still further reductions in the initial launch schedule, cuts which would again fall most heavily on commercial customers. Another would be to make deeper cuts in the space science programme, perhaps delaying or even cancelling the Galileo mission to Jupiter, scheduled for 1986.

With the White House talking of further substantial reductions in public expenditure next year, NASA's prospects look bleak. None of this augurs well for the International Solar Polar Mission (ISPM), planned jointly with the European Space Agency. Congress is still debating whether to replace funds for the spacecraft which the United States is contributing to the two-vehicle mission, which was axed by the Reagan Administration in March.

Publicly NASA officials are continuing to express enthusiasm about maintaining the US involvement in the mission. But internal budget proposals sent up to NASA administrator James Beggs for the next year are said not to include any funds for the ISPM spacecraft (nor for a mission to Halley's comet, now being pushed as a sample-return mission to distinguish it from the European Space Agency's Giotto programme). This could change before the final budget proposals are agreed to either by the NASA hierarchy or with the Office of Management and Budget; but nobody is David Dickson being too optimistic.

Judy Redfearn adds:

Arianespace says that potential customers have been heartened by the success of the test flight from Kourou, French Guiana, in June. Those already holding launch reservations are apparently beginning to negotiate term contracts. One official guesses that three of the 14 reservations now on the books will be made firm by the end of December. The Ariane rocket will put a satellite into geostationary orbit for between \$27 and \$28 million. Thor Delta launches now cost up to \$7 million more. The attractions of the shuttle. originally estimated to cost between \$15 and \$16 million per launch, have diminished with the uncertainty about the project and the threat that the cost will increase by more than half in 1985 and perhaps by three times that.

Deep sea research

Watery grave?

Washington

In a compromise designed to meet conflicting pressures on oceanographic research, the National Science Foundation (NSF) has decided to incorporate its current Deep Sea Drilling Project (DSDP) into its new Ocean Margin Drilling Program (OMDP), the latter to be financed jointly with the oil industry using the drilling ship Glomar Explorer to investigate the geology of the ocean bed.

The decision will mean phasing out Glomar Challenger, the ship from which DSDP currently operates. Several scientific goals of the programmes will continue in drilling from the Explorer. To accommodate them, part of Explorer's own research programme, to be directed at the ocean margins beyond the edge of the continental shelf, will be delayed.

NSF officials hope that by combining the two programmes they will significantly reduce the total costs of scientific ocean drilling programmes and provide a "realistic framework" within which both can be pursued. In addition to the financial advantages, they concede that the decision should reduce some of the friction that had been caused by the scientific community's concern that commercial goals would squeeze out scientific objectives.

Ten oil companies have so far agreed in principle to contribute towards the costs of the Ocean Margin Drilling Program, announced last year by NSF as a jointly-funded venture (*Nature* 283, 321; 1980). The project had been enthusiastically promoted by the Office of Science and Technology Policy as a model for future collaboration between the public and the private sector.

Many scientists, however, feared that too much of the OMDP activity would be concentrated on areas of the ocean floor of greater interest to the oil industry than to the academic community; also that the series of experiments currently being conducted from Glomar Challenger, which can drill holes more cheaply and quickly but is more restricted in its performance, than Glomar Explorer, would be brought to a premature conclusion.

Dr John Slaughter, director of NSF, has now announced a compromise. Glomar Challenger will be retired in about 1983, when experiments would be transferred to Explorer. Plans to equip Explorer with a "riser" — a casing around the drill used to prevent blow-outs from occurring if pockets of oil or gas are hit — would be carried out 2 or 3 years later. After that the Explorer would divide its time between OMDP (to which industry will contribute about \$18 million a year) and an extension of the current non-riser drilling being carried out by Challenger, a solution which seems acceptable to both scientists and **David Dickson** industry representatives.

A Press Council adjudication

This is the text of an adjudication issued by the UK Press Council on 26 August:

The Press Council finds the author was invited to submit an article but not that there was an undertaking that the article would be published. Notwithstanding the invitation, whether it would be published remained a matter for the editor's decision. Alterations which met the criteria laid down by the editor had not been made by the time he told Dr Miller he had decided after all not to publish the article and suggested he send it elsewhere.

The editor did, as Dr Miller complains, submit the article to another scientist mentioned in it, but the Press Council is satisfied he did so with Dr Miller's consent and no complaint can be made at this.

The council is not satisfied the editor used material from the unpublished article in his editorial comment dealing with the same subject. The council, however, believes that the timing of the editorial comment so soon after the rejection of the article was unfortunate. It shares the view put to it by the editor that he ought to have given Dr Miller a fuller answer sooner — and thus an opportunity to submit his article to another scientific journal for consideration before publication of Nature's editorial on the subject. The complaint against Nature is not upheld.

The explanatory statement issued by the UK Press Council is as follows:

Although a scientist was invited to submit a magazine article, the decision whether to publish it was for the editor, the Press Council has ruled.

Soon after an invited article was rejected, the magazine commented editorially on the same subject. Despite the unfortunate timing, the Press Council said, it was not satisfied the editor used material from the submitted article.

The council did not uphold a complaint against *Nature* by Dr Jeffrey H. Miller, professor in the Department of Molecular Biology at the University of Geneva that, having refused to publish an invited article submitted to him in confidence and amended by the author according to requirements stipulated by the editor, the editor submitted the article to a person mentioned in it and used some of the material in the article as the basis of his own editorial comment.

Dr Miller suggested an article to *Nature* and was asked to submit it. In it he forecast growing controversy over mixed industrial and academic research into applied microbiology. The article contained criticisms of Professor Charles Weissmann, of the University of Zurich.

Mr John Maddox, who then became editor of *Nature*, sent Dr Miller an amended copy of the manuscript, saying charges against individual scientists should be justifiable and temperate. Dr Miller sent a second version including

many suggested changes but which was still critical of Professor Weissmann.

The editor phoned Dr Miller saying he was ready to publish the article but would like to show it to Professor Weissmann. Dr Miller gave permission. Mr Maddox later said he could not publish the article: it was too directly concerned with Professor Weissmann's work.

Dr Miller then sent a third version, saying he had agreed to the professor seeing the article because he assumed it was agreed in principle to publish it. Although he asked for an explanation if the article was not published, he did not receive one.

Soon afterwards *Nature* commented editorially on the propriety of academics being involved with commercial interests, saying attempts to exploit new genetic manipulation techniques could sour the atmosphere of academic research.

After the solicitors for Dr Miller complained to the Press Council, the editor said it was a convention of the scientific press that personal criticism by outside contributors was not published without warning. He would have replied to Dr Miller if the latter had not told a colleague that he would complain to the council if his third draft were not published.

Dr Miller said the editor's plagiarism of themes not before similarly expressed made it difficult to publish his article elsewhere. Mr Maddox argued that Dr Miller's comment covered the whole academic community while the leading article dealt primarily with molecular biology.

Mr Maddox said there were incorrect and unpublishable charges against Professor Weissmann in the first version. He had had two long phone conversations with Dr Miller. In the second he said he would publish the article if the professor could see it. Discovering that Professor Weissmann acted with propriety had undermined his confidence in Dr Miller's reporting.

Dr Miller commented he had understood the article was accepted for publication and only the Weissmann references were in doubt. The reasons given for withdrawal had no substance and the editor drew heavily on his article.

Dr Miller could not attend an oral inquiry. Mr Maddox told the complaints committee he showed the second version to Professor Weissmann, who explained he had a formal agreement regulating his commercial activities. This was not mentioned in Dr Miller's drafts. Mr Maddox said that his reaction to the third draft was that Dr Miller could have made the amendments earlier but that on reflection he regretted his rather hottempered reaction that there was no point in further correspondence.

Nature has no further comment to make except to hope that Dr Miller (and Professor Weissmann) will continue to contribute to its columns.

CORRESPONDENCE

Coping with cuts

SIR — The sad plight of UK universities facing increasing costs and diminishing financial support, possibly even the elimination or demotion of "redundant" universities, moves me to suggest an arrangement not uncommon in American universities, whereby members of university faculties have 9-month rather than 12-month appointments.

This means that faculty members have 3 months in the year free for other activities. Scientists and engineers can frequently find summer employment in government and industrial laboratories, where the broader experience may well be beneficial to their university teaching and research. In any case, the experience may be valuable in combating the ivory tower outlook and maintaining contact with the real world. Colleagues in the humanities may find it more difficult to obtain alternative summer employment, but they would be free to write books, paint pictures, compose music and thereby practice the arts they teach.

In many cases, it may not be necessary for the "9-month scientist" to forsake his laboratory. A large part of American (meaning US) university research is funded by grants from government agencies and from industries. It is common practice for a research proposal to include not only support for a post-doc associate or a graduate student assistant, but also an item for 50 per cent or even 100 per cent of the time of a faculty member for two or three months of the year. Such support is by no means automatic but depends on the quality of the proposal, that is, on the quality of the person making the proposal.

Thus in one way or another the really capable people on university staffs can add usefully to their salaries and those who have "retired" from active productive scholarly work and have little or no standing in their subjects are left where they belong, at a lower level on the financial ladder.

Much good can come from the operation of the free enterprise system, and those who prefer the old, easy-going ways must accept lower annual remunerations.

G.W. BRINDLEY

Pennsylvania State University, Pennsylvania, USA

Nuclear electricity

Sir - In commenting on the analysis of relative costs of nuclear and coal-fired electricity given in Appendix 3 of the Central Electricity Generating Board (CEGB) 1979/80 Annual Report, I stated that "the full calculation (of costs corrected for inflation) can only be undertaken when the CEGB decides that its present policy of withholding the data . . . is counterproductive" (Nature 287, 674; 23 October 1980). I had first asked in specific detail for the data at the beginning of September 1980 and had pursued the matter through both the Department of Energy and the CEGB to the extent of getting a discussion meeting with both department and board officials present. In spite of this the detailed figures of capital cost only became available at the end of March 1981 and then only as the result of a parliamentary question put down on 2 February.

The results of the present calculations, utilizing the newly available data are summarized in the table below. These calculations are significantly different in method from those used in an earlier paper (Energy Policy, December 1980, p.344-6) and referred to in my letter of 23 October. The retail price index (RPI) has been used to correct for inflation and a uniform interest rate of 5 per cent has been applied instead of the historic National Loans Fund rate.

However, as the table below shows, even with these modifications the inflation corrected cost of nuclear electricity from Magnox stations is still 18 per cent above that of comparable coal fired stations, instead of 17 per cent below as given by historic costs, and if a previously unconsidered effect of inflation on nuclear fuel costs is included, the margin becomes 34 per cent above that for coal.

Calculations similar to those for the stations of Table 1, Appendix 3 have been done for Hinkley Point B and Drax (first half) (Table 2). The results are also given below. The full paper giving the details of the calculations and dealing also with stations under construction and future stations (Tables 3 & 4, Appendix 3) is in preparation (to be submitted to *Energy Policy*).

Generation costs at nuclear and conventional stations (pence per kWh 1979/80 prices)

Stations commissioned between 1965 and 1979

	1	2	3
Nuclear	1.30	2.06	2.34
Coal-fired	1.56	1.75	1.75
Nuclear/Coal %	83	118	134

Hinkley Pt B (AGR) and Drax (first half) (coal-fired)

	4	5	6	7
Hinkley Pt B (nuclear)	1.35	1.80	2.06	2.38
Drax (first half) (coal-fired)	1.52	1.69	1.69	1.69
Nuclear/Coal %	89	107	122	141

Notes to columns: (1) Historic costs as given by CEGB in Table 1, Appendix 3, 1979/80 Annual Report. (2) RPI corrected capital costs, 5 per cent interest rate and fixed costs corrected for load factor equal to availability. (3) As 2, but with the addition of an estimated minimum inflation correction for nuclear fuel costs. (4) Historic costs as given by CEGB in Table 2, Appendix 3, 1979/80 Annual Report. (5) RPI corrected capital costs and 5 per cent interest rate. (6) As 5, but with the addition of an estimated minimum inflation correction for nuclear fuel costs. (7) As 6, but estimated probable fuel cost correction.

Finally may I take up one point in Dr Jones's letter (Nature 288, 638; 1980) in which he criticizes my use of the combined coal and oil fuel costs in Fig. 1 of my letter of 23 October. Separate coal fuel costs in p/kWh similar to the combined ones of Table 9 of the CEGB Statistical Yearbook are not available, but figures of coal prices to the CEGB in p/GJ from 1960/61 to 1979/80 have been supplied by the Commercial Controller. When the real price index is normalized to equality with the coal and oil figures for 1972/73 and plotted on the same graph there is no significant difference between the points. I understand from Dr Jones that he was considering National Coal Board production cost data which may well give a different picture, but it is the price to CEGB and specifically the coal fuel cost in p/kWh which I was dealing with and my graph is an accurate reflection of this.

J.W. JEFFERY

Birkbeck College, Malet Street, London WC1, UK

Attack on Tamuz

SIR — Your recent editorial "Making Israel atone for Tamuz" (Nature 18 June, p.523) leaves out many facts which are important in considering the reasons for Israel's attack and the reality of the situation.

As you and I are aware, nations do what is in their best interest regardless of what treaties they do or do not sign. Just because one nation is a signatory of the Non-Proliferation Treaty does not mean that it will not produce a nuclear weapon. Iraq has been one of Israel's most aggressive and belligerent neighbours, and in fact these two countries are still at war with one another, as no peace treaty has ever been signed. What could this or any other country do if indeed Iraq or any other nation had a nuclear weapon and threatened to use it against an enemy? We could not stop the Soviet Union from invading Afghanistan, nor prevent the problems of Iran. We cannot even clear up the fighting in Lebanon. The world needs Iraq's oil, so "international pressure" would never work

I agree with your statement concerning the use of oil to generate hydroelectric power, but why did Iraq refuse to accept lower grade uranium that would work very well in a nuclear reactor to produce electricity, but would not be able to produce atomic weapons? Iraq demanded from France weapons grade uranium or their oil supply treaty would be null and void. In addition, one of the inspectors from the International Atomic Energy Agency stated during an interview that the reactor at Tamuz could easily have produced nuclear weapons and that such production could have been shielded from any inspector, thus making the whole question of on-site inspection ludicrous. Although Iraq was supposed to return the spent uranium to France for reprocessing, Iraq also had uranium already purchased from Third World nations, thereby bypassing France completely. You also do not mention that Iraq had contracted with Italy to build a "hot cell" plant that would also allow for enriching and processing plutonium for no other purpose than to build an atomic bomb. M.S. PASCAL

Englewood, New Jersey, USA

SIR — Israel's attack on the Iraqi reactor may have been politically unwise, even immoral (although I suspect Benthamites might be able to generate some fairly strong Utilitarian arguments in support of the raid). It is, however, something approaching hyperbole to characterize the raid as a flagrant violation of international law (Nature 18 June, p. 523), as Iraq remains in a technical state of war with Israel, and has repeatedly called for the destruction of the "Zionist entity".

It is also somewhat naive, to be gentle, to assert that Iraq's acceptance of the principles of the Non-Proliferation Treaty assures compliance with its provisions. Soviet citizens who seek the enforcement of the principles of the Helsinki accord often must pack for very cold climates on very short notice.

On judgement day when we must all atone, there may be many whose need for absolution will be greater than that of the Israelis.

New York, USA

ELLIOTT B. GROSSBARD

Neutron bombs

SiR — Neutron weapons, I was astounded to read in your journal, "are a blessing and not a curse". With blessings like that, who needs curses?

That surely is the point about the theory of nuclear deterrence. Its insane logic presents every new cursed twist of the nuclear arms race as a blessing. The 50 and 100 megaton weapons were supposed to be a blessing because politicians would hesitate more before unleashing Armageddon. You now argue that neutron bombs are a blessing because they obviate the need to use the big bombs first. Next you will argue, if a low-radiation highblast bomb is developed, that this is also a blessing since it will destroy property but not people. And a Doomsday bomb that would blow the world asunder would also be a blessing because of the fear of starting a war that it would put in the enemy.

In the last analysis, those who support the nuclear deterrent theory will admit that these weapons are all a curse. That's why one wants nuclear disarmament. But that sane admission cannot co-exist with the dogma that new weapons of death are a blessing. If you allow it to, you become guilty of a form of double-think which is scientifically dishonest and politically and militarily disastrous.

As for the "cynical" (as you call it) argument that neutron weapons will help disarmament negotiations, it is not so much unrealistic, as you argue, as just plain stupid. It is surely obvious that balanced mutual force reductions are more difficult to obtain agreement over the more numerous become the weapons systems deployed on both sides. To argue the opposite is perverse.

I am a "lay" reader of *Nature*. Your support of neutron weapons seems to me to be a betrayal of the efforts of your contributors to understand — and improve — the world we live in. Your arguments do disservice to the entire scientific community and the human values that give it purpose.

MARTIN RABSTEIN

London N5, UK

THE phrase complained of appears in the following sentence: "In the bizarre logic of the nuclear battlefield, in which strategic nuclear weapons are intended to stay forever in wonderland, neutron weapons are a blessing and not a curse." — Editor, Nature.

Shielding tanks

SIR — The United States Government and the British Ministry of Defence claim that the neutron bomb is a weapon developed for use against tanks. You have stated (Nature 13 August, p.571) that these weapons are more easily (than "ordinary" nuclear weapons) directed against military personnel.

By making reasonable assumptions about neutron weapons concerning the neutron energy spectrum and the ratio of the neutron and gamma energy fluxes it can be shown that a radiation shield could be incorporated into the armour of a tank which would reduce the radiation dose to the crew by a factor of over 100. Such a shield would negate the enhanced radiation properties of a neutron weapon. The neutron bomb would become no more effective than normal nuclear weapons of similar explosive power.

The precise constituents of such a shield would depend on whether it would be attached on the outside of the tank or added as a lining to the crew compartment. Let us assume that for the tank armour possibly 5 cm of steel can be used as the outer component of the shield. A lining of 10 cm of polythene or other hydrogenous material would thermalize the neutrons and a millimetre of boron or other neutron absorber would remove the neutrons. The inside layer would be a gamma shield and 1 cm of lead would be adequate. Such a shield would add about 5 tons to the weight of a 30 ton tank.

This type of shield, which could be widely incorporated into tanks and military vehicles, would make neutron bombs no more effective against tanks than the small fission nuclear weapons which are reputed to be widely distributed to United States forces throughout the world.

The United States insistence that neutron bombs are for use against tanks in the face of widely available information which enables tanks to be protected against the special effects of neutron bombs, forces the impartial observer to attribute the real reason for the production of neutron bombs to their potent ability to kill people whilst causing only the minimum of collateral damage.

J.E.F. BARUCH:

The University, Leeds, UK

Local reaction

SiR — Apparently, Lord Rutherford's maxim that there is mathematics and physics and that all other activities are stamp collecting, is still valid in Ms Rich's writings on Yugoslav scientific institutes. The result is a biased assessment of the scope of their competence, specifically in problems related to nuclear power (Nature 11 June, p.446-447). A quick check with the Science Citation Index would show the scientific activities of the two cited "physics" institutes to cover a broad range in Rutherford's "philatelistic" category.

Contrary to the statements in *Nature*'s article, there is no "local misunderstanding of the distinction between the charging of a reactor and its start-up". There is no misunderstanding or lack of knowledge with respect to reactor or nuclear fuel technology, much less on the importance of additional power sources in a power-hungry country with few significant energy resources still available for exploitation. Nor has there ever been any misunderstanding based on ideological doctrinarism, contrary to what could have been perceived from another article by the same author (*Nature* 288, 5; 1980).

The dispute is over the technological and organizational discipline necessary in building, starting and operating high technology units like nuclear reactors. The one mishap, in 1958, was the result of just such a typical breach of work discipline.

The dispute is rather between the narrow, mission-oriented technocrats and the concerned scientists on the consequences of siting large facilities in an already ecologically strained region. Scientists insist on careful planning and broadly based environmental impact assessment. Their concern reaches beyond the narrow, albeit possibly correct, advice of "no technological obstacles", offered by a reputable international expert to our supposedly less developed country. The dispute is well within the framework of questions raised in reviewing the Final Safety Analysis Report, and concerns another problem — that of the reference plant for the

Krško unit.

The scientific community is not beyond reproach either. Its lack of credibility, or "weight", with the decision-making social strata stems from some recent and not so recent failings. But it should not be blamed for either disciplinary monoculture or primitive ecological extremism.

I could not refrain from voicing my displeasure with these articles, although this letter might prove just another exercise in futility. Ms Rich's articles remind me of a statement made by one of your great historians. Writing about an infamous propaganda minister of recent European vintage, he claimed that the points in the minister's propaganda items were made so well, that even the opposite of it was still not the truth.

VELIMIR PRAVDIĆ

Ruder Bošković Institute, Zagreb, Yugoslavia

VERA RICH WRITES — I cannot accept Dr Pravdic's statement that there is no "local misunderstanding". During my visit to Zagreb in March and April of this year, I spoke with a number of members of the public who interpreted the announcement of the imminent "charging" of the reactor to mean that it would "start working" immediately. I fully agree that the main dispute lies between the "concerned scientists" and the technocrats. Perhaps the undoubted local apprehension arises from a fear that in such a conflict, the technocrats may win.

Mystery genre

SIR — In reading Edmund Leach's review of the book Genes, Mind and Culture by C.J. Lumsden and E.O. Wilson (Nature May 21, p.276) it strikes me that comments such as "crass idiocy", "parody of science", "gibberish" and "phoney" might be a trifle intemperate coming from a reviewer who admits that he cannot comment on the bulk of the work, which is in neuroscience and psychology.

What is far more intriguing is the question of what Leach could have had in mind when he referred to the "genre" to which Genes, Mind and Culture belongs. At first it went right by me, but I did a double take later when I recalled that Leach had once written a review on the genre of "popular ethology" books, such as those of Lorenz and Ardrey. It was hilariously entitled "Don't say 'Boo' to a Goose" and appeared in the New York Review of Books.

That was 15 years ago. Is it possible that Leach is still referring to the same genre? I ask, because — significantly — he does not mention the major theoretical work Sociobiology in his list of E.O. Wilson's credentials. Worse, he calls Wilson a "popularizer". Surely there is not a regular reader of Nature anywhere — on either side of the well-known floor — who conjures up the image of "popularizer" when Wilson comes to mind.

I urge that Leach be invited to identify the mysterious genre in which he has classified the Lumsden-Wilson book and if it is ethology—there is virtually no ethology in Genes, Mind and Culture—then clearly his review must be rescinded from the pages of Nature as having been written by a uniquely unqualified party.

N. JACKSON

Belmont, Massachusetts, USA

Nobel Physics Prize in perspective

Robert Marc Friedman*

WITH the time for the announcement of the 1981 Nobel Prizes approaching, it is timely to consider the nature of these awards. There is a need for a wider understanding of the process by which these honours are determined: a realistic appreciation of the prizes can serve only to enhance their value¹. So far, however, little has been written about the decision making process. By drawing on historical research, I shall seek to elucidate some of the issues which have been raised, especially relating to the scope of the prize in physics.

To what extent does Nobel's will define the field of physics that can be considered for a prize? Little was specified in Nobel's will and, while drawing up the statutes, the prize-awarding institutions resisted all attempts to delineate or define the broad terms "physics", "chemistry" and "physiology and medicine" used in the will². Hence, when the members of the committee for the prize in physics met for the first time in 1901, they were faced with

B. Hasselberg, wrote that the prize could be awarded for great achievements not only in "pure Physics properly so-called", but also in "the sciences most closely connected with Physics and for the cultivation of which physical methods are employed[;] as [such] Astrophysics and physical chemistry are also to be taken in account. This I suppose will certainly answer to the proper meaning of the testator. Whereas thus ... pure Astronomy or celestial dynamics or Astrometric [sic] will scarcely be included, any work on Astrophysics of great importance deserves certainly a thorough consideration"5.

Moreover, the inclusion of meteorology raised little, if any, objection; as well as acting as host for this meeting, the Meteorological Institute's director, Professor H. H. Hildebrandsson, served on the original committee. At the meeting, committee members called for strengthening the expertise in "cosmical physics" (meaning

The history of the award of the Nobel Prize in Physics, which is partly the history of interpreting the statutes, also depends on the development of physics in Sweden. The now traditional restricted scope of the prize has its origin in past controversies, not formal rules. Einstein's relativity and Bjerknes's meteorology were both casualties.

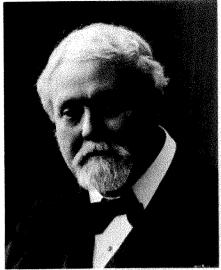
the same ambiguities in the statutes that we recognize today. How ought they interpret and reconcile such clauses as "have conferred the greatest benefit on mankind" and "most important discovery and invention within the field of physics"? The provision in the will that works "during the previous year" should be interpreted to mean "for the most recent achievements . . . and for older works if their significance has not become apparent until recently", created further opportunity for uncertainty and arbitrariness.

During the early years, both the committee and the executors of the estate understood that physics ought to be defined as broadly as possible³. At the first committee meeting, held at the Meteorological Institute of the University of Uppsala, the committee agreed that astrophysics was eligible while astronomy was not⁴. More specifically, in responding to an inquiry, the head of the committee,

the physics of the macro-world) on the grounds that nominations from this branch of physics would surely increase⁶. In fact, during the next thirty years, nominations to honour various astrophysicists, geophysicists and meteorologists were not and could not have been formally disqualified for being outside "physics" (for example, G. E. Hale, H. Deslandres, K. Birkeland, C. Størmer, N. Shaw, J. Hann, V. Bjerknes). In short, neither Nobel's will nor the statutes restricted the definition of physics to a narrowly defined group of specialties.

Narrow scope

I shall argue that the subsequent restriction of the scope of the physics prize stemmed from a determined effort within the Swedish physics discipline. Not surprisingly, Nobel Prize deliberations became enmeshed in the process by which various factions within the Swedish physical science community attempted to define and to legitimize their particular notions of physics: its objects, methods and goals. The history of prize decisions, being also the history of interpreting the



H. H. Hildebrandsson, professor of meteorology and member of the committee for physics (1901–1910), hosted the committee's first meeting.

statutes, cannot be divorced from the development of the physics discipline in Sweden. Consequently, various traditions have been established for interpreting the statutes, which arose from past conflicts, debates and strategies for attaining authority, that have little relevance for the present. Traditions grounded in past contingencies ought not be mistaken for immutable, formal rules and regulations. Unfortunately, literature concerning the Nobel Prizes tends to discuss the awards in the passive voice, and thereby tends to reinforce the mystique of anonymity and objectivity in the decision process. This frequent use of the passive voice also slights the committee members, the difficulty of whose task is easily overlooked.

The Nobel Committee

Committee members play critical roles by evaluating nominees and selecting nominators. During the period for which committee archives are accessible (1901-30), the committee's five members judged annually the nominations received and then proposed to the physics section (Klass) of the Royal Swedish Academy of Sciences how the prize should be allocated. Following approval from the Klass, or suggestion of an alternative proposal, the full Academy then voted on the award. On some occasions, the Klass, and even the Academy, disagreed strongly with the original choice, so that the committee had to justify its actions carefully, especially when these were controversial or not unanimous. The committee members also drew up each year a list of institutions and individuals who would be invited to send nominations for the following year. Thus the committee regulated to some extent the nominators who might supplement the fixed group entitled to make nominations - members of the Academy, former prize winners, and professors of physics at Scandinavian universities and technical colleges.

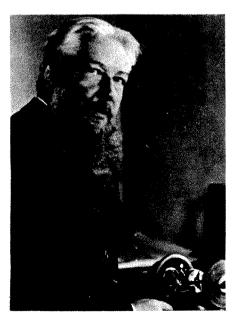
^{*}Institute for Studies in Research and Higher Education, Norwegian Research Council for Science and the Humanities, Wergelandsveien 15, Oslo 1, Norway.

Dissent over Einstein

The influence of the Swedish physics discipline on Nobel Prize decisions is well illustrated by Albert Einstein's prize. In 1922 the Academy voted to award Albert Einstein the previously "reserved" 1921 Nobel Prize "for his services to theoretical physics, and especially for his discovery of the law of the photoelectric effect". Both the timing and the citation have long raised questions among Nobel Prize watchers. If the opinions of the scientific community at large, or more specifically of the nominators, had been decisive, Einstein would certainly have received a prize earlier, and for his theories on special and general relativity.

As early as 1910, W. Ostwald began nominating Einstein for his work in special relativity. During the First World War and immediately thereafter, many nominations for Einstein arrived specifying his research on relativity, brownian motion and specific heats of solids and/or quantum theory in general. Following the famous 1919 solar eclipse experiment which, according to many, confirmed Einstein's prediction of the bending of light by the gravitation of a massive body, a virtual flood of nominations for Einstein ensued.

Yet, in 1920, the committee and the Academy agreed to award the prize to Charles Guillaume. One year later, claiming that they could not find any grounds for awarding a prize to Einstein, the committee, followed by the Academy, voted to reserve the 1921 prize⁷. Finally, in 1922 Einstein received a prize, specifically for the law of the photoelectric effect—not for his quantum theory to explain the law, nor for relativity in any form. Of the approximately fifty nominations of



W. Ostwald nominated Einstein for the 1910 physics prize, specifying work on "the relativity principle". By 1922 many physicists had proposed awarding a Nobel Prize for Einstein's relativity theories; these included: Bohr, Eddington, Haas, v. Laue, Lorentz, Planck, Sommerfeld and Zeeman.

EINSTEIN'S RELATIVITY THEORY OF GRAVITATION

The debate on Einstein's theory was in full flow in *Nature* of 4 December 1919. E. Cunningham wrote:

THE results of the Solar Eclipse Expeditions announced at the joint meeting of the Royal Society and Royal Astronomical Society on November 6 brought for the first time to the notice of the general public the consummation of Einstein's new theory of gravitation. The theory was already in being before the war; it is one of the few pieces of pure scientific knowledge which have not been set aside in the emergency; preparations for this expedition were in progress before the war had ceased.

The question from which Einstein began the great advance now consummated in success was this. If energy and intertia are inseparable, may not gravititation, too, be rooted in energy? If the enrgy in a beam of light has momentum, may it not also have weight?

The mere thought was revolutionary, crude though it be. He asked for an out-and-out relativity of space and time. He would have it that there is not ultimate criterion of the equality of space intervals or time intervals, save complete coincidence. All that is asked is

that the order in which an observer perceives occurrences to happen and objects to be arranged shall not be disturbed.

This, then, was the mathematical problem. The pure mathematics required was already in existence. An absolute differential calculus, the theory of differential invariants, was already known. In pages of pure mathematics that the majority must always take as read, Riemann, Christoffel, Ricci, and Levi-Civita supplied him with the necessary machinery. It remained out of their equations and expressions to select some which had the nearest kinship to those of mathematical physics and to see what could be done with them.

Although in the letter immediately before Cunningham's report, Alexander Anderson of University College Galway concluded:

I THINK it is quite likely that if the refraction of the atmosphere of the Earth due to density changes during an eclipse could be accurately obtained and allowed for, it would be found that there is no Einstein effect at all.

Einstein over the years, the law of the photoelectric effect was specified by only one nominator. Certainly, a familiarity with the committee must be sought to begin understanding these events.

Experimental bias

At the time, three of the committee's five members belonged to the Uppsala tradition of experimental physics — B. Hasselberg, G. Granqvist, and A. Gullstrand. The others were S. Arrhenius (physical chemistry) and V. Carlheim-Gyllensköld (mathematical and cosmical physics). The committee's strong experimentalist bias proved significant from the start in determining Nobel Prize decisions and for interpreting the statutes.

Debates on the role of mathematics and theory in physics that had begun in the 1890s carried over into deliberations on the prize. Hence, even in the case of a worldwide campaign for Henri Poincaré, in which his mathematical physics qua physics was delineated from his purely mathematical accomplishments, the committee did not see fit to recommend him for the prize8. In a protest note, Carlheim-Gyllensköld pointed out the committee's general unwillingness and inability during the first ten years to evaluate nominations in mathematical physics such as those of Boltzmann, Heaviside, Kelvin and Poynting9. Arrhenius had commented that Uppsala physicists consider "spectral analysis . . . the only part of physics worth pursuing '10. Indeed, Hasselberg did "all in my power to procure the prize" (1907) for A. A. Michelson, who had recieved but two nominations, not for his role in the aether-drift experiment but, rather, primarily for his work in precision spectroscopy and metrology11. Hasselberg admitted that he found a "sympathy for an

area closely connected with my own speciality...I cannot but prefer works of high precision"¹².

Other examples abound. Thus the proposal for the 1908 prize was sabotaged partly by those who believed it "unjust" to award a prize to a theoretician, M. Planck, without dividing the honour with an experimentalist¹³. Apparently, to ensure the defeat of the Planck nomination, the Academy was informed of Planck's questionable "hypothetical molecules of energy", which were not mentioned in the committee's report14. Not surprisingly, the majority's stringent demands that theory be completely verified by experience virtually precluded a prize for Einstein's various theoretical endeavours. Yet, did the 1919 eclipse experiment have any impact on the committee's evaluation?

Although often hailed as a "crucial



B. Hasselberg. "Icannot but prefer works of high precision" — on A.A. Michelson's spectroscopic measurements. "It is highly improbable that Nobel considered speculations such as these to be an object for his prizes" — on Einstein's relativity theories.

experiment" that proved Einstein's general relativity, the 1919 test left many reasonable physicists unconvinced, among them the members of the Nobel committee for physics. In 1920 Arrhenius prepared a special report on the subject for the committee in which he noted that objections could be made against the accuracy of the observations, which were therefore not a proof of the prediction¹⁵. In any case the prize for 1920 seemed already determined. Hasselberg then lay gravely ill. Having championed precision measurement, especially in spectroscopy, Hasselberg now favoured his colleague on the International Bureau of Weights and Measures, Guillaume, whose work in metallurgy was of importance for metrology. To express appreciation for Hasselberg's work on the committee since 1901, the Academy could pay homage to him in this manner.

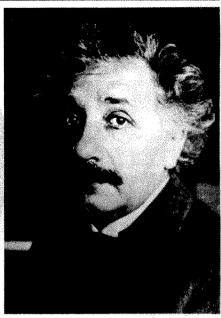
Nevertheless, the Einstein campaign continued to grow in 1921, with strong support for the relativity theories. To ensure a sound evaluation, the committee assigned the task of writing a special report focusing on Einstein's relativity and gravitational theories to its esteemed member Gullstrand, who would have received the 1911 prize in physics for his contributions to dioptrics had he not first been named for a prize in medicine¹⁶. In his fifty-page report, Gullstrand concluded that neither the general nor the special theory of relativity warranted a Nobel Prize¹⁷. In 1922 he brought his report up to date and came to the same conclusion. Acceptance of these theories remained simply "a matter of faith (trossak)"18. He resolved that Einstein must never receive a prize19.

The 1922 Prize

In general the committee supported Gullstrand. Hasselberg asserted from his sickbed that "it is highly improbable that Nobel considered speculations such as these to be an object for his prizes"20. Nor was there much sympathy for honouring Einstein's other theoretical contributions. Even his theory of the photoelectric effect was not considered significant enough to warrant a prize, since much of the elaboration of the quantum theory entailed others' endeavours²¹. Yet, when the Klass met to vote on the committee's proposal to reserve the prize, mathematical physicist C. W. Oseen suggested that Einstein's law, rather than the theory, ought be considered for a prize since this law forms the foundation for all the recent remarkable achievements in atomic physics²². The Klass then voted to acknowledge the significance of Einstein's discovery of the law of the photoelectric effect, but resolved that it was not worthy of a prize²².

Oseen did not intend to let the issue end there. He was concerned not so much with Einstein but with Niels Bohr, who had been repeatedly nominated since 1917²³. He regarded Bohr's atomic model as "the





A. Gullstrand (left) prepared special reports on Einsten's relativity and gravitational theories in which he concluded that they did not warrent a Nobel Prize. The acceptance of these theories remained, even after 1919, "a matter of faith (trossak)".

most beautiful of all the beautiful' in theoretical physics, and was thus determined that this work should receive a prize²⁴. Moreover, he had already begun on a campaign to strengthen Swedish physics, especially by introducing theoretical physics. For Oseen, atomic physics offered the challenge of theoretical investigation while remaining tightly disciplined by experiment. Previously, the experimentalists on the committee balked at approving Bohr's atom model which, they claimed, stood "in conflict with physical laws" and hence reality²⁵.

Now, in 1922 Oseen proposed Einstein for the discovery of the law, and then joined the committee as a special extra member. Oseen wrote special reports on Einstein's law and on Bohr's atomic theories. He carefully argued that experimental investigations had confirmed Einstein's law so thoroughly that it must be considered among the "soundest propositions physics now possesses"26. Hence Bohr's models, which he argued were built on this law, must be regarded as being in solid agreement with physical reality²⁶. Finally, not without some difficulty, Oseen prevailed. Although further study is needed, this episode demonstrates the importance of the committee membership's interests and philosophies of science for Nobel Prize decisions.

The Einstein deliberation came at a turning point in the development of Swedish physics and of the committee. By 1923, after the deaths of Hasselberg and Granqvist, Oseen was elected to the committee as a regular member and was ioined by his Uppsala colleague, atomic physicist Manne Siegbahn. Together with Oseen's friend, Gullstrand, the three Uppsala physicists commanded a majority on the committee and could plan and act together on Nobel Prize matters. Oseen's broad knowledge, international reputation

and aggressive determination allowed him to assume leadership in the committee. His vision called for an overhaul of Swedish physics: stronger links with major foreign research centres, and greater visibility and prestige to ensure adequate research funds and university positions²⁷. By promoting atomic physics, in which experiment and theory intimately progress together, he hoped to overcome traditional prejudices and to institutionalize a theoretical physics standing apart from abstract mathematical physics and mechanics. Yet, to be successful in this "new epoque"28, which he declared after the election of Siegbahn to the committee, they not only had to promote and to legitimize their preferred research programmes, but would also have to eliminate what they considered insignificant specialities. Starting in the 1920s, the Uppsala group began a determined campaign to restrict the definition of physics within the Academy and with regard to the Nobel Prize. To pursue this strategy they could try to change the statutes formally, control membership in the Klass and committee and/or establish traditions for interpreting the statutes by honouring particular specialities and blocking others.

Arrhenius and astrophysics

Change of statutes entails considerable debate and great difficulty, since the entire Academy must vote on such motions. Thus the committee members tried to avoid formal motions to change the statutes since these inevitably breed conflict within the committee and the Klass, which in turn weakens their posture in the Academy. This became clear when, in 1923, the new Uppsala group and Arrhenius attempted to eliminate astrophysics from the scope of the prize in physics. Arrhenius, who wrote the proposal, seems to have feared that the establishment of new Nobel institutions



S. Arrhenius, member of the committee for physics, had originally supported cosmical physics, but in 1923 he tried unsuccessfully to eliminate formally astrophysics from the scope of the Nobel Prize for physics.

would threaten his own Nobel Institute for Physical Chemistry. (According to the statutes, Nobel funds can be used to establish research institutes where nominations can be evaluated.) Previously, only Arrhenius's institute had been supported in this way, but during the war Carlheim-Gyllensköld, suggested a department for cosmical physics that was later followed by detailed plans for an astrophysics centre^{29,30}.

Consequently, Arrhenius proposed that astrophysics no longer should be considered part of physics. Although previously a strong supporter of cosmical physics, he now argued that astrophysics had progressed so dramatically, that it now incorporated all physical astronomy31. Thus, he concluded, astrophysics is astronomy, and therefore not part of physics. Carlheim-Gyllensköld vigorously opposed this action and, predictably, the statute change was defeated³². A much easier strategy entails dismissing outright nominations in cosmical physics as "not being significant for physics", the phrase commonly used by writers of the general report to the Academy. Hence, Hale and Deslandres, previously considered certain winners of the prize, could now be quickly dismissed once Hasselberg had died and Arrhenius no longer supported these sciences.

To proceed in this way requires a committee and a Klass that does not object too strongly—so that membership must be controlled when possible. When the physics section of the Academy expanded from 6 to 10 members in 1904, it was given the title "Physics and Meteorology". Having established strong research traditions in meteorology during the nineteenth century, Swedish meteorologists received a number of places in the Klass. By 1919, however, the promise of Swedish meteorology had waned and a

long, bitter and inconclusive feud concerning atmospheric thermodynamics raised doubts over the personal and/or scientific reliability of some of the meteorologists. Oseen was prompted to express concern whether any branch of "terrestrial physics" could become rigorous, given the scale of geophysical phenomena and lack of laboratory testing possibilities³³; he sensed a "relativity of all knowledge" within these sciences33. Uppsala experimental physicists shared this belief. They attempted to reduce the meteorological influence in the Klass and prevent the awarding of a Nobel Prize to this science, as this would legitimize meteorology as part of physics in the Academy.

Meteorology out of favour

The campaign against meteorology in the Klass seems to have begun after the death of foreign member J. Hann, an Austrian meteorologist. The election of foreign members has a double significance. On occasion, such an election is a stepping stone for prospective Nobel Prize candidates; in general, all foreign members have the right to propose candidates for the prize. In this instance, members of the committee tried to obtain before the meeting the signatures of a majority of the Klass for the appointment of a physicist, thereby precluding replacing Hann with another meteorologist34. Arrhenius sketched a nomination for Planck and travelled up to Uppsala; however, rather than speaking with meteorologist Hildebrandsson as promised, he carefully avoided him while meeting the other physicists. The elderly Hildebrandsson felt betrayed³⁵. But his subsequent request at the meeting of the Klass that they promise to elect a meteorologist next time, was defeated36. And, when foreign member Röntgen died the following year, Gullstrand organized a comparable campaign against electing a meteorologist, saying he would refuse to vote if a meteorologist were proposed³⁷.

Similarly, when Klass members Hasselberg, Granqvist, and Bäcklund died, they were rapidly replaced by physicists Siegbahn, Pleijel, and Oseen. After the death of meteorologist N. Ekholm in 1923, Hildebrandsson feared the worst and decided to take the offensive by bringing the touchy issue into the open and up to the entire Academy. Now that physicists had twice been elected to succeed meteorologists, "should this happen yet again, then it must be regarded as a wish of the physicists to get rid of meteorology within the Third Klass"38. He pleaded that a meteorologist should now be elected because, out of the ten members of the Klass, the two remaining meteorologists were so old that there was a real danger that meteorology would soon lose its representation in the Academy. To refuse Hildebrandsson would have been a personal insult, so that his candidate, A. Wallén, was elected³⁹. Nevertheless, when

the other meteorologist, Hamberg, died shortly afterwards, Gullstrand warned members of the committee that "under all circumstances we must act [ställa oss], so that we do not get in yet another weak meteorologist".

This time they acted quickly. Led by committee members Oseen, Siegbahn, Gullstrand and Arrhenius, a majority signed a proposal for physicist C. Benedicks. Carlheim-Gyllensköld, a cosmical physicist, opposed these efforts. When he next came up for re-election on the Nobel committee, Oseen, Gullstrand, and Siegbahn tried to remove him. First, they tried to reduce the number of committee members from five to four, thereby eliminating his position. When this tactic failed, they used their right to elect a foreign member - and proposed Niels Bohr. Both committee and Klass approved the Oseen-Siegbahn nomination, but the Academy again sensed an Uppsala grab for control, and voted to retain Carlheim-Gyllensköld41.

Similar tactics were used within the committee to avoid awarding Nobel Prizes for meteorological research. Occasional nominations for a meterologist were dismissed as "not significant", or "not confirmed by experience"; but, one nominee reappeared repeatedly, with increasing support from both physicists and meteorologists. Oseen and his Uppsala colleagues were determined that he — Vilhelm Bjerknes — should not receive a prize.

The Bjerknes group

Between 1918 and 1924 a group of Norwegian and Swedish scientists working in Bergen (Norway) under the leadership of Bjerknes transformed theoretical and practical meteorology. Their various conceptual achievements included a new model of the extratropical cyclone, the type of low-pressure system common in the mid-latitudes. Claiming that these atmospheric disturbances are composed of three-dimensional surfaces of discontinuity - fronts - they began conceiving of a cyclone as a wave that forms and evolves along pre-existing polar fronts separating polar and tropical air masses. Their theory provided the first clear physical model of cyclonic evolution, which in turn provided for direct interaction between theory and practice.

The Bergen meteorologists constructed their models over a period of time partly as a result of changes in forecasting methods and partly in conjunction with their efforts to forge new forecasting techniques to meet challenges arising from agriculture, aviation and fishery. Earlier, Bjerknes had for almost two decades worked on an ambitious project to "transform the inexact science of meteorology into an exact physics of the atmosphere". As such, theoretical and physical reasoning informed both the forecasting effort and the new concepts⁴². Having been a

professor of Stockholms högskola from 1894 to 1907, Bjerknes had various supporters — and opponents — within the Swedish scientific community. In particular, the national rivalry that occurs when Norway outshines her traditionally richer and culturally superior neighbour provoked a general reaction of "humbug" from some Swedish scientists⁴³, making it easier for Oseen and others to block Bjerknes's candidature in 1923, 1924, 1926, 1929 and repeatedly in the 1930s.

During the 1920s, several reasons were given for excluding Bjerknes. In brief, the general reports by the committee claimed that the various "empirical" discoveries and improvements in forecasting had arisen simply as a consequence of an improved network of observation stations44. Moreover, Bjerknes's baroclinic circulation theorems for fluids in which density is a function of both pressure and temperature (1898, 1903), should not be considered a discovery or invention; according to Oseen, these innovations and their application to atmospheric and oceanic phenomena were already implied in Helmholtz's earlier circulation theorem for ideal fluids45. Moreover, Oseen and Gullstrand repeatedly asserted that the wave-theory of



C. W. Oseen. His goal of promoting theoretical physics and of improving the international stature of Swedish physics motivated him to attempt restricting the definition of physics within the Academy and for the Nobel Prize.

cyclones and the polar front theory are not proven by experience⁴⁶. Lastly, they held that the Bergen meteorology had been accepted only by a limited number of scientists⁴⁷. How valid were these objections to the Bjerknes nominations?

First, the claim that the Bergen models and improved forecasting arose simply because of the refinement of the observation network cannot be substantiated. Comparable networks had been introduced during the war without similar fruitful results; moreover the Bergen successes did not all immediately ensue. More telling in intent is Oseen's claim that Bjerknes's discovery of the baroclinic circulation theorems did not qualify as a discovery in the sense that Nobel had specified. Yet, as a hydrodynamicist, Oseen knew full well that Helmholtz had never considered the possibility of

baroclinic fluids; indeed, several years earlier, he had declared that Helmholtz received too much credit in this area48. When L. Silberstein derived these theorems independently of Bjerknes, he dismissed such fluids as pure theoretical constructs. Bjerknes's achievement was that he recognized their profound implications for the study of atmospheric and oceanic motions. Revealingly, shortly after Oseen and Gullstrand argued that Bjerknes's work cannot be considered a discovery or invention, they urged the Klass to recall that Nobel's meaning of "discovery and invention" must be interpreted as broadly as possible⁴⁹; on that occasion they were defending a candidate for the 1924 prize against objections of the sort they had levelled against Bjerknes, the nominee here being their colleague, Manne Siegbahn.

Uppsala and geophysics

The cavalier manner in which Bjerknes's work was dismissed was apparent to those who were close at hand. Otto Pettersson, who had sat on the Nobel committee for chemistry and who believed that Bjerknes deserved a prize, recognized the pattern and noted in dismay that apparently, "Uppsala doesn't consider geophysics to be physics" 50. He also wondered whether the committee was competent to appreciate Bjerknes's work or whether Oseen and Gullstrand would ever bother to read enough of it to be able to judge it fairly 51.

Oseen provided other reasons to confirm Pettersson's fear that scientists with great authority all to often misuse their power⁵². After Hildebrandsson's death in 1925, Oseen found an opportunity to use meteorologists against Bjerknes. For the election to the Klass he and other Uppsala scientists backed F. Akerblom, an opponent of the Bergen school and of Bjerknes personally. But when a letter from Bjerknes was distributed suggesting that some of Akerblom's research results seemed to have been borrowed from others, Åkerblom was dropped, leaving as potential candidates two former Bjerknes assistants, V. W. Ekman and J. W. Sandström⁵³. When Sandström was eventually nominated, Oseen almost exploded with rage claiming he would show that Sandström did not even understand the basic physics behind meteorology and oceanography⁵³. He demanded and received a postponenment of several weeks. But instead of demolishing Sandström's candidature, Oseen and Siegbahn had the original proposal replaced by one of their own. Apparently Oseen had learned that Ekman — the alternative — was planning to campaign for Bjerknes and therefore opposed him in spite of an earlier declaration that Ekman's work stood clearly above Sandström's54.

Oseen also made use of an attack by the volatile Sandström against his friend Bjerknes, written in 1923 at a moment of great personal despair. Sandström's harangue followed an interview with

Royal Meteorology Society: Symons Gold Medal

From Nature of 6 January 1940:

THE decision of the Council of the Royal Meteorological Society to award the Symons Gold Medal for 1940 to Dr J. Bjerknes will be very popular among British meteorologists, to whom he has become well known during his frequent visits to this country. In 1932 the medal was awarded to his father, Prof. V. Bjerknes, and it is fitting that the son, who shared the work, should also share the honours.

In 1933 the accumulated research of the Norwegian school of meteorologists into the dynamics of the atmosphere was published in book form under the title "Physikalische Hydrodynamik" by V. and J. Bjerknes, H. Solberg and T. Bergeron. Dr Bjerknes is still young, and we look forward to further important research in future from these brilliant Norwegians.

Bjerknes carried in the Stockholm daily Dagens Nyheter entitled, "Norway, meteorology's leading nation"55. In a published response, Sandström said that the polar front theory has not been effective in Sweden nor elsewhere, and that the wave-theory of cyclones conflicted with empirical experience in Sweden. He added that "Norwegians are almost too vigorous when it comes to creating new hypotheses and theories, and it is neither desirable nor practical that we follow them in all their capers and flights of fancy [krumsprång och hugskott] of this sort"56. Oseen in his support for Sandström's election to the Klass, claimed therefore that Sandström had during the past few years tested empirically Bjerknes's theories for the polar front and for cyclones, finding that "the first theory is not confirmed by the meteorological phenomena in Sweden while the Bjerknes equations for cyclones do not stand in agreement with the actual [observed] conditions"57. Sandström was elected and Oseen now had a basis for arguing against Bjerknes's candidature. Yet, by the 1930s, most meteorologists were forecasting with the Bergen school's methods and working on theoretical problems associated with them. So Oseen and other committee members who preferred a restricted definition of physics had to find another strategy.

By 1936 the case for awarding a prize to Bjerknes and his chief assistants was strong. Ekman, who supported Bjerknes and who was now a member of the Klass, requested that the committee prepare a special report on Bjerknes, even though C. D. Anderson was "waiting in turn"58. Unable to avoid the issue, Oseen began a detailed report on the Bjerknes school's activities. Apparently, he concluded that regardless of the value of their work, meteorology could not be considered as part of physics, and hence not in the domain for the prize⁵⁹. Ekman protested, asserting that meteorology and weather forecasting, when based on solid physical

principles such as those of Bierknes, perfectly meets Nobel's intentions of both being significant in physics and benefiting mankind60.

Knowing that Oseen dared not try to change the statutes formally to exclude meteorology, Ekman suggested that Oseen should take the matter up before the Academy⁶⁰. Should the committee and the Klass believe Bjerknes worthy, they could propose him for a prize and, also, suggest an alternative candidate within Oseen's "restricted definition of physics". Thus, should the Academy decide that meteorology is not eligible, the committee would still have control over the alternative candidate. In short, Ekman suggested that the Bjerknes case could be a precedent for interpreting the statutes60. But, Oseen did not want to risk having a precedent for meteorology being part of physics. To ensure that the Bjerknes nomination did not reach the Academy, by having it first rejected in the committee or in the Klass, Oseen apparently included in his special report a disparaging personal attack. According to Sandström, who was in the Klass, Bjerknes would have probably received a prize had Oseen not written his 'perfidious Bjerknes biography''61. Oseen seems to have chosen Bjerknes's outmoded interests in physics to discredit him as a scientist. Bjerknes had found it impossible to abandon his father's search for analogies between hydrodynamic and electromagnetic fields of force. As a young man Oseen had also worked in this area. but he now considered these methods and goals of the 1890s to be totally absurd and even contemptible⁶². By portraying Bjerknes as a hopelessly plodding

- Nature 287, 667-668 (1980).
- 2. Crawford, E. The Beginnings of the Nobel Prize Institution: The Science Prizes, 1901-1915 (Work in progress). 3. Protokoll. Nobelkomittéen för fysik, 26 September 1910.
- Protokoll, Nobelkomittéen för fysik, 8 January 1901.
- Letter, Hasselberg, B. to Newcomb, S., 26 October 1900 (Newcomb papers, Library of Congress, Washington, D.C.).
- 6. Nobelprotokoll, K. Vetenskapsakademiens 4de Klass, 29 November 1902.
- 7. Protokoll, Nobelkommittéen för fysik, 7 September 1921; Nobelprotokoll, K. Vetenskapsakademiens 3de Klass, 29
- 8. Protokoll, Nobelkommittéen for fysik, 26 September 1910.
- 9. Protokoll, Nobelkommitten för josik, 20 September 1911.
 10. Arrhenius, S. Levnadsrön, 168 (unpublished autobiography, 1927) Arrhenius papers, K. Vetenskapsakademien,
- Stockholm.
 11. Letter, Hasselberg, B. to Hale, G.E., 5 July 1907 G.E. Hale papers.
- 12. Letter, Hasselberg to Hale, 29 December 1907.
- Protokoll, Nobelkommittéen för fysik, 24 September 1908.
 Letter, Ekholm, N. to Arrhenius, S., 10 March 1910 Arrhenius papers.
- 15. Protokoll, Nobelkommittéen for fysik, 8 September 1920.
- Protokoller, Nobelkommittéen for fysik, 16 March 1921;
 September 1911.
- 17. Protokoll. Nobelkommittéen för fysik, 7 September 1921
- Protokoll, Nobelkommittéen för fysik, 6 September 1922 Mittag-Leffler, G. Dagbok: Resan sommaren 1922, 27 July 1922 Kungliga Biblioteket, Stockholm.
- Letter, Hasselberg to Hildebrandsson, H.H., 9 November 1921 Hildebrandsson papers, Universitetsbiblioteket,
- 21. Protokoll, Nobelkommittéen for fysik, 7 September 1921. Nobelprotokoll, KVA's 3de Klass, 29 October 1921.
- 23. Letter, Oseen, C.W. to Arrhenius, 20 November 1922 Arrhenius papers; Oseen, "Den Einsteinska lagen" Kosmos. Fysiska uppsatser utgivna ar 1922 105-131 (Stockholm, 1922).
- 24. Oseen, C.W. Atomistiska föreställningar i nutidens fysik. Tid, rum och materia 15 (Almqvist and Wiksell, Uppsala, 1919).

physicist, who neither accepted nor understood modern physics. Oseen tried to convince the committee and Klass that Bjerknes would be an embarrassment for the Academy. Oseen related how with 'special satisfaction" he could use against Bierknes the latter's belief that Einstein's relativity theory marked the end of classical physics, rather than the start of the new⁶³. Finally, to finish off the Bjerknes candidature Oseen solicited comments from vet another Bierknes assistant, who was then attempting to establish a rival school of meteorology in the United States: "For practical weather forecasting . . . Bjerknes's calculations have absolutely no significance whatsoever''64. Although effective in preventing Bjerknes from receiving a Nobel Prize, this statement, in this form, is false.

Oseen died in 1942 having successfully blocked the awarding of a Nobel Prize for work in the geophysical sciences. His goal of promoting theoretical physics and of improving the international stature of Swedish physics had led him to attempt restricting the definition of physics within the Academy and for the prize. Yet, there is no formal reason based on Nobel's will for excluding geophysics and astrophysics from the prize. Rather, there is only a tradition of interpreting the statutes that has been informed by past personalities and contingencies. Oseen and other committee members recognized this to be true. When Bjerknes confronted him with solid evidence that Nobel considered meteorology and geophysics to be part of physics, Oseen replied that "the interpretation of words changes from person to person. What do we mean by

- Protokoller, Nobelkommittéen för fysik, 15 September 1919; 7 September 1921.
- Protokoll, Nobelkommitteen für fysik, 6 September 1922. 27. Letters, Oseen to Mittag-Leffler, 15 November 1918, 17 October 1918 Mittag-Leffler papers; Oseen to Bjerknes, V., 12 November 1920, 3 February 1923 Bierknes papers, Universitetsbiblioteket, Oslo; Oseen to Arr-
- henius, 29 September 1920 Arrhenius papers. Letter, Oseen to Bjerknes, 3 February 1923.
- Protokoll, Nobelkommittéen för fysik, 24 March 1917.
- Protokoller, Nobelkommittéen för fysik, 1922, 6 December 1922.
- Protokoll, Nobelkommitteen för fysik, 5 September 1923.
- Protokoll, Nobelkommittéen för fysik, 31 January 1924.
- Letter, Oseen to Bjerknes, 18 June 1919 Bjerknes papers Letter, Granqvist, G. to Arrhenius, 28 December 1921
- Arrhenius papers. 35. Letters, Hildebrandsson to Hasselberg, 22 January 1922 Hasselberg papers, Universitetsbiblioteket, Uppsala; Hildebrandsson to KVA's 3de Klass, 4 January 1922
- Nobelprotokoll, KVA's 3de Klass, 7 January 1922
- 37. Letter, Gullstrand, A. to Arrhenius, 28 March 1923 Arrhenius papers.
- Letters, Hildebrandsson to KVA, 6 September 1923; Hildebrandsson to KVA's 3de Klass, 6 September 1923
- Protokoll, KVA's 3de Klass, 3 September 1923
- Letter, Gullstrand to Arrhenius, 25 December 1923 Arrhenius papers.
- etters, Oseen to Gullstrand, 1 November 1928, 5 November 1928 Gullstrand papers, Universitetsbiblioteket, Uppsala; Nobelprotokoli, KVA's 3de och 4de Klasser. 16 November 1929; Nobelprotokoll, KVA's 3de Klass, 30 November 1929.
- 42. Friedman, R.M., "Vilhelm Bjerknes and the Bergen School of Meteorology, 1918-1923. . . " (Thesis,
- Johns Hopkins University, 1978) (book in progress).
 43. Letters, Sandström, J.W. to Bjerknes, 22 September 1918 Bjerknes papers; Ryder, C. to Hildebrandsson, 28 July 1922 Hildebrandsson papers.
- Protokoller, Nobelkommittéen för fysik, 5 September 1923, 8 September 1924.
- 45. Letter, Oscen to Ekman, W.V., 3 December 1924 Ekman

physics? What do we mean by 'during the preceeding year' or 'recently'? . . . Do any words exist that have a fixed significance?"65. As soon as one finds an interpretation "consistent with one statute, one finds another inconsistent"66.

The summing up

Inevitably, the interpretation of the statutes rests on personal judgement and prejudice. When in 1926, the Klass rebelled against the committee's long-standing rejection of J. Perrin for a prize, Gullstrand insisted, in an attempt to block him once again, that Perrin's work on brownian motion has not benefitted mankind⁶⁷. Recognizing the inconsistent standards used by the committee, Benedicks responded by calling attention to the fact that the committee seemed to use the statutes arbitrarily to dismiss persons they a priori did not want to honour with a prize⁶⁸. Summing up the whole controversy, one member of the committee for chemistry remarked in despair "that to sit on a N[obel] C[ommittee] is like sitting on a quagmire [gungfly] — one doesn't have firm footing under one's feet"69. Similarly, to discuss and to assess the significance of the Nobel Prizes without taking into account the Swedish context is, as well, "att sitta på en gungfly"!

I thank Elisabeth Crawford (CNRS, Paris) for criticism and for access to the manuscript of her book in progress, the Royal Swedish Academy of Sciences for admitting me to their archives, the Norwegian Research Council for Science and the Humanities and Maison des Sciences de l'Homme (Paris) for financial support.

- papers (private).
- 46. Protokoll, Nobelkommitteen for fysik, 5 September 1923. 47. Letter, Pettersson, O. to Ekman, 17 March 1925 Ekman
- Letter, Oseen to Bjerknes, 16 January 1919 Bjerknes papers.
- Nobelprotokoll, KVA's 3de Klass, 29 October 1925.
- 50. Letter, Pettersson to Arrhenius, Midsommar 1926 Arrhenius papers.
- 51. Letter, Pettersson to Ekman, 17 March 1925, 23
- November 1930, n.d. Ekman papers. Letter, Pettersson to Ekman, 17 March 1925
- Letter, Arrhenius to Bjerknes, 5 November 1925 Bjerknes
- papers; Protokoll, KVA's 3de Klass, 29 October 1925. Letter, Oseen to Ekman, 5 May 1923 Ekman papers. Dagens Nyheter (Stockholm), 27 December 1923.
- Dagens Nyheter 28 December 1923. Protokoll, KVA's 3de Klass, 20 November 1925.
- Letter, Ekman to Oseen, 6 April 1936 Oseen papers,
- Letters, Oseen to Bjerknes, 4 February 1938 Bjerknes papers; Ekman to Oseen, 7 November 1937, 15
- November 1937 Oseen papers. 60. Letters, Ekman to Oseen, 15 November 1937, 25
- January 1938 Oseen papers.
- 61. Letter, Sandström to Bjerknes, 4 April 1939 Bjerknes papers; Dagens Nyheter 3 March 1939.

 Oseen, 'Albert Viktor Bäcklund'' in Vetenskapsakademiens
- Arsbok 1924 303-304, 306-307 (Uppsala, 1924).
 63. Letter, Oseen to Bohr, Niels, 25 June 1935, Bohr Scientific
- Correspondence Niels Bohr Institute, Copenhagen.
 64. Letter, Ekman to Oseen, 18 October 1939 (quoting from "Kompletterande utredning om V. Bjerknes") Oseen
- papers. Letters Rierknes to Oseen 17 January 1938 Oseen papers: Oseen to Bjerknes, 4 February 1938 Bjerknes papers.
- Letter, Oseen to Bierknes, 4 February 1938
- Letter, Oseen to Arrhenius, 3 November 1926 Arrhenius papers; Nobelprotokoll, KVA's 3de Klass, 23 October 1926.
- Nobelprotokoll, KVA's 3de Klass, 23 October 1926. Letter, Widman, O. to Palmer, W., 19 November 1826 [sic] Palmer papers KVA.

NEWS AND VIEWS

The search for proton decay

from Frank Close

THE discovery that at ultra-high energy, the strong, electromagnetic and weak forces may take on similar behaviours has led to the development of grand unified theories (GUTs) which unite them at 10¹⁵ GeV. The theories predict that, at extreme energies, quarks and leptons assume similar characteristics and can transmute back and forth. At very low energies, such as are present today, these exotic processes are frozen but not totally eradicated. Transmutation of quarks (in the proton) into leptons is predicted to occur, albeit very rarely, causing protons to decay with a half life of about 10³⁰ yr.

The search for evidence of proton decay is one of the most popular topics in highenergy physics and the opening talk at the biennial European Physical Society conference* held in Lisbon last month outlined ten independent such experiments around the world. Three are still proposals, five are being set up, one has put a limit of ≥1030 yr on the lifetime and a Bombay-Japan collaboration has three possible candidate events for proton decays. If these survive, then the future will be very exciting as the proposed experiments should see many scores of events which will not only confirm the proton decay phenomenon but, by studying the nature of its decay products, yield profound insights into the relationship between quarks and leptons.

Studying rare phenomena, such as the cold residue of a high-energy unity, is one way of testing the unified theories. More direct of course is to push laboratory experiments to ever higher-energy regimes and so push nearer to that unity.

H. Schopper, the director-general of CERN, surveyed the planning for high-energy physics facilities until the 1990s. Towards the end of this decade it is hoped that very high-energy annihilations of electrons and positrons may be achieved at Stanford University, California or at the large electron-positron accelerator (LEP)

to be built at CERN, Geneva, which will be able to probe the energy region where the weak force has merged in strength with the electromagnetic. Great excitement was generated by Carlo Rubbia's (CERN) announcement that the first collisions of protons and antiprotons had been achieved in the CERN SPS accelerator, paving the way for producing W and Z bosons there. The optimists hope that the first few events producing these weak interaction quanta may occur by early next year.

The theories of electromagnetic and nuclear forces that have spawned the GUT were reviewed by several speakers. On the phenomenological front, P. Landshoff (University of Cambridge) surveyed the field, concluding that all the data continue to support the Glashow-Weinberg-Salam SU, ×U, model of electroweak forces and the SU, quantum chromodynamic theory (QCD) of quark forces. The obvious qualitative successes of the latter are proving hard to make more quantitative because perturbation theory of quarkgluon interactions converges very slowly. Landshoff reviewed some theoretical attempts to construct approaches to the renormalization of QCD which would improve the convergence properties. This is an area of study that is still in some flux.

R. Marshall (Rutherford Laboratory, UK) exhibited some of the most compelling evidence yet for gluons manifesting themselves as parents of particle jets in electron-positron annihilation data. The electron and positron annihilate producing a quark in one direction and an antiquark and glue centred on the opposite direction. A shower of particles induced by the quark is balanced by two showers symmetric about the 180° axis — the products of (anti) quark and gluon.

F. Close (Rutherford Laboratory) pointed out how just as coloured quarks cluster to form 'white' hadrons, so should coloured gluons cluster to give white glueballs. There has been some speculation that a glueball may have been seen with a mass of about 1,400 MeV (Nature, News and Views 292; 195, 1981). It has also been

suggested that a 2-GeV state seen decaying into two \$\phi\$ mesons may be a glueball; more data from this experiment may elucidate the possibility. At the moment the 1,400-MeV state looks the best bet but, as outlined in *Nature* (292; 195, 1981), there is still much work to be done.

The search for white glueballs as opposed to individual coloured gluons is based on the belief that QCD confines colour in such clusters. G.'t Hooft (University of Utrecht) gave a detailed review of current attempts to prove this and R. Feynman (Caltech) reported his own attempts to prove confinement in a world of two-spatial dimensions.

But are quarks confined? A confrontation took place between W. Fairbank (Stanford University), who has claimed to observe fractional electrical charges on niobium spheres, and G. Morpurgo (University of Genoa), who finds no such phenomenon on iron. The issue was not resolved — Morpurgo's experiment cannot levitate niobium, and Fairbank's cannot levitate iron. Morpurgo may be able to study an 80:20 iron/niobium mixture soon; whether this helps resolve the question remains to be seen. (See Lyons Nature, News and Views 291; 534, 1981 for a discussion.)

Another interesting confrontation between groups with data in prima facie conflict took place. A Bologna-CERN-Frascati collaboration has claimed to produce a baryon containing a bottom (or 'beauty' quark). This state has a mass of 5.4 GeV and, if verified, would be the most massive baryon ever found. The experiment occurred in the split-field magnet at the CERN intersecting storage rings (ISR) and the claimed mass is in excellent agreement with theoretical predictions. However, a collaboration from Annecy-CERN-Paris-Dortmund-Heidelberg and Warsaw reported that they had performed a similar experiment in the same conditions and found no signal.

Frank Close is at the Rutherford and Appleton Laboratories, Didcot, UK.

^{*}The High-Energy Physics European Physical Society conference was held in Lisbon from 8 to 15 July.

Transposable elements and proviruses

from D.J. Finnegan

THE discovery that both prokaryotic and eukaryotic genomes are less stable than classical genetic analysis had indicated is one of the many important developments made in molecular genetics over the past few years. Application of recombinant DNA techniques to the study of eukaryotic genome organization has revealed the presence of transposable elements within the genome of Drosophila melanogaster1-3. These comprise about thirty families of repeated sequences, together making up approximately one-half of the moderately repetitive DNA (5-10 per cent of the total genome) in this species. The best studied families (known as copia, 412, 297, mdgl and mdg3 after the first member of each to be studied) have several properties in common, although there is no detectable homology between them. The members of each family (often referred to generically as 'copia-like' elements) are well conserved and are located at 20-40 sites distributed throughout the genome. The number and locations of elements in each family vary between embryonic and tissue culture cell DNA where there may be up to 250 copies per haploid genome^{2,4}. The number of potential sites must be high and elements may even insert at random.

At both ends of a transposable element are direct repeats (1 in Fig. 1a) a few hundred base pairs long, the length and sequence of which are specific for each family of elements^{5,6}. At the extreme ends of each element are short (about 10 base pairs) inverted repeats (2 in Fig. 1a) which, except in the mdg3 family, occur at both ends of the long direct repeats. Immediately before and after each element is a short direct repeat (3 in Fig. 1a), the length, but not sequence, of which is constant for all members of a particular family (5 base pairs in the case of copia elements). Comparisons between particular sites in the genome from different sources, one containing and one lacking (an 'empty site') a tranposable element, indicate that the bases of the short direct repeat occur only once at the site into which an element inserts7.

The properties of *Drosophila* transposable elements described above resemble those of bacterial transposons⁸. This raises the possibility that, despite no evidence for a direct relationship between the two, *copia*-like elements might be responsible for the same range of genetic events (stable and unstable mutations, deletions, inversions and translocations) in *D. melanogaster* as are transposons in *Escherichia coli*. The most interesting

Fig. 1 a, A transposable element. The various components are: 1, long direct repeat; 2, inverted repeat; 3, short direct repeat. b, Relationship between viral RNA and proviral DNA. U3, unique sequence from the 3' end of the viral RNA; R, sequence repeated at both ends of the viral RNA; U5, unique sequence from the 5' end of the viral RNA.

EMPTY SITE

example of this to date concerns the white eye gene (w). Bingham, Judd and Rubin (Rubin, personal communication) have shown that the white apricot mutation, w^a , is due to insertion of a copia element at the white locus. Some unstable w alleles are also due to insertions, although the sequences involved do not belong to any of the well characterized families of transposable elements (Rubin, personal communication).

The abundance of transposable elements in the genome of *D. melanogaster*, and the fact that their activity can have recognizable genetic consquences, prompt one to ask whether sequences of this type might be found in other eukaryotes. Indeed, a family of transposable elements, *TyI* elements, has been discovered in the yeast *Saccharomyces cerevisiae* and these have properties very similar to those of *copia*-like elements. The same is true of the DNA

proviruses of vertebrate retroviruses.

Cells which have been infected by retroviruses may have one or more copies (proviruses) of the viral genome integrated into their chromosomes. At each end of a provirus are direct repeats, known as long terminal repeats (LTRs). At the left-hand end of a LTR is a unique sequence from the 3' end of the viral RNA. Adjacent to this is the sequence repeated at both ends of the RNA and a unique sequence from its 5' end. These are shown in Fig. 1b where they are designated U3, R and U5 respectively. After infection of a host cell, viral RNA molecules are first copied into linear double-stranded DNAs with a LTR at each end. These are then converted into circular molecules with either one copy of the LTR or two copies in tandem. It is not known which of these DNAs integrates into host chromosomes but some evidence favours a circular form (for detailed

TRANSPOSABLE ELEMENT

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D.J. Finnegan is in the Department of Molecular Biology, University of Edinburgh.

discussion, see ref. 9). The sequence organization of proviruses resembles that of Drosophila transposable elements in several ways10. They have short inverted repeats at their ends and are flanked by a direct repeat of a few base pairs which occurs only once at the site of insertion in their absence (Fig. 1b).

Vertebrates do not have to be infected by retroviruses to acquire sequences of this type. The genomes of most, if not all, vertebrates contain endogenous proviruses, which can make up a substantial proportion of their DNA (about 0.1 per cent in primates and rodents)11. Like Drosophila transposable elements, the chromosomal locations of endogenous proviruses differ between individuals in populations of mice and chickens. The topographical similarities between proviruses and transposable elements suggest that they may be related and Temin¹² has argued that proviruses evolve from transposable elements. If this is the case, one might expect to find within D. melanogaster cells circular DNAs similar to extrachromosomal proviral DNA. Circular DNAs were detected in D. melanogaster embryos and tissue culture cells some time ago and have been shown to be largely comprised of repetitive sequences¹³. In a recent issue of *Nature* (292; 591, 1981), Flavell and Ish Horowicz described their attempts to find molecules with copia-specific sequences amongst these circles. They have been able to purify three different circular DNAs of this type from D. melanogaster tissue culture cells using ethidium bromide-caesium chloride density centrifugation and DNA cloning techniques. On the basis of their restriction site maps two of them do appear analogous to circular proviral DNA, having all the sequences from the body of a copia element plus one copy of its long direct repeat or two repeats in tandem. The third class appears to be a deletion derivative of one of the other two.

Stimulating though these observations are, they do not clarify the relationship between transposable elements and retroviruses since (as Flavell and Ish Horowicz point out) these circular molecules need not be produced by reverse transcription but could be formed by

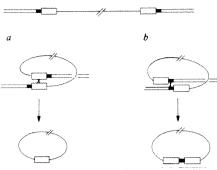


Fig. 2 Recombination in a transposable element: a, between the long direct repeats at the ends of an element, giving a circular molecule with one copy of the direct repeat, and b, between the 5 base pairs on either side of the element giving a circular molecule with two copies of the direct repeat.

recombination. Recombination between the long direct repeats at the ends of a transposable element would yield a circular molecule with one copy of the direct repeat (Fig. 2a) whereas recombination between the 5 base pairs repeated on either side of a copia element would yield a circular molecule with two copies of the direct repeat in tandem (Fig. 2b). These possibilities should be distinguished by DNA sequence analysis of the circular molecules with two copies of the long direct

repeat. If they are derived recombination, then the two repeats should always be separated by 5 base pairs but a different 5 base pairs in each case; if they are derived by reverse transcription of full-length copia RNA, then all circles with two repeats should be identical. Whatever their origin these circles could be intermediates in transposition, a possibility which Flavell and Ish Horowicz are investigating.

The sequences discussed so far are not the only ones involved in DNA rearrangements. Potter and his colleagues have found transposable sequences in D. melanogaster with properties distinctly different from those of copia-like elements and specific, rather than random or semirandom, rearrangements have been demonstrated for genes controlling mating type in S. cerevisiae, surface antigens in trypanosomes and immunoglobulins in mammals. Others may exist, but one should be wary of getting too carried away by these results. Eukaryotic genomes are more labile than has been generally supposed but wholesale rearrangements do not occur. In Drosophila, genes still retain their order in linkage groups, this order is the same in somatic and germ-line cells, and the banding pattern of polytene chromosomes is remarkably constant.

The expanding use of potential-sensitive dyes

from a correspondent

THE recent paper in Nature reporting the use of a voltage-sensitive dye to localize pacemaker activity in early embryonic heart is a good example of the expanding interest in and application of potentialsensitive dves for biological, electrophysiological and biophysical studies. Cyanine, merocyanine and oxonol dyes respond spectrally to potential changes in, for example, squid giant axon^{2,3}, red blood cells⁴⁻⁶, black lipid membranes7, frog skeletal muscle8 and frog heart9

The available probes are of two general types: those which permeate the cell membrane and have both slow and fast responses (for example, carbocyanines) and non-penetrating dyes with only fast signals, of which merocyanine-rhodanine used by Kamino et al. is a good example. As reviewed recently by Waggoner¹⁰, the slow dyes, with their large responses, seem ideally suited for following the kinetics of potential changes that occur in times of a few seconds, whereas the fast probes can resolve adequately events associated with membrane excitation during action

potentials, although their absorption and fluorescence changes are usually only 0.001 to 1 per cent. The slow (or redistribution or accumulation) dyes operate by potential-dependent mechanisms redistributing the charged dye between extracellular and intracellular environments of the cell, organelle or vesicle, whereas optical signals from rapidly responding dyes occur through potentialdependent changes of dye molecules located on (or very near) the membrane.

There are obvious attractions in using these optical probes, especially in cells which are too small to be examined by classical microelectrode techniques, or in complex cellular networks where multiple and accurate impalements would be very laborious and difficult. However, it has long been recognized that care has to be taken in both interpreting experimental data, hence the comforting corroboration in squid axon where parallel membrane potential measurements can be made with confidence, and ensuring that introduction of the dye does not in itself perturb the preparation. Permeant dyes can inhibit

Potter, S.S., Brorien, W.J., Dunsmuir, P. & Rubin, G.M. Cell 17, 415 (1979).

Tchrikov, N.A., Ilyin, Y.V., Ananiev, E.V. & Georgiev, G.P. Nucleic Acids Res. 6, 2169 (1978).

Rubin, G.M. et al. Cold Spring Harb. Symp. quant. Biol.

^{4.} Strobel, E., Dunsmuir, P. & Rubin, G.M. Cell 17, 429

^{5.} Levis, R., Dunsmuir, P. & Rubin, G.M. Cell 21, 581

Bavev, A.A. et al. Nucleic Acids Res. 8, 3263 (1980)

Dunsmuir, P., Brorien, W.J., Simon, M.A. & Rubin, G.M. Cell 21, 576 (1980).

Calos, M.P. & Miller, J.H. Cell 20, 579 (1980). Gilboa, E., Mitra, S.W., Goff, S. & Baltimore, D. Cell 18, 93 (1979)

Shimotohno, K., Mizutani, S. & Temin, H.M. Nature 285, 550 (1980)

Todaro, G.J., Callahan, R., Rapp, U.R. & De Larco, J.E. Proc. R. Soc. B210, 367 (1980)

Temin, H.M. Cell 21, 599 (1980).

Stanfield, S.W. & Lengyel, J.A. Proc. natn. Acad. Sci. U.S.A. 76, 6142 (1979).

intracellular metabolic processes and the binding of charged dve molecules might interfere with important membrane processes mediating biological responses. In addition, dyes may react with the exogenous reagents, whose effects are being examined, to form non-fluorescent complexes. This would make doseresponse curves difficult to interpret and would complicate kinetic calculations. Furthermore, even with modest illuminations, some dves can cause cellular photodynamic damage. These considerations have prompted intensive work to produce dyes with better responses and biological inertness.

It is possible to end on an optimistic note, for recent reports (to quote but two examples) have described how potentialsensitive dyes can be used to advantage to monitor either mitochondrial membrane potentials¹¹ or radial propagation along muscle fibre T-tubules¹². These, together with the elegant studies of Kamino, Hirota and Fujii¹, suggest that such techniques have an important future in many investigations involving molecular mechanisms at the cellular level.

- Kamino, K., Hirota, A. & Fujii, S. Nature 290, 595 (1981).
- Cohen, L.B. et al. J. Membrane Biol. 19, 1 (1974).
 Ross, W.N. et al. J. Membrane Biol. 33, 141 (1977)
- Hoffman, J.F. & Laris, P.C. J. Physiol., Lond. 239, 519 (1974).
- 5. Sims, P.J. et al. Biochemistry 13, 3315 (1974).
- Hladky, S.B. & Rink, T.J. J. Physiol., Lond. 263, 287 (1976).
- 7. Waggoner, A.S. et al. J. Membrane Biol. 33, 21 (1977). 8. Baylor, S.M. & Chandler, W.K. Biophys. J. 25(2),
- 9. Morad, M. & Selema, G. J. Physiol., Lond. 292, 267 (1972).
- 10. Waggoner, A.S. A. Rev. Biophys. Bioengny 8, 47 (1979).
- 11. Johnson, L.V. et al. J. Cell Biol. 88, 526 (1981).
- 12. Nakajima, S. & Gilai, A. J. gen. Physiol. 76, 751

The evolution of sedimentary basins

from C.A. Williams

THE selection of 'The evolution of sedimentary basins' as the topic for a Royal Society discussion meeting* reflects the impetus given to the field by new models of basin formation, in particular the crustal stretching model of McKenzie, and by the availability of quantitative data on crustal loading and flexure which are allowing models to be tested. The McKenzie model (Earth planet, Sci. Lett. 40; 25, 1978) proposes that sedimentary basins are formed by rapid stretching of the continental lithosphere by normal faulting, instigated by a passive upwelling of hot asthenospheric material beneath the crust. Once the thermal stage has passed, the lithosphere thickens as the heat is conducted to the surface and undergoes a slow subsidence which is not associated with faulting and which may be amplified by sediment loading.

The meeting made no attempt to cover all types of basin, but concentrated on those where good-quality data are available. Discussion of continental margin basins was dominated by contributions on the Biscay margin (presented by D.G. Roberts, IOS, and J.-P. Foucher, CNEXO, Brest), largely based on the results of IPOD drilling on the North-west Biscay margin and on associated seismic reflection and refraction data. Both Roberts and Foucher showed seismic reflection evidence of listric faults dipping consistently oceanwards. These two

studies, whilst producing the most detailed information we yet have on this margin, also indicate the limitations of fragmentary evidence in reassembling faulted strata. Robert's interpretation requires infinite stretching of the lower crust but less stretching in the upper crust than that required by Foucher's interpretation, yet both studies are based on essentially the same data set. The range of disagreement is such as to encourage the acquisition of more and better data to allow more accurate estimation of fault displacements.

The continental margin basin section was completed by an exposition of the North-west Australian margin by D.E. Powell (Hudbay), a margin remarkable in that the basins seem to be related to the late Palaeozoic phase of abortive rifting with little or no modification during the eventually successful rifting in the early Cretaceous.

An intriguing puzzle came from discussion of intracontinental basins. There appear to be two types of basement beneath basins, one in which normal faulting occurs as expected; but in cratonic shelf basins, for example, Witwatersrand, Transvaal and Michigan Basins, the basement does not appear to be normally faulted and some other mechanism of basement subsidence, other than extension by faulting, must be evoked. The Michigan Basin, nonetheless, shows the same exponential subsidence as other basins and the existence of COCORP seismic reflection data across it suggests a clear line of future study into the apparent lack of basement faulting.

In the session on basin formation, A.B. Watts (Lamont-Doherty Geological Observatory) discussed the role of crustal flexure, showing that this becomes a major factor in basins more than 200 km wide and that the best overall fit to the observations is an elastic plate model with crustal strength increasing with age. This predicts a sedimentary geometry with coastal onlap and it seems that Vail's eustatic sea-level changes based on evidence of onlap may in reality be a record of basin subsidence. The thermal effects of subsidence were discussed by D.L. Turcotte (Cornell University) (all basins appear to be initiated by a thermal event), while C. Beaumont (Dalhousie University) compared foreland and Atlantic-type basins and concluded that the major contrast is the thermal age of the lithosphere. The old, cooler and thicker lithosphere of the foreland compressive area will respond to loading with greater rigidity and depression wavelength than the younger, hotter, thinner and less rigid rifted continental margin basin.

Some of the outstanding problems in understanding sedimentary basin subsidence, in particular the origin of tensile stresses in the crust which initiate the normal faulting, were discussed by M.H.P. Bott (Durham University). Their origin may be found in stresses at convergent plate boundaries, hence, for example, the origin of the Northern European Carboniferous basins may be related to stress fields in operation during the subduction of the Hercynian Ocean.

In summarizing the meeting, A.W. Bally (Shell) cautioned that the folded belts, for example Zagros, Franklinian Basin and the Precambrian basins, are too complicated for modelling with currently available data. The complexity of listric faulting causes inherent problems in estimating fault displacement. While in some spectacular cases the sole of the listric fault has been observed, more high-quality seismic data across basins are required for better evidence of the actual existence and shape of listric faults. The question of whether symmetric graben are purely a function of geological imagination was also raised, since observed graben are either asymmetric or exist as half graben.

The general conclusion was that the stretching model gives a reasonable explanation of the formation of many basins. It was a little disappointing that among the excellent and often complicated stratigraphical histories described in oil-company presentations, studies on subsidence, flexure, fault displacements and so on were not emphasized, leaving one to wonder whether the oil companies are way ahead in considering these effects in a quantitative way, though refrained from divulging same, or whether in fact it is the academics who are leading in this area.

^{*}The meeting was organized by Sir Peter Kent, M.H.P. Bott, D.P. McKenzie and C.A. Williams and the proceedings will be published in the *Philosophical Transactions of the Royal Society*, January 1982.

C.A. Williams is in the Bullard Laboratories, Department of Earth Sciences, University of Cambridge.

Membrane manoeuvres in Marseille

from R.C. Hider

Most animal and bacterial toxins are directed against membranes, the more toxic interfering with critical receptor proteins while others are highly efficient lytic agents. The former, by virtue of their high-affinity interactions, have been used as probes in developmental and physiological studies and the latter, because of their affinity for lipids, are used in the elucidation of the molecular organization of membranes. A number of recent developments in these fields were reported at a meeting of the International Society of Toxinology* where discussions centred on the sodium channel, the cholinergic synapse and cytolytic toxins formed the framework of the meeting.

The sodium channel

The voltage-dependent sodium channel, a prime constituent of excitable membranes, remained firmly out of the biochemist's reach until the discovery of channel-specific toxins. Since 1970 however, the concerted application of tetrodotoxin, saxitoxin, scorpion and sea anemone toxins has given reliable information on the distribution, density and unit ion flux of

*The Fourth European Symposium on Animal, Plant and Microbial Toxins (24-27 June 1981) was organized by Hervé Rochat, Faculté de Medécine Sectear Nord, Marseille. The proceedings of the meeting will be published by Pergamon Press in 1982.

various sodium channels. As reported by V. Berwald-Netter (Collège de France, Paris) and J.F. Renaud (Centre de Biochimie, Nice), the development of these channels in embryonic tissue can also be monitored as sea anemone and scorpion toxins bind to both silent and functional channels1 and can give a quantitative index of neuronal maturation². W.A. Catterall (University of Washington, Seattle) reported the isolation of a functional sodium channel from mammalian brain³ but instability of the preparation — its lifetime is limited to 3 days — is a major problem. As with other membrane protein isolation procedures, the presence of a protease inhibitor cocktail has been essential, Catterall's laboratory including o-phenanthroline. This powerful chelator removes essential cationic cofactors from proteolytic enzymes and yet leaves calcium in solution to enhance the stability of the isolated channel. Catterall reported that the membrane channel consists of two proteins of molecular weights 270,000 and 37,000, both being specifically labelled by photoaffinity-tagged sea anemone toxin. The nature of the large subunit, which has also been reported elsewhere4, is of great interest. Does it consist of subunits crosslinked by functional groups other than disulphide fuctions? Does calcium

modulate its conformation? Whatever the answers to these questions, its isolation in a functional state marks the beginning of a new era which is likely to be centred on precise electrophysiological experiments with well defined reconstituted bilayer membranes.

There was general agreement at the meeting that the 'old world' scorpion neurotoxins bind to the sodium channel in a voltage-dependent manner and thus interact with a component capable of existing in two conformational states, the relative proportions of which depend on membrane potential⁵. However, the 'new world' scorpion toxins lack this property and bind to a different site on the sodium channel⁶. The three-dimensional structure of one such toxin (from Centruroides sculpturatus) was shown by J.C. Fontecilla (Aarhus University) to possess an extensive backbone of secondary structure crosslinked by four disulphide bridges7. Emerging from this relatively rigid structure are two peptide loops which are believed to interact with the receptor. On the basis of secondary structure predictions, Fontecilla suggested that both

R.C. Hider is in the Department of Chemistry, University of Essex.

100 years ago

"How I crossed Africa, from the Atlantic to the Indian Ocean" By Major Serpa Pinto (London: Sampson Low & Co., 1881)

To cross Africa has almost ceased to be an extraordinary feat. Indeed it seems evident, the more we know of the Portuguese native traders, that even before Livingstone's memorable first journey, it was no uncommon thing for the Pombeiros to do in the ordinary way of business. Of course some routes are more dangerous than others, and that by which Stanley made his famous march was perhaps the most difficult and dangerous that could be selected. Still the journey performed by Major Serpa Pinto was in many ways remarkable, and perhaps not its least remarkable feature is the characteristic manner in which he tells his story.

One of our illustrations gives a good idea of an antelope which was met with in the Cuchibi, which the Major thus describes:

"At one of the turns of the river I perceived three antelopes of an unknown species, at least to me; but just as I was in the act of letting fly at them they leaped into the water and disappeared beneath its surface. The circumstance caused me immense surprise, which was increased as I went further on, as I occasionally came across several of these creatures, swimming; and then rapidly diving, keeping their heads under water, so that only the tips of their horns were visible. This strange animal bears among the Bihenos the name of Quichôbo. Its life is in a great measure passed in the water, it never straying far



The Quichôbo

from the river banks, on to which it crawls for pasture, and then chiefly in the night-time. It sleeps and reposes in the water. Its diving-powers are equal, if not superior, to those of the hippopotamus. During sleep it comes near to the surface of the water, so as to show half its horns above it. It is very timid by nature, and plunges to the bottom of the river at the slightest symptom of danger. It can easily be captured and killed, so that the natives hunt it successfully, turning to account its magnificent skin and feeding off its carcase, which is however but poor meat. Upon leaving the water for pasture its little skill in running allows the natives to take it alive; and it is not

dangerous, even at bay, like most the antelope tribe. The female, as well as the male, is furnished with horns. There are many points of contact between the life of this strange ruminant and that of the hippopotamus, its near neighbour. The rivers Cubangui, Cuchibi, and the upper Cuando offer a refuge to thousands of Quichôbos, whilst they do not appear either in the lower Cuando or the Zambesi. I explain this fact by the greater ferocity of the crocodiles in the Zambesi and lower Cuando, which would make short work of so defenceless an animal if it ventured to show itself in their waters."

From Nature 24, 7 July, 215, 1881.

classes of toxin have similar shapes but differ in the relative lengths of the peptide loops emerging from the conserved framework. Why these relatively minor differences are associated with the presence or absence of voltage-sensitive binding is not clear but it would be interesting to study their relative affinity for the 37,000-molecular weight protein identified by Catterall.

The cholinergic synapse

There is now general agreement on the structure of the Torpedo (electric ray) acetylcholine receptor. In his review, J.P. Changeux (Pasteur Institute) reported that the basic functional unit, as judged by reconstitution experiments, is composed of four polypeptide subunits of molecular weights 40, 50, 60 and 65×10^3 respectively, the latter three being extremely susceptible towards proteolysis. The 40,000molecular weight protein is the site of agonist (acetylcholine) and antagonist (curare and elapidae snake toxin) binding. Exactly how the snake neurotoxins interact with the 40,000-molecular weight subunit is unknown and this uncertainty severely limits their use as affinity ligands. However the problem is being energetically attacked by the Russian group led by F. Bystrov and V. Tsetlin (Shemyakin Institute), who are applying ESR, NMR and photoaffinity labelling techniques to monitor the toxinreceptor interaction8. A 43,000-molecular weight subunit, which is also associated with the receptor, has been shown not to be essential for acetylcholine receptormediated cation translocation9 but is thought to have a structural role, crosslinking receptor rosettes into the characteristic double rows often seen in electron micrographs 10.

Presynaptic phenomena are not so well characterized at the molecular level as are the corresponding postsynaptic events, but J.O. Dolly (Imperial College, London) and A.L. Harvey (University of Strathclyde) reported two new approaches designed to tackle this problem. Dolly described a method for the purification and iodination of botulinum toxin which specifically inhibits the release of acetylcholine¹¹ and Harvey demonstrated that Kunitz-type protease inhibitor homologues, isolated from the venom of *Dendroaspis* snakes (mambas), dramatically stimulate acetylcholine release¹². Significantly, as judged from pharmacological studies, these inhibitor-like polypeptides compete with β -bungarotoxin (from krait venom) for a common binding site on the presynaptic membrane. β -bungarotoxin is used extensively by physiologists and consists of two polypeptides, the A chain, a phospholipase, and the B chain, a protease inhibitor homologue. Thus it seems likely that the B chain directs the phospholipase activity of the A chain to the presynaptic membrane. Both botulinum toxin and protease inhibitor homologues would appear to have potential as specific presynaptic probes, as unlike the toxins currently used, they lack phospholipase activity. Development of new antivenins

In many meetings devoted to neurotoxins, there is often a tendency to forget that snakes and scorpions can present serious health hazards. Each year 1,000 people die in Mexico from scorpion stings and 2,500 people die in South-east Asia from snake bites¹³. Some of the symptoms associated with such poisoning were vividly presented in a session devoted to the clinical aspects of envenomation which was chaired by H.A. Reid (Liverpool School of Tropical Medicine and see ref. 14). The efficiency of antivenoms is likely to improve now that new techniques have been developed to produce antibodies specifically directed against the most toxic components of the venom. One such example was reported by J.C. Boulain and A. Menez (CEN Saclay, France) where the production of a monoclonal antibody directed against a cobra neurotoxin was described. Combinations of such antibodies directed against different snake neurotoxins should produce efficient antivenoms for specific geographical localities and thus, in principle, a comprehensive range of such antivenoms could become available for world-wide distribution.

Cytolytic toxins

Cobra venoms, in addition to containing postsynaptically acting neurotoxins, possess an even higher content of so-called 'cardiotoxins'. This term, although widely used, provides too narrow a label as these polypeptides depolarize all muscle types and many also possess lytic properties. Indeed, as described by D.S. Chapman (Ashington, Northumberland, UK), cytotoxicity and not neurotoxicity is the dominant symptom of Naja nigricollis (spitting cobra) envenomation. J. Dufourcq (Talence, France) eloquently described the interaction of these hydrophobic proteins with negatively charged lipids, showing how they induce lipid phase separation, and R. Hider (University of Essex) demonstrated that some toxins which possess powerful cardiotoxic properties lack lytic properties, arguing against the view that the muscle-depolarizing ability is directly associated with non-

- Catterall et al. Proc. natn. Acad. Sci. U.S. A. (in the press)
- Agew & Raftery Biochemistry 18, 1912 (19 Catterall A Rev Pharmac Toxical 20, 15 (1980)
- 6. Jover et al. Biochem. biophys. Res. Commun. 95, 1607 (1980).
- Bugg et al. Proc. natn. Acad. Sci. U.S.A. 77, 6496 (1980).
- Bystrov et al. FEBS Lett. 106, 47 (1979)
- Wu & Raftery Biochemistry 20, 694 (1981)
- 10. Changeux et al. FEBS Lett. 121, 327 (1980). Dolly et al. J. Physiol., Lond. (in the press)
- 12. Harvey et al. Naunyn-Schmiedebergs Arch. Pharmac. 312.
- 13. Progress in Characterization of Venoms and Standardization of Anti-venoms (W.H.O., 1981)
- Taxicon 18, 129 (1980).
- 15. Delori & Tessier Biochimie 62, 287 (1980)
- 16. Mollby & Thelestam Biochim. biophys. Acta 557, 156
- 17. Latorre & Alvarez Physiol, Rev. 61, 77 (1981).
- 18. Engelman et al. Proc. natn. Acad. Sci. U.S.A. 77, 2023 (1981).
- 19. Duncan Experienta 34, 1531 (1978); Schanne et al. Science 206, 700 (1979).

specific lytic properties. In the past much of this work has been bedevilled by contaminating traces of phospholipase, which can endow the preparation with lytic activity. H. Veheij (University of Utrecht) reviewed the chemistry of phospholipase-A, molecules, indicating how the increased content of surface aromatic side chains is associated with improved powers of membrane penetration. Presumably the tight association between these enzymes and the hydrophobic cardiotoxins results from a similar interaction. Immunoaffinity chromatography is an excellent method for removing traces of phospholipase¹⁵ and probably should be used routinely in the isolation of this class of protein.

R. Mollby and M. Thelestam (National Bacteriology Laboratory and Karolinska Institute, Stockholm) described how their fibroblast-based analytical system permitted the classification of cytolytic toxins16. Their data formed a useful background for the classification of toxins isolated from Aeromonas hydrophila (Gram-negative rod bacterium) and the socalled thiol-activated cytolysins. However it was clear from the meeting that the mode of action of many of these toxins is not understood. The colicins A, E, I and K were reported by F. Pattus and C. Lazdunski (University of Marseille) to form voltage-dependent channels in bilayer membranes, in a similar manner to the lytic peptide alamethicin¹⁷. In this context, J.H. Freer (University of Strathclyde) tentatively suggested the existence of an oligomeric pore structure for staphylococcal δ-lysin and R. Hider (University of Essex) made a similar suggestion for bee venom melittin. The lipophilic portion of most plasma membranes is 3 nm thick and thus can be spanned by a 21-residue α -helix. As alamethicin (20 residues), δ-lysin (26 residues) and melittin (26 residues) can all form amphiphilic helices, toxin aggregates could readily form hydrophilic pores capable of spanning a membrane. Significantly, the average number of residues in each of the seven bacteriorhodopsin α -helices is 25 (see ref. 18). Thus in principle, cytolysin oligomers could form bacteriorhodopsin-like structures. If calcium entered or magnesium left the cell via such channels, intracellular proteases would be activated, inducing dramatic effects on the cell membrane¹⁹. Ion specificity of such a channel would be controlled by both the diameter of the aqueous channel and its net charge, and as such could be related to the Mollby-Thelestam classification scheme. Ion specificity of such oligomeric structures will be of considerable interest.

The success of this meeting was, to a large extent, due to the International Society of Toxinology attracting, and thus facilitating, interaction between scientists from an extremely wide range of disciplines. As toxin chemistry and biochemistry continue to expand, so will this Society.

Romey et al. Biochim. biophys. Acta 556, 344 (1979). Berwald-Netter et al. Proc. natn. Acad. Sci. U.S.A. 78,

ARTICLES

Three myosin heavy-chain isozymes appear sequentially in rat muscle development

Robert G. Whalen, Susan M. Sell & Gillian S. Butler-Browne

Département de Biologie Moléculaire, Institut Pasteur, 25, Rue du Dr Roux, 75724 Paris, France

Ketty Schwartz & Pierre Bouveret

INSERM Unité 127, Hôpital Lariboisière, 41, Bd de la Chapelle, 75010 Paris, France

Ingrid Pinset-Härström

Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91190 Gif sur Yvette, France

Three different heavy-chain isozymes of myosin appear sequentially in rat muscle during the period from late gestation to about three weeks of age. In part these changes can be related to changes in innervation taking place during this period.

THE myosins found in both muscle and non-muscle cells comprise a family of proteins having similar native structures and subunit compositions¹⁻⁴. The myosin isoenzymes in adult skeletal muscle can be classified into two major types. A myosin capable of rapid ATP hydrolysis is characteristic of fast contracting muscle fibres while a myosin of lower ATPase activity is found in slow-contracting fibres⁵. These fast and slow isoenzymes differ in both their heavy- and light-chain subunits. It has been shown⁵ that the maximum speed of shortening of adult muscles is closely correlated to the rate of hydrolysis of ATP by myosin. Therefore the type of isoenzyme present in a muscle can be of physiological importance^{5,6}.

The muscles of newborn animals contract slowly although they may become fast-contracting in the adult⁷⁻⁹. Previous results¹⁰⁻¹⁶ have suggested that slow myosin heavy chain was a major constituent of developing muscle, whereas others report the presence of fast myosin¹⁷⁻²¹ (see also discussion in refs 22, 23).

Recent biochemical and immunological studies have however shown that fetal and newborn skeletal muscles contain certain myosin subunits distinct from the adult forms 24-27. In the case of the myosin heavy chain, the adult forms are not detected as major components of fetal muscle or cultured muscle cells 25-27. To characterize the developmental changes in more detail, we have investigated the types of myosin heavy chains found at various times after birth in rat skeletal muscle destined to become the fast-contracting type. We used various approaches, including polypeptide mapping, complement fixation, immunocytochemistry, gel electrophoresis of native myosins, and the study of synthetic myosin thick filaments by electron microscopy. Our results provide evidence for the sequential appearance of at least three heavy-chain isozymes during muscle development.

Polypeptide mapping of the myosin heavy chains

The large size of the myosin heavy chain $(200,000\,M_{\rm r})$ allows it to be cleaved into many polypeptide fragments which are conveniently analysed by standard one- or two-dimensional gel electrophoresis (ref. 27 and refs therein). However, partial proteolytic cleavage of native myosin is influenced by divalent cations²⁸, by phosphorylation of the LC2 light chain²⁹, and by interaction with actin³⁰ and presumably other proteins. It is thus important to denature the myosin preparations before partial proteolysis to minimize influences other than that of the primary structure on the cleavage of the heavy chain. Proteolysis of SDS-denatured myosin suggests that neither the presence of the light chains nor the presence of another myosin species

influences the cleavage of a given heavy-chain type (ref. 27 and S.M.S. and R.G.W., in preparation). This approach has therefore been used to examine the myosin heavy chains present in rat muscle development.

Figure 1 (upper) shows the heavy-chain degradation pattern produced by chymotryptic treatment of various myosins from cultured cells (L6) or fetal, newborn and adult muscle tissue. From about 19 days after birth, the myosin heavy-chain cleavage pattern is indistinguishable from that shown by adult fast myosin. As described previously²⁷, a distinct embryonic myosin heavy chain (MHC_{emb}) can be found in cultured muscle cells and fetal muscle tissue. However, during the first two weeks after birth another heavy chain isozyme appears which differs in its degradation pattern from both the embryonic form and the adult fast and slow forms. We will refer to this isozyme appearing in the neonatal period as MHC_{neo}.

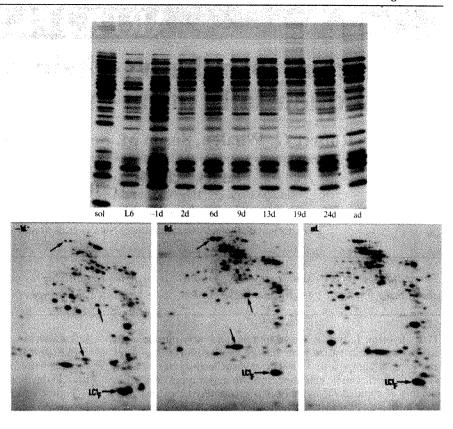
The cleavage pattern produced by the neonatal myosin preparations is unique, as the two-dimensional gel analysis of the chymotryptic polypeptides illustrates (Fig. 1, lower half). This high-resolution analysis shows that the cleavage pattern of neonatal heavy chain is qualitatively different from that of either MHC_{emb} or fast myosin heavy chain (MHC_F). Some characteristic MHC_{neo} polypeptides are shown by arrows in Fig. 1 (lower half). The myosin from fetal tissue (-1 day) contains some MHC_{neo} (arrows; see also Fig. 4). The two-dimensional polypeptide map of MHC_{neo} bears no resemblance to that of the adult slow heavy chain (compare with Fig. 3 of ref. 27). The cleavage pattern of MHC_{neo} is unlike that of any other adult skeletal or cardiac muscle myosin that we have examined nor is it homologous to non-muscle heavy chain (S.M.S. and R.G.W., unpublished observation).

Immunological studies on developmental isozymes of myosin

Complement fixation has been used as a means of measuring the extent of cross-reactivity of a given antibody with different myosins^{27,31-35}. Previous work has shown that the myosin subfragment, heavy meromyosin (HMM), denatured with SDS before injection, produces antibodies with considerable muscle-type specificity. This specificity is greater than when the HMM is injected in its native state^{27,35}. Thus antibodies to SDS-denatured HMM prepared from 8-day old rats were raised in guinea pigs.

In our experiments we used antisera from two different guinea pigs. These anti-neonatal myosin sera react predominantly with the heavy-chain component; this was verified by testing the sera against myosin or crude myosin-containing extracts after separation by SDS-gel electrophoresis and transfer to nitrocel-

Fig. 1 Polypeptide mapping of various myosins. Myosins were prepared as previously described2 , the final step being purification on a Sepharose 2B column in the presence of ATP. Myosin was prepared from the adult soleus muscle (sol) as an example of slow myosin, and from L6 cells as an example of a myosin containing the embryonic heavy chain (see text). The remaining myosins were prepared from the hind leg muscles of adult rats (ad), the soleus muscle being deliberately excluded, or from rats at various stages of development (day -1 is 20 days of gestation; the other numbers indicate days after birth). The proportion of slow myosin in the adult hind leg muscle preparations did not exceed 5%, as determined by electrophoretic analysis of native myosin (see Fig. 4 legend). In the upper part of the figure, analysis was carried out on a one-dimensional SDS gel composed of 12.5% (w/v) acrylamide and 0.1% (w/v) bisacrylamide. Myosins (0.4 mg ml^{-1}) were digested with chymotrypsin (108 µg ml⁻¹) for 30 min at 37 °C as described previously²⁷, and 16 µg of each degraded myosin was loaded on to the gel. In the lower part of the figure, 60 µg of chymotryptic cleavage products were analysed on twodimensional gels. For these experiments, the concentration of myosin was 1.2 mg ml that of chymotrypsin 300 µg ml⁻¹. ampholytes in the isoelectric focusing gel were composed of 1.6% (w/v), pH 5-8 and 0.4% (w/v), pH 3-10, and the second dimension slab gel was 12.5% (w/v) acrylamide and 0.1%



(w/v) bisacrylamide. Two-dimensional gel electrophoresis was carried out as in ref. 24 with the modifications described in ref. 51. The position of the highest molecular weight light chain, LC1_F, is shown for reference as the extent of migration varied slightly among the different samples. Unlabelled arrows indicate characteristic polypeptides of the neonatal heavy chain which are also present in small quantities in the -1 day myosin sample.

lulose paper³⁶ (A.-M. Lompré and K. Schwartz, unpublished results). The myosins to be used as test antigens in the complement fixation reaction were purified on Sepharose 2B columns²⁴, denatured with SDS and mercaptoethanol by heating, and the excess SDS removed as described previously³² These myosins were reacted with the antisera at dilutions chosen to give $\sim 60-70\%$ complement fixation with the homologous antigen (myosin from 10-day old animals). When the antisera were tested with myosin from adult fast muscle, fetal muscle tissue or from L6 cells, the amount of complement fixed was considerably less than in the case of the neonatal myosin (Fig. 2). This result illustrates that there are quantitative antigenic differences between the various myosins, although they share some common determinants. The absence of a reaction for L6 myosin at the dilutions shown does not indicate a total lack of cross-reactivity; if the neonatal myosin antibodies are simply concentrated then a positive reaction can be obtained for the L6 myosin which differs quantitatively from that given by the homologous antigen (result not shown; see also ref. 27). The myosin from fetal muscle tissue gives greater complement fixation than does L6 myosin due to the presence of MHC_{neo} in the tissue preparations (see Figs 1, 4).

These results show that neonatal myosin differs immunologically from both adult myosin and the embryonic form found in fetal muscle tissue and L6 cells. Together with previous results which show immunological differences between L6 and adult myosin²⁷, this approach distinguishes between the three myosins containing MHC_{emb}, MHC_{neo} and MHC_F.

Antibodies to adult fast and slow myosin were also used in indirect immunofluorescence assays on sections of the gastrocnemius muscle of 7-8-day old rats. Most of the fibres in such muscles are stained when reacted with the antibody to adult fast myosin (Fig. 3a). When this antibody is absorbed with myosin extracted from neonatal muscle (see Fig. 3 legend for details), the fluorescence reaction with the neonatal muscle sections is diminished as expected (Fig. 3b). When used at the same dilution, this absorbed antibody will still react with the fast fibres

of the adult diaphragm (Fig. 3c). These results show that the unabsorbed anti-fast myosin antibody cross-reacts with neonatal myosin but that these cross-reacting antibodies can be removed without totally abolishing the reaction with adult fast myosin. An antibody to adult cardiac myosin which cross-reacts extensively with slow-twitch skeletal myosin^{27,35} was used as a probe for slow myosin-containing fibres. Few fibres stained brightly (Fig. 3d); the proportion of positive fibres varied according to the region of the muscle examined. These immunocytochemical results thus completely agree with the

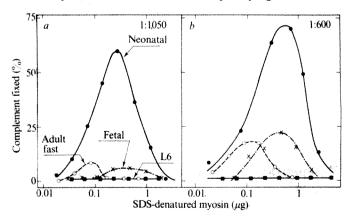


Fig. 2 Complement fixation reactions using antisera to neonatal myosin. Heavy meromyosin (HMM) was prepared from the myosin extracted from the hind leg muscle of 8-day old rats³³. The HMM was denatured with SDS and the excess SDS removed on a G-10 column as described elsewhere³² before injection into five guinea pigs. Four animals developed antibodies; the results shown here are for two of these antisera. The test antigens were prepared from adult muscle (adult fast, ○), muscle of 10-day old animals (neonatal, ♠), muscle from animals at 20 days of gestation (fetal, ×) and from fused cultures of L6 cells (L6, ■). Each test antigen was denatured with SDS and the excess SDS removed on a G-10 column³² before reaction in the complement fixation test^{33,35}.

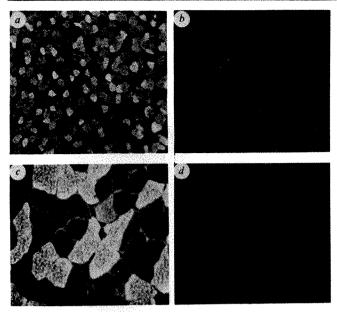


Fig. 3 Immunocytochemical reactions of neonatal and adult muscles. Indirect immunofluorescence was carried out using antibodies prepared in guinea pigs. A fluorescein-labelled goat antiguinea pig immunoglobulin antiserum (Nordic) was used as the second antibody. In a, an antibody to adult fast myosin³⁵ was reacted at a dilution of 1:100 with a section of 7-day gastrocnemius muscle. The anti-fast antibody was also absorbed with myosin prepared from neonatal muscle by first mixing the myosin and antiserum in 0.6 M NaCl and then dialysing to low ionic strength to precipitate the myosin. In b, this absorbed antibody was reacted at a dilution of 1:40 with the 7-day gastrocnemius muscle. In c, the 1:40 diluted absorbed antibody was reacted with a section of adult diaphragm; it reacts with those fibres that react as fast fibres in histochemical staining (results not shown). d, The 7-day gastrocnemius muscle was stained with an antibody to adult cardiac myosin^{27,35}

polypeptide mapping and complement fixation experiments in that they show differences between neonatal and adult myosins.

Electrophoresis of native myosins

The various myosin preparations were further investigated by gel electrophoresis in native conditions^{37,38}; L6 myosin (containing MHC_{emb}) gave only one band (Fig. 4). This same band is found in extracts of fetal tissue at 3 days before birth. Two days later, and in the first few days after birth, another band is present which is distinct from that containing MHC_{emb} (see also the results for the mixture of L6 myosin and a 10-day preparation); this corresponds to the appearance of myosin containing MHC $_{\rm neo}$ (Fig. 1). In the second week after birth, three bands are seen for neonatal myosin; the presence of three bands at this time is probably due to the formation of isoenzymes based on light chain content^{25,39}. From about 14 days onward, the adult fast myosin bands can be seen. The adult slow myosin isozymes are clearly separated from the embryonic, neonatal and adult fast forms (Fig. 4). This analysis confirms the time course of the developmental sequence observed using the polypeptide mapping approach (Fig. 1).

Formation of synthetic myosin filaments

The size and structure of synthetic thick filaments formed from adult fast skeletal myosin depends critically on the relative concentrations of ATP and Mg²⁺ in the dilution buffer⁴⁰. We have therefore compared the effect of these parameters on the formation of synthetic filaments obtained from myosins containing the embryonic, neonatal and adult fast and slow heavy chains.

Synthetic filaments were formed by controlled dilution of myosin and were examined by electron microscopy as previously described⁴⁰. In these experiments, the concentration of ATP in the diluting buffer was kept constant at 0.1 mM and the

 ${\rm Mg}^{2^+}$ concentration varied between 0.1 and 10 mM. Electron micrographs of the various types of filaments obtained from neonatal myosin are shown in Fig. 5a-c. Depending on the concentration of ${\rm Mg}^{2^+}$, three types of filaments were observed. At intermediate concentrations bipolar filaments with tapering ends and constant diameters of 15-20 nm were found (Fig. 5a). This type of filament is structurally similar to natural ones isolated from adult fast skeletal muscle 40.41. At high concentrations of ${\rm Mg}^{2^+}$, thicker spindle-shaped filaments up to 50-60 nm in diameter were formed (Fig. 5b). At low concentrations of ${\rm Mg}^{2^+}$, thin branched structures, ~5-7 nm wide were observed (Fig. 5c). The neonatal myosin filament preparations thus behave qualitatively in the same way as those of adult fast myosin 40.

The concentration range over which these types of filaments were found differed for the several myosins tested (results shown schematically in Fig. 5, lower left). Both fast and slow adult myosins form bipolar filaments with constant diameters over a similar, large concentration range (0.5-10 mM Mg²⁺). The Mg² values at which such bipolar filaments are found is narrow for neonatal myosin and is particularly restricted with L6 myosin. In the latter case, homogeneous populations of bipolar filaments with constant diameters were rarely obtained. Furthermore, the lengths of the bipolar filaments formed in the presence of 2 mM Mg2+ differed for the various myosins (see histogram, Fig. 5, lower right). Both adult fast and slow myosin formed very long filaments (5-7 µm), longer in fact than natural filaments from fast muscle, which are 1.6 µm long41. Neonatal myosin formed filaments of intermediate length (2-4 µm) and L6 myosin only very short ones ($<1 \mu m$).

The formation of myosin thick filaments is due to the aggregation of the heavy-chain subunit, in particular the C-terminal α -helical portion. It has been suggested that the LC2 light chain might influence filament formation 42.43. In adult fast, neonatal and L6 myosins, the LC2 light chain seems to be the same (ref. 24 and R.G.W., results not shown). Thus the differences in filament formation between these three myosins are probably attributable to differences among the heavy chains, in agreement with our biochemical and immunological results.

Relationship to previous studies

We have demonstrated that development of rat skeletal muscle tissue is characterized by the presence of specific myosin isozymes differing from both the fast and slow adult forms. These results are consistent with several previous studies. Earlier work by Trayer and Perry¹⁰ has shown that the Ca²⁺-ATPase activity of myosin changes with development of the muscles of several animals, including rats. In addition, Drachman and Johnston⁹ have shown that actomyosin ATPase activity is low in the neonatal rat EDL (extensor digitorum longus) muscle until 5 days after birth. After this time the activity increases, reaching adult values by ~20 days. These changes in ATPase activities

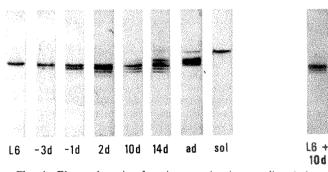


Fig. 4 Electrophoresis of native myosins in non-dissociating conditions. Myosin samples (1-3 μg) were run for 16 h at 14 V cm⁻¹ and 2 °C on cylindrical gels (5×60 mm) which consisted of 3.88% (w/v) acrylamide and 0.12% (w/v) bisacrylamide and contained 20 mM sodium pyrophosphate (pH 8.5), 10% (v/v) glycerol, 1 mM EDTA and 0.01% (v/v) 2-mercaptoethanol. Migration was from top (cathode) to bottom (anode). Abbreviations as in Fig. 1.

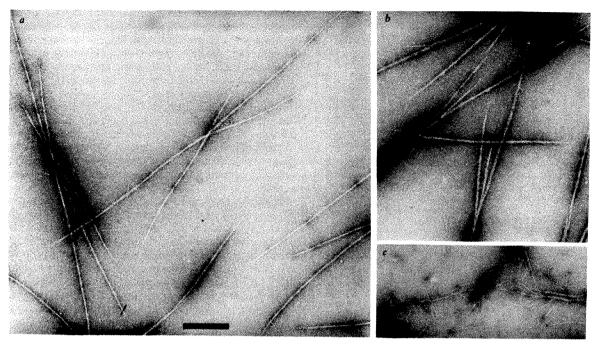
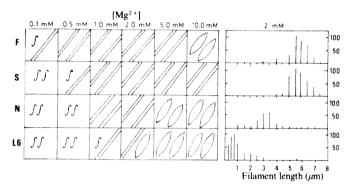


Fig. 5 Electron microscopy of synthetic myosin filaments. The upper part of the figure shows representative micrographs illustrating the overall appearance of filaments formed from neonatal myosin (10-day old muscle) in the presence of 0.1 mM ATP. The Mg²⁺ concentration in the polymerization medium was: a, 2 mM Mg²⁺; b, 10 mM Mg²⁺ and c, 0.1 mM Mg²⁺. Filaments were formed by controlled dilution and prepared for electron microscopic examination as previously described⁴⁰. The bar in a represents 0.5 μ m. The lower left part of the figure shows a schematic representation of the electron microscopic observations of the filaments formed from several types of myosins: adult fast (F), adult slow (S), neonatal (N) and embryonic myosin from fused L6 cells. Polymerization was carried out in the presence of 0.1 mM ATP and the Mg²⁺ concentration was varied over the values indicated. The parallel lines indicate the presence of long, bipolar filaments of 15-20 nm diameter (as in a; see text). The leaf-shaped



symbols indicate the presence of filaments of thicker diameter (as in b). The curved lines indicate the presence of short, branched structures (as in c). Two identical symbols in the same square indicate that the filament population was homogeneous; two different symbols indicate that the sample was apparently a mixture of filament types. The lower right part of the figure is a histogram showing the distribution of filament lengths (in μ m) observed at 2 mM Mg²⁺ for the four different myosins. In all the experiments shown here, the preparation of the myosin and the electron microscopy did not exceed 24 h to minimize any phenomena due to ageing of the myosin as noted previously⁴². A shortened protocol was therefore used to prepare the myosin. The muscle tissue was homogenized in a Polytron homogenizer in three volumes of a buffer containing 0.5 M NaCl, 0.05 M Tris-HCl (pH 6.8), 10 mM MgCl₂ and 3 mM ATP. After extraction for 20 min at 0 °C, the sample was centrifuged at 15,000g for 20 min. More ATP was added to the supernatant to give a final concentration of 6 mM, and the sample was centrifuged at 400,000g for 1 h or more to remove polymeric actin. The resultant supernatant was dialysed against a large volume of 40 mM NaCl, 30 mM Tris-HCl (pH 6.8). After ~3 h the precipitated myosin was collected by centrifugation and resuspended in 0.5 M NaCl, 20 mM Tris-HCl (pH 6.8). The myosin was stored on ice for several hours until the start of the electron microscopy experiments.

may be a manifestation of the transitions taking place among different myosin isozymes, as they occur in rats at about the same time as the observed transition from neonatal to adult myosin.

Trayer and Perry¹⁰ have also shown that changes in ATPase occur somewhat earlier in rabbit than in rat. Using polypeptide mapping, we have found that the predominant myosin heavy chain in newborn rabbits is homologous to the rat neonatal form (results not shown). Migration of the rat neonatal isozymes in native electrophoresis is also very similar to that of newborn rabbit myosin²⁵. The neonatal form of rabbit myosin persists until at least 10-15 days after birth (results not shown). This observation can be related to studies of amino acid sequence: Huszar⁴⁴ found that the myosin from 3-week old rabbits contains two different, yet homologous, histidine-containing tridecapeptides whereas adult myosin retains only one. From our results, the additional sequence found at 3 weeks post-natal probably corresponds to that of rabbit neonatal myosin and not embryonic or adult slow myosin. Both the sequence studies and the results of polypeptide mapping show that the isozyme transitions involve heavy chains that differ in primary structure. Our results are consistent with previous immunocytochemical studies on developing rat muscle 14,16 which used antibodies directed against the adult fast and slow myosins. It is likely that these antibodies cross-reacted with MHC_{emb}, MHC_{neo} or both. The use of antibodies to native myosin has demonstrated considerable antigenic homology among adult fast and slow as well as embryonic myosin heavy chains^{27,34}. Moreover the adult anti-fast myosin antibody used here (Fig. 3) clearly cross-reacts with neonatal myosin. However, it can be noted that Gauthier *et al.* 14 found that the adult pattern of antibody staining is established by the third week post-natal. This timing correlates well with the disappearance of the neonatal isozyme.

The conflicting results of immunocytochemical studies on developing chicken muscle^{13,15,19,21} may also be due to differing degrees of cross-reactivity of the antibodies with myosin types analogous to the rat embryonic and neonatal forms. It has been claimed²¹ that the myosin heavy chain of cultured chicken cells is identical to the adult protein, based on results of Ouchterlony double diffusion. It was found that if a mixture of radioactively-labelled, cultured myosin and adult fast myosin was diffused against antibodies to fast myosin then both proteins were found

MHCEMB	MHCNEO	MHC _F
LC1 _{EMB} - LC1 _F -	LC1 _F	◆ LC1 _F
LC2 _F	LC2 _F	LC2 _F
·	LC3 _F	LC3 _F
L6 CULTURES		
PRIMARY CULTURES	7-11 DAY	ADULT FAST
FETAL TISSUE	Muscle Tissue	Muscle

Fig. 6 Schematic representation of the myosin subunits present at various times during fast muscle development. This representation is meant to illustrate concisely the predominant polypeptides present in a given situation; it does not show explicitly the stoichiometry of the native molecule nor does it indicate presumably minor combinations of heavy and light chains (such as LClemb with MHC_{neo}).

in the same precipitation line²¹. Such a result is precisely that expected if the culture myosin is different from the adult fast protein but simple shares some antigenic determinants with the latter. Polypeptide mapping studies provide evidence for a distinct developmental form of myosin heavy chain in chickens (refs 26, 45 and S.M.S. and R.G.W., unpublished observations). Thus there may be parallels between avian and mammalian muscle development concerning the myosin heavy-chain isozvmes.

Innervation and myosin isozyme transitions in muscle development

A schematic representation of the myosin transitions occurring in rat muscle development is given in Fig. 6. Our data suggest that the first heavy-chain isozyme to appear after muscle cell fusion is that designated MHC_{emb}, based on results with cultured myotubes and fetal muscle tissue^{27,46}. The light-chain subunits associated with this heavy chain evolve as cultured myotubes then mature⁴⁶. The predominant myosin heavy-chain form at different stages of development is either the embryonic or the neonatal isozyme (Fig. 6). We cannot, however, rule out the presence of small amounts of the adult fast and slow types at these stages. Immunocytochemical studies of fetal rat muscles suggest the presence of a major fibre population containing slow myosin¹⁶. Whether this result is simply due to a cross-reaction with embryonic myosin is unknown. The precise sequence of appearance of these four isozymes within individual fibres can be determined when antibodies specific to each type become available. It is possible, for example, that different fibre types evolve differently16 and that MHCemb may be a component of one type and MHC_{neo} a component of another.

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- Clarke, M. & Spudich, J. A. A. Rev. Biochem. 46, 797-822 (1977).
- Burridge, K. & Bray, D. J. molec. Biol. 99, 1-14 (1975). Chi, J. C., Fellini, S. A. & Holtzer, H. Proc. natn. Acad. Sci. U.S.A. 72, 4999-5003 (1975).
- Scordilis, S. & Adelstein, R. A. Nature 268, 558-560 (1977). Pette, D. (ed.) Plasticity of Muscle (Walter de Gruyter, Berlin, 1980).
- Salmons, S. & Henrikson, J. Muscle & Nerve 4, 94–105 (1981).
 Buller, A. J., Eccles, J. C. & Eccles, R. M. J. Physiol., Lond. 150, 399–416 (1960).
 Close, R. J. Physiol., Lond. 173, 74–95 (1964).
 Drachman, D. B. & Johnston, D. M. J. Physiol., Lond. 234, 29–42 (1973).

- Trayer, I. P. & Perry, S. V. Biochem. Z. 345, 87-100 (1966). Brevet, A. & Whalen, R. G. Biochimie 60, 459-466 (1978).

- Brevet, A. & Whalen, R. G. Biochimie 60, 459-466 (1978).
 John, H. A. FEBS Lett. 64, 116-121 (1976).
 Masaki, T. & Yoshizaki, C. J. Biochem., Tokyo 76, 123-131 (1974).
 Gauthier, G. F., Lowey, S. & Hobbs, A. W. Nature 274, 25-29 (1978).
 Cantini, M., Sartore, S. & Schiaffino, S. J. Cell Biol. 85, 903-909 (1980).
 Kelly, A. M. & Rubinstein, N. A. Nature 288, 266-269 (1980).
 Dow, J. & Stracher, A. Proc. natm. Acad. Sci. U.S.A. 68, 1107-1110 (1971).
 Streter, F. A., Balint, M. & Gergely, J. Devl Biol. 46, 317-325 (1975).
 Rubinstein, N. A., Pepe, F. A. & Holtzer, H. Proc. natm. Acad. Sci. U.S.A. 74, 4524-4527 (1977).

- Rubinstein, N. A. & Kelly, A. M. Devl Biol. 62, 473-485 (1978).
 Rubinstein, N. A. & Holtzer, H. Nature 280, 323-325 (1979).
 Whalen, R. G. Plasticity of Muscle (ed. Pette, D.) 177-191 (Walter de Gruyter, Berlin,
- Whalen, R. G. Adv. physiol. Sci. 5, 63-69 (1981). Whalen, R. G., Butler-Browne, G. S. & Gros, F. J. molec. Biol. 126, 415-431 (1978).

- Whatel, R. S., Sultar Bowle, G. P. S. Nature 280, 321-323 (1979).
 Rushbrook, J. I. & Stracher, A. Proc. natn. Acad. Sci. U.S.A. 76, 4331-4334 (1979).
 Whalen, R. G., Schwartz, K., Bouveret, P., Sell, S. M. & Gros, F. Proc. natn. Acad. Sci. U.S.A. 76, 5197-5201 (1979).

Activity or trophic influences of the nerve are important in determining the phenotype of the muscle in the adult animal, and similar mechanisms might operate during development5.6 Muscle fibres of the newborn rat are in contact with several nerve axons⁴⁷. This polyneuronal innervation regresses so that by ~3 weeks after birth only one axon remains per muscle fibre. Results which suggest that both fast and slow adult myosins are present in the early developmental stages14 could be taken to indicate that a switching off of one or the other myosin type occurs in response to the establishment of the adult configuration of the neuromuscular junction. It has also been suggested 20 that only fast myosin is present in developing muscle fibres and that the induction of slow myosin can occur as a specific nerve impulse pattern is established during the regression of polyneuronal innervation.

However, our results have shown that the predominant myosin heavy-chain isozymes in developing muscle are neither adult fast myosin nor a mixture of the adult fast and slow types. One possibility is that the absence of well defined nerve activity or trophic pattern at these stages is responsible for the absence of adult myosin types. Embryonic myosin is synthesized by cultured myotubes in the absence of innervation 27,46. Whether the neonatal heavy-chain form is actively induced as a consequence of polyneuronal innervation, or whether it can be synthesized by the muscle fibre in the absence of any nervous input is unknown.

This is the first demonstration of three myosin heavy-chain isozymes occurring sequentially in fast muscle development. There have been other demonstrations of the sequential appearance of proteins of related structure and function, for example, the embryonic, fetal and adult forms of globin⁴⁸, or α -fetoprotein and serum albumin⁴⁹. The class switch of immunoglobulin molecules in the maturing lymphocyte is also an example of the sequential expression of protein isoforms. This immunoglobulin switching occurs via deletions of the DNA coding for the constant-region domains of the antibody molecule (see ref. 50). Myosin is a protein also composed of several clearly different domains1. Whether the isozymic transitions of the myosin heavy chain reported here are related to gene deletions and fusions can be tested at the protein level, by comparing the various subfragments of the different isoenzymes, and at the DNA level, using cloned gene fragments.

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- Weeds, A. G. & Pope, B. J. molec. Biol. 111, 129-157 (1977).
 Ritz-Gold, C. J., Cooke, R., Blumenthal, D. K. & Stull, J. T. Biochem. biophys. Res.
- Commun. 93, 209-214 (1980).
 30. Mornet, D., Pantel, P., Audemard, E. & Kassab, R. Biochem. Biophys. Res. Commun. 89, 925-932 (1979)
- Bruggmann, S. & Jenny, E. Biochim. biophys. Acta 412, 39-50 (1975)
- 32. Lompré, A.-M., Bouveret, P., Leger, J. & Schwartz, K. J. immunol. Methods 28, 143-148
- 33. Schwartz, K., Bouveret, P., Sebag, C., Leger, J. & Swynghedauw, B. Biochim. biophys. Acta 42, 24-36 (1977).
- Schwartz, K., Bouveret, P. & Sebag, C. FEBS Lett. 87, 99-102 (1978).
 Schwartz, K., Lompré, A.-M., Bouveret, P., Wisnewsky, C. & Swynghedauw, B. Eur. J. Biochem. 104, 341-346 (1980).
- 36. Bowen, B., Steinberg, J., Laemmli, U.K. & Weintraub, H. Nucleic Acids Res. 8, 1-20
- Hoh, J. F. Y., McGrath, P. A. & White, R. I. Biochem. J. 157, 87-95 (1976)
- Ton, J. F. L., McChaut, F. A. & White, R. L. Bickern, J. 131, 6 (279).
 d'Albis, A., Pantaloni, C. & Bechet, L. J. Eur. J. Biochem. 99, 261–272 (1979).
 Lowey, S., Benfield, P. A., Silberstein, L. & Lang, L. M. Nature 282, 522–524 (1979).
 Pinset-Härström, I. & Truffy, J. J. molec. Biol. 134, 173–188 (1979).
 Huxley, H. E. J. molec. Biol. 7, 281–308 (1963).
 Pinset-Härström, I. & Whalen, R. G. J. molec. Biol. 134, 189–197 (1979).

- Scholey, J. M., Taylor, K. A. & Kendrick-Jones, J. Nature 287, 233-235 (1980).
 Huszar, G. Nature new Biol. 240, 260-264 (1972).

- Huszar, C., Nature new Biol. 240, 200-204 (1912).
 Benfield, P. A., LeBlanc, D. D. & Lowey, S. Fedn Proc. 39, 2169 (Abstr. 2966) (1980).
 Whalen, R. G., Butler-Browne, G. S., Sell, S. & Gros, F. Biochimie 61, 625-632 (1979).
 Brown, M. C., Jansen, J. K. S. & Van Essen, D. C. J. Physiol. Lond. 261, 387-425 (1976).
 Weatherall, D. J. & Clegg, J. B. Cell 16, 467-479 (1979).
 Law, S. W. & Dugaiczyk, A. Nature 291, 201-205 (1981).
 Molgaard, H. V. Nature 286, 657-659 (1980).

- Whalen, R. G. & Sell, S. M. Nature 286, 731-733 (1980).

LETTERS TO NATURE

Distinguishing between a white dwarf and a neutron star in an X-ray binary

Joseph Patterson

Harvard-Smithsonian Center for Astrophysics, Cambridge, Massachusetts 02138, USA

It is proposed that the single most useful clue to the nature of the accreting compact star in an X-ray binary is its ratio of X-ray to visual luminosity $L_{\rm x}(2-10~{\rm keV})/L_{\star}$. Present data indicate that typical $L_{\rm x}/L_{\star}$ ratios are ~4 for white dwarfs, and ~5000 for neutron stars. These numbers are straightforward consequences of the energetics of accretion, and are approximately independent of the distance, stellar mass, and mass transfer rate. The data suggest that the ratios are also not very sensitive to any of the other system parameters, except the radius of the compact star.

Remarkably, there is still no general and reliable method for determining whether the accreting component in a galactic X-ray binary is a white dwarf or neutron star. Davidson and Ostriker¹ and Lamb et al.² argued that the pulsation periods of Cen X-3 and Her X-1 are too short to represent white dwarf pulsation or rotation periods. Lamb et al.² and Rappaport and Joss³ used the observed spin-up time scales in X-ray pulsars to establish the presence of a neutron star in most or all of these systems. Joss4 identified X-ray bursters as neutron stars, and Kylafis and Lamb⁵ suggested that all sources with $L_x >$ 5×10^{36} erg s⁻¹ contain neutron stars. None of these criteria are generally applicable, however, as most sources do not pulse, burst, or reveal their distances. The X-ray spectrum was once thought to be a useful discriminant, when the first detection of an accreting white dwarf showed a very soft spectrum⁶. But subsequent observation has shown that accreting white dwarfs are generally quite hard sources, with bremsstrahlung temperatures of ~10-30 keV (ref. 7). I propose here that the most sensitive and useful indicator of the identity of the compact star is its ratio of X-ray to visual luminosity L_x/L_y . This is easily measured; approximately independent of the distance, stellar mass and mass transfer rate; easily understood in terms of the underlying energetics of accretion; and seems to give the right answer. Figure 1 shows all available data on optically identified compact \bar{X} -ray sources. These divide neatly into two classes.

(1) The filled squares in Fig. 1 are sources discovered by scanning X-ray telescopes (hence 'X-ray selected'), and represent a nearly complete sample down to a flux level of $F_{\rm x}(2-10~{\rm keV}) \simeq 4 \times 10^{-11}~{\rm erg~cm^{-2}~s^{-1}}$. Bradt *et al.*⁸ presented data on $L_x(2-10 \text{ keV})/L_y$ for 54 sources, and seven subsequent identifications have been added: $2A0311-227 (L_x/L_v=5.5)$ (refs 9, 10), 2A0526-328 (1.6) (ref. 11), H2252-035 (3.2) (ref. 12), V1223 Sgr (2.2) (ref. 13), U Gem (1.4) (ref. 14), GK Per (5.2) (refs 15, 16) and 4U1556-60 (2,300) (ref. 17). Although Bradt et al.8 presented their data in terms of 'optical luminosity' (after applying the best available bolometric correction), we have used instead the visual flux and apply only their correction for visual absorption A_v . Thus we avoid working with large and uncertain bolometric corrections. No correction to the 2-10 keV X-ray flux is necessary, because the X-ray cutoff energies are known to be outside this bandpass. To determine the $L_{\rm x}/L_{\rm v}$ ratio of the accreting component (including an accretion disk if present), we must subtract the visual luminosity of the secondary. Where both components are visible (HZ Her, 4U2129+47, H2252-035, Cyg X-2, SS Cyg, GK Per, 2S0921-63, possibly SMC X-1), this can be done with reasonable precision; but in 23 of the 61 stars, the luminosity of the secondary is completely dominant at visual wavelengths, and hence only a lower limit to L_x/L_y can be obtained. Consequently, we exclude these 23 stars from Fig. 1. The remaining 38 stars are shown as the filled squares in Fig. 1.

The open squares in Fig. 1 are Einstein Observatory imaging

proportional counter (IPC) measurements of 25 additional optically selected cataclysmic variables, drawn from available lists (refs 7, 18, unpublished data, and P. Szkody, personal communication). We use the IPC counting rate in the 0.55-4.0 keV energy channels and the visual magnitude m_{ν} as a measure of $L_{\rm X}/L_{\rm v}$. Because eight of the X-ray-selected objects are cataclysmic variables which have been observed with the IPC, we may calibrate this ratio by requiring that the Einstein $L_{\rm X}/L_{\rm v}$ equals the ratio deduced from the data of Bradt et al.⁸. This converts roughly to: $L_x/L_y = 1$ for $m_y = 13.7$, IPC count rate = 0.22 c.p.s. (counts per s). This conversion should be fairly reliable as all these objects are nearby (~100 pc, rendering interstellar absorption unimportant), have similar UBV colours, and have similarly hard X-ray spectra. In addition to the 33 positive detections (including the eight X-ray-selected objects), there are six objects not detected, corresponding to $L_{\rm X}/L_{\rm v} < 0.1$; these are not shown in Fig. 1.

A biomodal distribution in the L_x/L_y ratios is visible in Fig. 1: the sources cluster around mean values of $L_x/L_y = 4$ and 4,900. Because there are two known stable configurations for compact stars with radii differing by a factor of $\sim 10^3$, the neutron-star and white-dwarf population of the two classes should be investigated. The existence of neutron stars in systems containing X-ray pulsars with short spin-up times, and neutron stars or black holes in systems containing X-ray bursters or with $L_x >$ 5×10^{36} erg s⁻¹ can be certified, as can the existence of white dwarfs in classical novae, dwarf novae, AM Herculis stars, and X-ray pulsars with long spin-up times. To avoid optical selection effects only X-ray selected objects are considered. All certified white dwarfs and neutron stars are labelled W or N respectively in Fig. 1. In the group near $L_{\rm X}/L_{\rm v}=5{,}000$, we find 12 neutron stars, no white dwarfs, and 17 uncertain cases. In the group near $L_{\rm X}/L_{\rm v}=4$, we find no neutron stars, seven white dwarfs, and two uncertain cases. The natural inference is that accreting neutron stars typically have $L_x/L_v \sim 5{,}000$, white dwarfs typically have $L_{\rm X}/L_{\rm v}$ -4, and the lack of overlap between the two classes emphasizes the value of $L_{\rm X}/L_{\rm v}$ as a signature of the presence of a white dwarf or neutron star. The Einstein observations of cataclysmic variables (which are known to contain white dwarfs) confirm that our nine objects near $L_x/L_v = 4$ are not aberrant cases, but are fairly typical of accreting white dwarfs (with high $L_{\rm X}/L_{\rm v}$ objects preferentially detected in X-ray surveys, as would be expected).

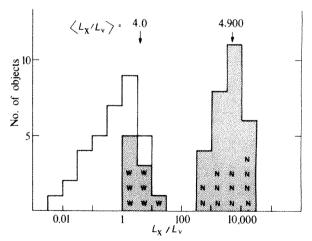


Fig. 1 Distribution of $L_{\rm X}/L_{\rm v}$ ratios for the accreting component of optically identified compact X-ray sources. \square , Sources identified from scanning X-ray telescopes; \square , Einstein observations of optically selected cataclysmic variables (the X-ray and optical measurements during quiescence were used for dwarf novae). The average values of $L_{\rm X}/L_{\rm v}$ are indicated for the group near $L_{\rm X}/L_{\rm v}=1$ and = 5,000. Systems known to contain white dwarfs and neutron stars are labelled W and N respectively.

This result is easy to understand in terms of the energetics of disk accretion, as most of the optical light emerges from large radii in the disk (where there is no dependence on the nature of the compact star), but the X rays emerge from gravitational potential wells which differ in depth by a factor of $\sim 1,000$. For a self-luminous steady-state accretion disk19 surrounding a 1 Mo neutron star, (or black hole, which is for present purposes equivalent to a neutron star), with $\dot{M} = 10^{-10} M_{\odot} \text{ yr}^{-1}$, only 5% of the optical luminosity is produced at radii less than a typical white dwarf radius of 6×108 cm. Thus, for accretion rates near $10^{-10} M_{\odot} \text{ yr}^{-1}$, we have approximately

$$L_{\rm v} = 3 \times 10^{32} \,{\rm erg \, s^{-1}} \left(\frac{M_{*}}{1M_{\odot}}\right) \left(\frac{\dot{M}}{10^{-10} M_{\odot} \,{\rm yr^{-1}}}\right)$$
 (1)

independent of the nature of the compact star. But the bolometric luminosity (L_{bol}) depends on the radius R_* of the compact star:

$$L_{\text{boi}} = 3 \times 10^{33} \text{ erg s}^{-1} \left(\frac{\dot{M}_{*}}{1M_{\odot}} \right) \left(\frac{\dot{M}}{10^{-10} M_{\odot} \text{ yr}^{-1}} \right) \left(\frac{R_{*}}{6 \times 10^{8} \text{ cm}} \right)^{-1} (2)$$

If a fraction k of the accretion energy emerges in hard X rays, then $L_x = kL_{bol}$, and therefore

$$L_{\rm x}/L_{\rm v} = 10 \ k \left(\frac{R_{*}}{6 \times 10^8 \ {\rm cm}}\right)^{-1}$$
 (3)

Now for both neutron stars and white dwarfs, the observed bremsstrahlung temperatures are in the range 10-30 keV, hence our adopted 2-10 keV window will typically miss ~50% of the X-ray flux. For white dwarfs, a well-formed accretion disk may radiate at optical and UV wavelengths much of the total gravitational energy available, lowering the value of k still further. Reasonable values are therefore k = 0.5 for a neutron star and k = 0.2 for a white dwarf. Then the expected values of $L_{\rm X}/L_{\rm y}$ become 2 and 3,000 for white dwarfs and neutron stars respectively.

Refinements to this plausibility argument—especially the consideration of detailed emission mechanisms—will be worth adding in the future. Two of the stars in the X-ray-selected sample are AM Her stars, in which accretion is thought to proceed by radial infall onto the white dwarf, without the mediation of an accretion disk. Yet their L_x/L_y ratios are typical of the entire class of accreting white dwarfs (4.0 for AM Her, 5.5 for 2A0311-227). Similarly, some fraction of the accreting neutron stars may produce most of their visual luminosity by reprocessing X- rays in the disk, which ought to lower the $L_{\rm X}/L_{\rm v}$ ratio from that expected in a pure accretion model. However, there is no obvious signature of the reprocessors in Fig. 1. We can speculate that the AM Her stars will be found to inhabit the high end of the $L_{\rm X}/L_{\rm v}$ distribution for accreting white dwarfs, and the reprocessors will prefer the low end of the $L_{\rm X}/L_{\rm v}$ distribution for accreting neutron stars. Good candidates for the latter include A0620-00, 2S0921-63, HZ Her, 4U1626-67, 4U1659-49, 4U1822-37, Aql X-1, and 4U1957+11. But the data do not yet demand this segregation. Presumably, the great difference in the depths of white dwarf and neutron star gravitational potential wells overpowers effects arising from the local geometry and the detailed emission mechanisms. Because $L_{\rm X}/L_{\rm v}$ is simple to measure and depends strongly only on the radius of the compact star, it seems to be the most useful and reliable indicator of the nature of the compact star.

How will discarding our selection criteria affect Fig. 1? The Einstein observations of cataclysmic variables show that the mean value of L_x/L_y for white dwarfs will decrease to ~ 1 . The mean value for neutron stars will probably increase, as there are many unidentified sources with small error boxes containing only very faint stars (whereas X-ray sources with bright counterparts have probably not been missed). In addition, our deduced values of $L_{\rm X}/L_{\rm v}$ must be increased if a significant fraction of the optical light is produced by means other than accretion—a very real possibility for neutron-star sources. Thus it seems likely that the empirical gulf between white dwarfs and

neutron stars in Fig. 1 will remain sufficiently wide to provide an unambiguous discriminant.

The use of the L_x/L_y discriminant should help to classify newly discovered X-ray binaries. Among presently known sources, there are controversies concerning the nature of the compact star in Sco X-1 ($L_{\rm X}/L_{\rm v} = 5{,}000$) (ref. 20), Cyg X-2 (11,000) (ref. 20), 4U1822-37 (300) (ref. 21), 4U2129+47(1,900) (ref. 22), and H2252 – 035 (3.2) (refs 23, 24). According to our criterion, the first four should be classified as neutron stars, and the last as a white dwarf. Of course, our criterion does not distinguish neutron stars from black holes. Finally, it should be emphasized that 38% of the identified sources have not been considered, in which the existence of a luminous secondary prevents the use of the L_x/L_y discriminant.

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- Davidson, K. & Ostriker, J. P. Astrophys. J. 179, 585 (1973)
- Lamb, F. K., Pethick, C. J. & Pines, D. Astrophys. J. 19, 383 (1973).
 Lamb, F. K., Pethick, C. J. & Pines, D. Astrophys. J. 484, 271 (1973).
 Rappaport, S. A. & Joss, P. C. Nature 266, 683 (1977).
- Joss, P. C. Astrophys. J. Lett. 225, L123 (1978)
- Kylafis, N. D. & Lamb, D. Q. Astrophys. J. Lett. 228, L105 (1979).

- Rappaport, S. A., Cash, W. & Doxsey, R. Asrophys. J. Leu. 187, L5 (1974).
 Cordova, F. A., Mason, K. O. & Nelson, J. E. Preprint (1981).
 Bradt, H. V., Doxsey, R. E. & Jernigan, J. G. in COSPAR Symp. Ser. Vol. 3 (eds Baity, W. A. & Peterson, L. E.) 3, Pergamon, New York, 1979)
 9. Griffiths, R. E. et al. Astrophys. J. Lett. 232, L27 (1979).
 10. Williams, G. et al. Nature 281, 48 (1979).

- 11. Charles, P., Thorstensen, J., Bowyer, S. & Middleditch, J. Astrophys. J. Lett. 231, 1.131
- 12. Griffiths, R. E. et al. Mon. Not. R. astr. Soc. 193, 25P (1980).
- Steiner, J. et al. Astrophys. J. Lett. (in the press).
 Swank, J. S., Boldt, E. A., Holt, S. S., Rothschilld, R. E. & Serlemitsos, P. J. Astrophys. J. Lett. 226, L133 (1978)
- King, A. R., Ricketts, M. J. & Warwick, R. S. Mon. Not. R. astr. Soc. 187, 77P (1979).
 Forman, W. et al. Astrophys. J. Suppl. 38, 357 (1978).
 Charles, P. et al. Bull. Am. astr. Soc. 11, 720 (1979).
 Becker, R. H. & Marshall, F. E. Astrophys. J. Lett. (in the press).

- Pringle, J. E. & Rees, M. J. Astr. Astrophys. 22, 1 (1972).
 Branduardi, G., Kylafis, N. D., Lamb, D. Q. & Mason, K. O. Astrophys. J. Lett. 235, L153
- White, N. E. et al. Astrophys. J. (in the press).
- Charles, P. A. & Thortensen, J. Preprint (1980).
- Patterson, J. & Price, C. Astrophys. J. Lett. 243, L83 (1981).
- White, N. E. & Marshall, F. E. Preprint (1981)

Pulsar slow-down epochs

H. Heintzmann

Institut für theoretische Physik der Universität zu Köln, 5000 Köln 41, FRG

Theoretical arguments about the progenitors of pulsars suggest that all neutron stars may have essentially the same mass¹ and the same magnetic moment^{3,4} M. Direct determinations^{5,6} support this view and give $m \approx 10^{33.5}$ g (the Chandrasekhar mass) and $\vec{M} \simeq 10^{30.5}$ G cm³ and theoretically inferred values for accreting binary systems^{7,1} show a surprisingly small scatter. Can this apparent uniformity of binary pulsars be reconciled with the timing data for (single) radio pulsars, many of which may have also been binaries for some time? In most theories it is assumed that the neutron star is slowed-down by the combined action of a plasma-current torque and a vacuum-wave torque. This has led to the conclusion that there is a deficiency in 'old' pulsars (as measured by their 'slow-down age' P/P) and that the inferred magnetic moments vary by two orders of magnitude. A worse result is the derived pulsar birth rate² of one per 10 yr if the half life of a pulsar is 106 yr as follows from the standard slow-down theory. From these facts we present a new model for pulsar slow-down. The present model predicts four different slow-down epochs from evolutionary changes of the magnetosphere, which are dominated by different braking mechanisms. The model assumes that the masses, magnetic moments and initial rotation periods of all neutron stars are equal. We show that no direct relationship can exist between the 'slow-down age' and the true age of a pulsar and that the pulsar birth rate is one per 100 yr.

Although radio pulsars have been identified with rotating, magnetized neutron stars8 and it has been shown that they must be surrounded by a magnetosphere 9,10 which is independent of the neutron star's surface 11,12 the progress towards understand-

ing the aspects of the magnetosphere, which determine the slowing-down ('braking') of the neutron star's rotation, has been slow¹³. The theoretical analysis of pulsar braking is hampered by two facts: no self-consistent solution for a pulsar magnetosphere has been found and that the pulsar timing data seem to reveal more about the neutron star's interior 14 than about its magnetosphere. The profusion of radio observations can at best be used as a diagnostic13 for the slow-down process. The present model is based on the following assumptions. (1) 'Young' pulsars produce so much plasma by means of 'sparking' 11,12 that within the plasma no low-frequency wave can propagate 15,16. (2) As pulsars 'grow older' sparking becomes less effective so that low-frequency waves can eventually be emitted from within the plasma. (3) The Goldreich-Julian model as extended to the oblique rotator by Mestel describes the long-term aspects of the magnetosphere out to the velocity-of-light cylinder (that part of the magnetosphere which, were it to corotate rigidly. would rotate at the speed of light). Minor modifications such as a current-regulating net charge and discharges due to radiation friction will be worked into the model later. (4) Considerable counteralignment between magnetic moment and the spin of the neutron star occurs during the first epoch, where the torque is dominated by currents in the plasma (the term 'counteralignment' is used here in the sense of perpendicularity it does not mean magnetic moment and angular momentum becoming antiparallel). The present model accounts for this counteralignment if the star can be treated as a sphere so that free nutation is not possible 17,18. However, any other (internal) mechanism which leads to considerable counteralignment will lead to the same consequences (see ref. 18 and refs therein).

The Goldreich-Julian model of the pulsar magnetosphere predicts an excess charge-density in the magnetosphere

$$q \simeq -\frac{\vec{\Omega} \cdot \vec{\mathbf{B}}}{2\pi c} \tag{1}$$

and an average current

$$\vec{\mathbf{j}} \simeq qc\,\vec{\mathbf{b}}_0 \tag{2}$$

along the open field lines which flow away from the surface area ΔF centred on the magnetic poles (polar caps). Here $\vec{\Omega}$ is the spin angular velocity, \mathbf{B} the magnetic field, c the velocity of light and \mathbf{b}_0 a unit vector in the direction of \mathbf{B} . For the star not to charge up indefinitely there must be a back-current which flows along magnetic field lines further away from the centre of the polar cap: it will be regulated by a net charge. To close the current, charges must flow within the neutron star across the magnetic field lines and it is this current which breaks the neutron star's rotation. In the Goldreich-Julian model it is assumed that energy and angular momentum are dissipated beyond the velocity-of-light cylinder. Within the velocity-oflight cylinder the charges move along the magnetic field lines like beads on a wire and their current provides a 'magnetic spring' between the neutron star's surface and the matter beyond the velocity-of-light cylinder. Its torque \vec{T} is given by

$$\vec{\mathbf{T}} = \frac{1}{4\pi r} \int (\vec{\mathbf{r}} \cdot \vec{\mathbf{B}}) \vec{\mathbf{r}} \times \vec{\mathbf{B}} \, dF$$
 (3)

where \vec{r} is the radius vector counted from the centre of the star and the integral is taken over a sphere of radius r. The current of equation (2)—in fact any current along the magnetic field lines which brakes the rotation—leads to a counteralignment torque ¹⁸ between $\vec{\Omega}$ and \vec{M} , in distinction to the torque exerted

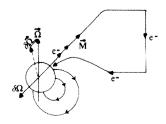


Fig. 1 Schematic current flow which leads to counteralignment. If $\vec{\Omega}$ and \vec{M} are parallel the current flow is metastable. The electron-flow is shown, the algebraic current \vec{j} is directed oppositely. The currents have axial symmetry about the dipole direction \vec{M} .

by low-frequency waves propagating in vacuo, which (if not impeded by nutation¹⁷) leads to alignment 19,20 . The counteralignment torque can be easily understood by noting that a current flowing through a magnetized sphere will set the sphere into rotation about the magnetic dipole axis $\tilde{\mathbf{M}}$ and the current of equation (2) is directed (Lenz' rule) so that it reduces the rotation about the original axis.

In the dipole approximation the polar cap surface area ΔF is given by $^{9,11}\Delta F\simeq 2\pi R^2$ ($\Omega R/c$), where $R\simeq 10^6$ cm is the radius of the star and in a coordinate system centred on the magnetic dipole-axis we find for the toroidal component of the magnetic field

$$B_{\phi} \simeq -\frac{\vec{\Omega} \cdot \vec{B}}{2\pi c} \cdot \int \frac{\mathrm{d}F}{R \sin \theta} \tag{4}$$

which leads by means of equation (3) to a torque

$$\vec{\mathbf{T}}_{pl} = -\alpha \frac{\Omega^2}{c^3} (\vec{\mathbf{\Omega}} \cdot \vec{\mathbf{M}}) \vec{\mathbf{M}}$$
 (5)

where $\vec{M} = R^3 \vec{B}$ is the magnetic dipole moment and $\alpha = 1$. From the corotating part of the magnetosphere we obtain an induced magnetic field parallel to the rotation axis which leads to an extra torque (by means of the magnetic dipole moment \vec{M}) on the star

$$T'_{pl} = \frac{\gamma}{Rc^2} (\vec{\Omega} \cdot \vec{M}) \vec{\Omega} \times \vec{M}$$
 (6)

where $\gamma \approx 1$. Equations (5) and (6) may be compared with the vacuum-wave torque 17,19,20

$$\vec{\mathbf{T}}_{w} = -\beta \frac{\Omega^{2}}{c^{3}} (\vec{\mathbf{M}} \times \vec{\Omega}) \times \vec{\mathbf{M}} + \frac{1}{Rc^{2}} (\vec{\mathbf{M}} \cdot \vec{\Omega}) \vec{\Omega} \times \vec{\mathbf{M}}$$
(7)

with $\beta = 2/3$. The common assumption that both plasma and low-frequency waves will contribute (more or less equally) to the slow-down torque, which leads to the large scatter in inferred magnetic dipole moments will now be shown to be wrong. If low-frequency waves cannot propagate within the plasma they cannot exist outside. Apart from a transition period where the plasma may just allow for low frequency wave propagation (the duration of which is difficult to estimate as it depends on the sparking mechanism) a pulsar is slowed-down therefore exclusively by either the plasma-current torque equation (5) or the vacuum torque equation (7).

The orthogonal rotator provides the most favourable conditions for low-frequency wave-emission and we assume that the plasma which flows out of the velocity-of-light cylinder fills the space about the equatorial plane, consequently the waves are emitted into a plasma dead zone centred on the rotations axis. Let us assume that the two zones are separated by a cone of half-angle ψ (counted from the rotation axis) and as a first approximation ¹⁶ that the plasma is infinitely well conducting.

This problem can be solved exactly. Taking the solution of the vector-Helmholtz equation from Morse and Feshbach²³ one finds that the TEM-mode dominates and the dominant radiation mode is given by the lowest n for which

$$\frac{n+1}{n}P_{n-1}^{1}(\cos\psi) - \frac{n}{n+1}P_{n+1}^{1}(\cos\psi) = 0$$
 (8)

$$P_n^1(\cos\psi) = \frac{n(n+1)}{2}\sin\psi F\left(1-n, 2+n|2|\frac{1-\cos\psi}{2}\right) \quad (9)$$

F in equation (9) is the hypergeometric function which for non-integer n is regular in the upper hemisphere where equations (8) and (9) hold. If we let the conducting cone shrink to the equatorial plane one obtains the Deutsch solution²⁴ with n = 1. For a thin plasma sheet $(\psi = \pi/2 - \varepsilon)$ one finds approximately

$$12 = (1 - \cos \psi) \cdot (n^2(n+2)(n+3) - (1-n^2)(1+n)(2-n)) \quad (10)$$

which shows that n is > 1. The radiated energy rate is

$$\dot{E} \simeq -\frac{2}{2cR^2} (\vec{\mathbf{M}} \times \vec{\mathbf{\Omega}})^2 \left(\frac{\Omega R}{c}\right)^{2n} \tag{11}$$

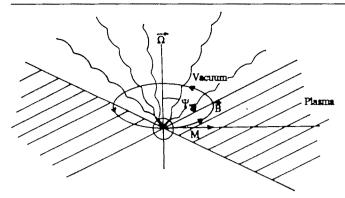


Fig. 2 Plasma-vacuum configuration for which an exact solution of the vector—Helmholtz equation is possible. Hatching indicates the area supposed to be filled with overdense outstreaming plasma in which no low-frequency waves can propagate. Equation (11) gives the energy-loss due to low-frequency waves radiated into vacuum and n is determined from equations (8) or (10)

For a finite thickness of the plasma sheet the emission of low-frequency waves is so strongly reduced $(n=2 \text{ for } \psi=\pi/4)$ that the wave pressure cannot balance the plasma pressure at the boundary and the plasma fills the whole space. Note, however, that if the plasma is asymmetrically distributed in the two hemispheres, such that one cone has $\psi > \pi/2$ (plasma swept backwards, for example, by the pulsar's proper motion?¹⁵) radiation emission is enhanced and such a state may be called superradiant. A young pulsar will therefore be slowed-down exclusively by the plasma current, an old one by the wave-torque and in the transition epoch we may have

$$\dot{\vec{\mathbf{J}}} = I \dot{\vec{\mathbf{\Omega}}} = (\beta - \alpha) \frac{\Omega^2}{c^3} (\vec{\mathbf{M}} \vec{\mathbf{\Omega}}) \vec{\mathbf{M}} - \beta \frac{\Omega^2}{c^3} \mathbf{M}^2 \vec{\mathbf{\Omega}} + \gamma \frac{(\vec{\mathbf{\Omega}} \vec{\mathbf{M}})}{Rc^2} \vec{\mathbf{\Omega}} \times \vec{\mathbf{M}}$$
(12)

where \vec{J} is the angular momentum and I the moment of inertia of the neutron star, $I \simeq 10^{45}$ g cm².

Together with the equation of motion for the dipole moment \vec{M} , which is frozen into the star

$$\vec{\mathbf{M}} = \vec{\mathbf{\Omega}} \times \vec{\mathbf{M}} \tag{13}$$

the evolution of the slow-down is easy to determine. Note that equation (12) takes into account only the convection current of equation (2). An additional conduction current due to sparking which may be larger than the convection current will only lead to a different α if the current flows along the magnetic field lines as depicted in Fig. 1. None of the conclusions regarding the first slow-down epoch will be affected as long as $\beta = 0$ as can be seen from equation (14) which becomes independent on α in this case. With the help of the first integral

$$\frac{(\Omega^2 \sin^2 \chi)^{\alpha}}{(\Omega^2 \cos^2 \chi)^{\beta}} = \text{constant}$$
 (14)

where χ is the angle between $\vec{\Omega}$ and \vec{M} we obtain for young pulsars, for which $\beta = 0$,

$$\sin \chi = \sin \chi_1 \left(\frac{\Omega_1}{\Omega}\right) \tag{15}$$

$$\Omega = \Omega_{1}(1 + ctg^{2}\chi_{1}(1 - e^{-\tau}))^{-1/2}$$
(16)

which for small times

$$\Omega = \Omega_i \left(1 - \frac{\tau}{2} ctg^2 \chi_i + \frac{\tau^2}{8} \left(2ctg^2 \chi_i + 3ctg^4 \chi_i \right) \right)$$
 (17)

where $\tau = 2\alpha \Omega_1^2 \sin^2 \chi_1 (M^2/Ic^3)t = t/t_0$ is a dimensionless parameter which measures the observer's time t in units of the e-folding time t_0 of the model, i refers to initial values.

Before discussing equation (16), we will consider briefly another important parameter for pulsar timing observations. The so-called braking index $N = (\Omega\Omega/\Omega^2)$ is given in our model by

$$N = 3 + 2 \frac{(\beta - \alpha)^2 \cos^2 \chi \sin^2 \chi}{(\alpha \cos^2 \chi + \beta \sin^2 \chi)^2}$$
 (18)

and is never < 3 due to torque minimization. Observationally N is reliably known only for the Crab pulsar^{26,27} where $N \simeq 2$, 5. Rewriting the energy balance equation in the form

$$\frac{1}{2}(I\Omega^2) = -\frac{\alpha}{cR^2}(\vec{\Omega}\vec{\mathbf{M}})^2 \left(\frac{\Delta F}{F}\right)^2$$
 (19)

where $F=4\pi R^2$ is the surface area of the neutron star. A braking index of <3 may be explained by the pulsar's crust shrinking^{21,22,28} at a rather large rate, or by a slightly larger polar cap $\Delta F/F=(\Omega R/c)^{2/3}$. In fact, some theories²⁹ have the pulse width ΔP and the period P related as $(\Delta P/P)^2=\Delta F/F$ and the observations of the Crab pulsar, where $\Delta P/P\sim 1/5$ is rather large²⁹, would fit better with $\Delta F/F\simeq (\Omega R/c)^{1/2}$ leading to a braking index $N=3-2/3+2tg^2\chi$.

The model is flexible enough to account for the available timing data even under the restriction that all pulsars have the same moment of inertia $I=10^{45}\,\mathrm{g}\,\mathrm{cm}^2$ and the same magnetic moment $M=10^{30.5}\,\mathrm{G}\,\mathrm{cm}^3$. To determine t_{\bullet} from the observations we identify those pulsars with anomalously low period derivative with the stars in our model which pass through the end of the first epoch. From the observations $\Omega \simeq 2\pi\,\mathrm{s}^{-1}$ which gives $\Omega_{\bullet} \sin \chi_{\bullet} = 2\pi\,\mathrm{s}^{-1}$ so that

$$t_{\bullet} \simeq 10^6 \text{ yr } I_{45} M_{30.5}^{-1}$$
 (20)

Observationally the two most extreme cases are the Crab pulsar and the binary pulsar. For them equation (20) would lead to $\sin \chi_1 = 10^{-1.5}$ and $\cos \chi_1 = 10^{-1.5}$ respectively if we assume that both are young objects. To explain the binary pulsar in this way a nearly orthogonal rotator is needed and one may worry whether equation (12) still applies. This case requires $\beta < 10^{-4}$ and $\cos^2 \chi_1 < 10^{-3}$. For the binary pulsar $(\Omega R/c)^2 < 10^{-5}$, which guarantees that $\beta < 10^{-4}$ and inspection of the current given by equation (2) shows that it can be closed along the magnetic field lines through the star so that it does not lead to a torque. The braking is then no longer effected by the current of equation (2) but comes about through secondary energy losses such as sparking. Ruderman's estimate12 of that energy for the (faster) Crab pulsar of 10³³-10³⁴ ergs s⁻¹ would lead to the observed braking of the binary pulsar. The first epoch, which lasts some 106 yr accounts for roughly a half of the pulsars assuming that all are born as fast rotators. The other half can be explained in the penultimate epoch of pulsar slow-down, where vacuum waves can be emitted in the presence of plasma so that the period derivative goes back to its normal value. Note that in the present model the slow-down age is not related to the true age. The mean active life as determined by Ohmic dissipation can easily exceed 107 yr (ref. 18) which brings down the pulsar birth rate by a factor of 10, in comfortable agreement with the more conservative estimates of supernova rates and the lack of discovered neutron stars at their centres36

We have so far only assumed that the current of equation (2) flows on average without demonstrating how it occurs. Of course, a rigorous demonstration requires a self-consistent solution of the magnetosphere problem, so only the following qualitative argument can be given. According to the Goldreich-Julian model particles cannot stay on open field lines within the velocity of the light cylinder for the same reason that they cannot stay within the star: large electric fields would pull them out. This causes the charge with the correct sign as given by equation (1) to be pulled out, charges with the opposite sign, however, are pulled in on the same field line. Hence a pulsar must have a net charge 30 Q to regulate the plasma outflow such that the star does not charge up indefinitely. Some of the charge will be distributed over the polar cap ΔF and most of it over the boundary of the corotating magnetosphere and because it must be able to influence the dynamics of the plasma at the velocity-of-light cylinder it must be of the order of

$$Q \simeq \frac{\vec{\Omega} \vec{M}}{c} \tag{21}$$

Such a charge reintroduces large electric fields parallel to B, so we have shifted the whole problem from the surface of the neutron star to its velocity-of-light cylinder, sufficiently far away, however, that the star does not get too heated 1,36. Note that the net charge as given by equation (21) will not give rise to a back current from the interstellar matter to the pulsar during its active life. This is because the pulsar is well shielded by the electromagnetic fields of the magnetosphere or the vacuum waves which both fall off like r^{-1} whereas the monopole field falls off like r^{-2} so that the force balance is in fact at the velocity-of-light cylinder. It follows that the exact current need not be that given by equation (2) but may be determined by the global magnetospheric structure. As pointed out by Mestel (personal communication) this need not have the symmetry about \vec{M} , which was crucial for deriving equation (5). In the general case one cannot therefore rigorously predict whether there will be counteralignment or alignment, whereas vacuum waves will always lead to alignment.

In the penultimate slow-down era, which is dominated by low-frequency waves this charge and the corotating (quadrupole) charge of the magnetosphere will also radiate leading to a friction force on the particles in the magnetic field with nonvanishing curl. To compensate for this, the particles must drift across magnetic field lines producing a current which empties the magnetosphere. For the quadrupole radiation from the corotating magnetosphere we get for the time scale of the ensuing discharges some 106 pulsar periods and a much shorter time scale for the dipole radiation due to the charge given by equation (21). These discharges may be related to the nulling phenomenon and may give rise to slow-down noise 27,28 but not to any directly observable speed ups as the inertia involved is too small. The present model does not explain why pulsars turn off unless sparking ceases to be regular enough to allow an observer to detect the object as a pulsar, but even accretion may apparently influence the final era31 especially if the pulsar has become an aligned rotator by then.

An attractive explanation is obtained, however, by combining the pulsar extinction hypothesis^{32,33} with the decay of the magnetic dipole moment is which regulates the physics at the velocity-of-light cylinder. The observed cutoff period $P \simeq 4 \text{ s}$ would then not mainly be a consequence of plasma inertia but rather reflect the time scale for ohmic dissipation in the pulsar's crust which may vary considerably from pulsar to pulsar depending on its thermal history at birth. According to the present model such neutron stars will slow-down in the final era by quadrupole radiation (or plasma currents) on a time scale exceeding the age of the Universe and only if they traverse dense interstellar matter may they be slowed-down more effectively by accretion³¹. The γ -ray transient³⁴ with a period of 8 s could in fact be such an old pulsar, whereas the γ -ray source CG195,5+ 4,5, if its periodicity of 59.35 s should be confirmed³⁵, should rather not be identified with an old pulsar according to the present model, which predicts ultimate periods around 10 s for dead pulsars, instead of 60 s as deduced by Michel³² assuming that the dipole field does not decay.

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- Pines, D. Science 207, 597 (1980); J. Phys. C2, 111 (1980).

- Kundt, W. Naturwissenschaften 64, 493 (1977).
 Ruderman, M. A. & Sutherland, P. A. Nature phys. Sci. 264, 93 (1973).
 Levy, E. H. & Rose, W. K. Nature 250, 40 (1974).
 Taylor, J. H., Fowler, L. A. & McCulloch, P. A. Nature 277, 437 (1979).
 Trümper, J. et al. Astrophys. J. Lett. 219, L105 (1978).
- Ghosh, P. & Lamb, F. K. Astrophys. J. (in the press); Proc. IAU Symp. No. 95, 303-320
- Gold, T. Nature 218, 731 (1968).
- Goldreich, P. & Julian, W. H. Astrophys. J. **157**, 869 (1969). Mestel, L. Nature phys. Sci. **233**, 149 (1971).
- Sturrock, P. Nature 227, 465 (1970); Astrophys. J. 164, 529 (1971). Ruderman, M. A. Proc. IAU Symp. No. 95, 87-98 (1980). Mestel, L. Proc. IAU Symp. No. 95, 9-24 (1980). Baym, G. & Pethick, Ch. A. Rev. Astr. Astrophys 17, 415 (1979).

- Asseo, E., Llobet, X. & Schmidt, G. Phys. Rev. D (in the press).
- Ozernoy, L. M. & Usov, V. V. Astrophys. Lett. 13, 151 (1973).
- Ozernoy, L. M. & Usov, V. V. Astrophys. Lett. 13, 151 (1973). Goldreich, P. Astrophys. J. Lett. 160, L. 11 (1970). Flowers, E. & Ruderman, M. A. Astrophys. J. 215, 302 (1977). Davis, L. & Goldstein, M. Astrophys. J. Lett. 159, L. 81 (1970). Michel, F. C. & Goldwire, H. C. Astrophys. Lett. 5, 21 (1970).

- Smoluchowski, R. *Phys. Rev. Lett.* **24**, 923 (1970). Smoluchowski, R. & Welch, D. O. *Phys. Rev. Lett.* **24**, 1191 (1970).
- 23. Morse, Ph. M. M. & Feshbach, H. Methods of Theoretical Physics Vol. 11, 1864 (McGraw-Hill, New York, 1953).
- Deutsch, A. Ann. Astrophys. 18, 1 (1955). Tademaru, E. & Harrison, E. R. Nature 254, 677 (1975).
- Groth, E. J. Astrophys. J. Suppl. 29, 453 (1975). Cordes, J. M. Astrophys. J. 237, 216 (1980).
- Cordes, J. M. Astrophys. J. 231, 216 (1980).
 Cordes, J. M. & Greenstein, G. Pulsar Timing IV. Cornell University Preprint NAIC 138 (1980); Proc. IAU Symp. No. 95, 291-298 (1980).
 ter Haar, D. Phys. Rep. 3C, 59 (1972).
 Jackson, E. A. Astrophys. J. 206, 831 (1976).

- Wright, G. A. E. Nature 280, 40 (1979). Michel, F. C. Astrophys. J. Lett. 195, L 69 (1975).
- Hill, T. W. Astrophys. Lett. 21, 11 (1980). Terrell, J., Evans, W. D., Klebesadel, R. W. & Laros, J. G. Nature 285, 383 (1980).
- Özel, M. E., Dickel, J. R. & Webber, J. C. Nature **285**, 645 (1980) Helfand, D. J. Nature **285**, 133 (1980); **283**, 337 (1980).

IR photometry of flat spectrum radio sources

C. D. Impey & P. W. J. L. Brand

Department of Astronomy, University of Edinburgh, Royal Observatory, Edinburgh EH9 2HJ, UK

IR measurements of optically faint, flat spectrum radio sources have implied the possible existence of a new class of quasar, with IR excess more extreme than that of any extragalactic object. In the present preliminary study, 18 Parkes flat spectrum sources have been observed in the IR without regard to optical morphology, and the red sources are found to be just the tail of the normal distribution of quasar colours. The results imply that a considerable fraction of flat spectrum sources from complete samples may have properties similar to the BL Lac objects.

Rieke et al.^{1,2} recently detected several objects at 2.2 µm in optically blank fields of flat spectrum radio sources^{3,4}, and derived such large IR/optical spectral indices ($\alpha = -d \ln flux$ density/d ln frequency) $\alpha_{KB} \approx 3$ that they proposed a new class of quasar, because other properties of the objects indicated that they were similar to violently variable quasars, or BL Lac objects. They further noted that at the largest (faintest) blue magnitudes, α_{KB} tended to be high, while at $m_B \le 18$, $\alpha_{KB} \approx 1$, a value typical for the optically bright quasars of Neugebauer et al^5 . The spectral index α_{KB} was calculated from measurements in the optical B band and the near-IR K band. To test the trend of α with $m_{\rm B}$, and to discover whether the red sources represent a new class of quasar, a sample of flat spectrum radio sources was observed in the IR without regard to optical identification. The candidates were selected from the list of 2.7-GHz sources of Condon et al.³ according to limits on spectral index (α < 0.5, 2.7 – 5.0 GHz), flux density ($S_{2.7} > 0.5$ Jy), RA (8 h < α < 15 h) and dec $(-30^{\circ} < \delta < -4^{\circ})$.

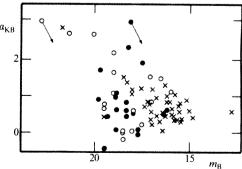


Fig. 1 Optical/IR spectral index versus optical magnitude, showing Rieke et al.'s objects $^{1.2}$ (\bigcirc), the optically bright quasars of Neugebauer et al. 5 (\times) and objects from the present study (\blacksquare). Trajectories show the effect of 1 mag brightening at B between epoch of Palomar plates and IR measurements.

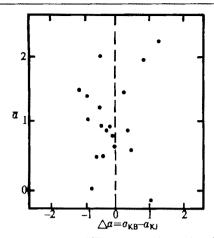


Fig. 2 Plot of mean optical/IR spectral index against the residual $\alpha_{KB} - \alpha_{KI}$. The deviation from the line $\alpha_{KB} = \alpha_{KI}$ is a measure of the curvature of the spectrum (either intrinsic or due to variability).

The IR observations were obtained with the Anglo-Australian 3.9-m telescope on 2-3 May 1980 using the InSb Infrared Photometry System. All the observations were made with the broad band $K(2.2 \,\mu\text{m})$ and $J(1.25 \,\mu\text{m})$ filters. Standard stars from the AAO list were observed to calibrate the photometry. The data have been corrected for near-IR extinction at Siding Spring according to recently determined coefficients (G. Gilmore and I. N. Read, personal communication). The data have also been corrected for the effect of different flux distributions on the broad band flux measurements. Only two of the sources lie at galactic latitude $|b| < 30^\circ$, and they were corrected for galactic extinction. The minimum error in the J and K magnitudes was determined from the repeatability of standard observations during a night. In two nights, 21 sources were observed, with 20 detected at K and 18 detected at J.

Table 1 shows the IR data, along with spectral indices calculated between J and K (α_{KJ}) and between the optical B band and K(α_{KB}). There are two major sources of error in these spectral indices; one is the effect of redshifted emission lines passing through the photometric bands. α_{KJ} may be affected, but over the long baseline from 2.2 μ m to the visible, α_{KB} is relatively insensitive to this effect⁶. The second is the fact that the optical magnitude is taken from Palomar plates, and variability might lead to errors in α_{KB} . The photographic fluxes have an uncertainty of ± 0.5 mag (ref. 3).

Figure 1 shows α_{KB} plotted against m_B , with the cluster of red, optically faint objects showing up clearly in the previous data. The 18 sources in our sample present a distribution of spectral indices covering the full range of α from 0 to 3. Very red sources are relatively common; four out of 18 lie in the top part of the

Table 1 IR data and spectral indices between the J and K, and B and K bands

Source	K(2 2 μm) (mag)	Flux (mJy)	J(1 25 μm) (mag)	Flux (mJy)	α _{JK}	a _{KB}	M ₃
0823 - 223	12.47 ± 0.03	6.79	14 90 ± 0 03	1.76	2.25	1.5	17.5
0919-260	1533 ± 0.07	0 49	16.73 ± 0.10	0.33	0.68	10	19.0
1032-199	15.58 ± 0.10	0.39	17.16 ± 0.10	0 22	0 96	0.8	190
1034 - 293	12.48 ± 0.03	6.69	14.42 ± 0.03	2.75	1.48	2.4	18.5
1124 - 186	15.01 ± 0.04	0 66	17.17 ± 0.09	0 22	1 85	0.9	18.5
1128-047	1533 ± 0.04	0.49	17 13 ± 0 10	0.23	1.29	1.6	20.0
1143 245	15.14 ± 0.04	0.58	16.29 ± 0.05	0.49	0 29	08	18.5
1145 - 071	16.59 ± 0.12	0.16	17.94 ± 0.14	0.11	0.68	02	18 5
1156 - 094	15.03 ± 0.10	0 65	15.42 ± 0.02	1 09 -	-0.85	0.3	17.5
1203 - 261	$18.3 (2\sigma)$	0 03	$19.5(2\sigma)$	0 02	0.32	-0.5	195
1243 - 072	15.34 ± 0.07	0 49	17.28 ± 0.10	0 20	1 51	1.0	19.0
1244-255	14.80 ± 0.04	0.80	16.32 ± 0.07	0.48	0.86	0.7	18.0
1256-220	1642 ± 011	0.18	$18.6 (4\sigma)$	0 05	2.12	0.9	20 0
1302 - 102	12.88 ± 0.03	4 71	14.26 ± 0.03	3 17	0 66	0.6	16.0
1335-1 2 7	11.43 ± 0.03	17.64	13.45 ± 0.03	6.72	1 61	30	18.5
1352 - 104	1536 ± 003	0 49	1677 ± 0.10	0.31	0 75	0.1	17.5
1354-152	15.23 ± 0.08	0.54	16.86 ± 0.08	0 29	1.03	07	18.5
1406-076	16.37 ± 0.11	0.19	18.28 ± 0.16	0 08	1.46	0.6	19.5
1511-100	>18.8	< 0.02	-	_			18 5
1519-273	17 9 (3σ)	0 05	*******				18.5

range of spectral indices for BL Lac objects and two are as red as any of those found by Rieke and Lebofsky¹. The data fill in the distribution of very red objects at brighter optical magnitudes, and shows that the trend of α_{KB} with m_B is a selection effect.

Figure 1 shows that much of the argument rests on the α_{KB} indices of the two reddest objects (1335-127 and 1034-293), which may be affected by variability. However, there are reasons to believe that these points are not spurious. First, direct observations on the AAT TV acquisition system confirmed that neither of the objects had varied by more than a magnitude from the epoch of the Palomar plates. The effect of 1 mag variability in B is shown as a trajectory in Fig. 1 both for 1335-127 from the present study and for the reddest object from refs 1, 2. While anomalous brightness at B would bring the two red objects down towards the bulk of the distribution, the same is true of the original red objects. As both sets of α_{KB} were calculated in the same way, the results are directly comparable. Second, there is no evidence from the cotemporaneous J and K measurements that these objects are abnormal. Figure 2 shows the mean spectral slope $\bar{\alpha}$ plotted against the difference $(\alpha_{KB} - \alpha_{KJ})$, where the steep spectrum sources lie at the top of the graph. Rieke et al. 1.2 noted that a roughly constant spectral index satisfied both their J, K and optical fluxes; this is represented by the line $\alpha_{KB} = \alpha_{KI}$ in Fig. 2. Figure 2 also shows that the objects with large α_{KB} do not have abnormally large residuals $\Delta \alpha$, and are not

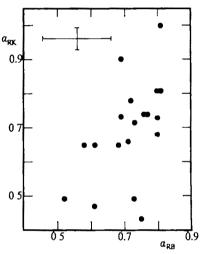


Fig. 3 Plot of radio/IR and radio/optical spectral indices. The dispersions in α_{RR} and α_{RB} are a measure of the relative IR and optical flux variations of the sample. Typical error bars are shown in the top left corner.

segregated from the main body of the distribution. While there is not a 1:1 correspondence between steep α_{KB} and steep α_{KJ} , the four reddest objects in α_{KB} are among the eight reddest objects in α_{XI} . The means and dispersions of both spectral index distributions are similar $(\bar{\alpha}_{JK} = 1.05 \pm 0.72, \ \bar{\alpha}_{KB} = 0.92 \pm 0.79)$. There is no evidence from either the optical or IR data that the red (α_{KB}) objects are other than the tail of the normal distribution of quasar colours. Objects which resemble BL Lac objects in their IR properties are relatively common. The four reddest sources are particularly interesting because (unlike the original 'red' sources) they are bright enough for follow-up spectroscopy and polarimetry. The reddest object (1335-127) has recently been confirmed as a BL Lac object. Our 2.2-um measurements with the UK Infrared Telescope show it to have varied by a magnitude over one year, and radio observations by Aller et al. show it to have variable linear polarization at frequencies from 4.8 to 14.5 GHz. In addition, the second reddest object (1034-293) shows evidence of variability $(\Delta m_{\rm B} > 1 \text{ mag})$ from UK Schmidt plates of the area, another indication that it is a BL Lac-type object.

Even for this relatively small sample it is possible to draw conclusions on the IR properties of flat spectrum radio sources,

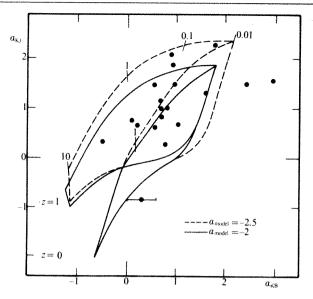


Fig. 4 Plot of near IR and optical/IR spectral indices with permitted zones from a two-component model. The error bars on the lowest point show the effect of a variation of $\pm \frac{1}{2}$ mag in B and typical 5% precision on the J and K flux measurements.

and compare them with the optical properties. Selection effects will not be important because the radio sample was completely identified optically, and with one exception was completely identified at 2.2 μ m. Figure 3 shows α_{RK} plotted against α_{RB} , where the spectral index is calculated between the 2.7 GHz measurement and the K and B data points. The range of radio fluxes is less than a factor of 3, while the range of IR fluxes is >500, thus the graph allows a comparison of the range of IR and optical fluxes in the sample. The mean spectral indices are $\bar{\alpha}_{RK} = 0.69$ and $\bar{\alpha}_{RB} = 0.71$, both very near to the canonical value for optically thin non-thermal radio sources. There is no significant difference between the range of IR and optical fluxes $(\sigma(\alpha_{RK}) = 0.15, \ \sigma(\alpha_{RB}) = 0.08$ and taking the relative standard error in the variance of n variates to be $1.4 (n-1)^{-1/2}$) and the even distribution of the very red objects on the α_{RB} axis is a confirmation of the fact that they do not tend to be optically faint. The objects of Rieke et al.² do not come from a separate population at better than the 1.5σ level; removing the lower four objects only reduces $\sigma(\alpha_{RK})$ by 0.03 to 0.12.

To see if a simple picture accounts for the range of optical/IR spectra, the energy distribution of a giant elliptical8 was added to a power law spectrum of slope α which flattened off $(\alpha = 0)$ at some wavelength λ_n (the knee). Figure 4 shows the data plotted on a two-colour diagram of α_{KB}/α_{KJ} . The extreme red objects lie at large values of α_{KB} . As the knee wavelength travels across the spectrum, the source traces a trajectory in the two-colour plane. As the proportion of non-thermal to thermal components is varied, the trajectory sweeps out an area on the diagram. The right-hand boundary represents the extreme non-thermal case of $S_{GAL}/S_{NT} = 0.01$, where S_{GAL} is the peak flux of the elliptical galaxy and $S_{\rm NT}$ is the flux at the point where the power law flattens. As the flux contribution of the elliptical increases, the trajectory sweeps across to smaller values of α_{KB} and α_{KJ} , and the left-hand boundary of the enclosed area shows the case where $S_{GAL}/S_{NT} = 10$ and the spectrum is dominated by stellar flux. The redshift distribution of flat spectrum radio samples is expected to peak around $z \sim 1$ (ref. 9), and Fig. 4 shows the case for z = 0 and z = 1. Given the luminosity of a giant elliptical, the flat spectrum sources would be expected to populate the nonthermal $(S_{GAL}/S_{NT} < 1)$ portion of the z = 1 area and the thermal $(S_{GAL}/S_{NT} > 1)$ part of the z = 0 area. Finally the areas have been plotted for two values of α ; $\alpha = 2.0$ and $\alpha = 2.5$ are the solid and dashed curves respectively.

Most of the data points lie well within the area described by the model; the $\alpha = 2.5$ case covering 16 of the 19 sources. Some of the scatter of the data in α_{KJ} will be caused by emission lines

(especially $H\alpha$ passing through the J band) and some of the scatter in α_{KB} may be caused by variability and the nonsimultaneity of the optical and IR flux measurements. However, the agreement is surprisingly good given the simple assumptions. A range of α from 2 to 3 is sufficient to cover even the most extreme sources. The assumption that $\alpha = 0$ for $\lambda > \lambda_n$ is not critical. Observations at longer wavelength will help confirm this picture.

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- Rieke, G. H. & Lebofsky, M. J. IAU Symp. 92, 263–267 (1980). Rieke, G. H., Lebofsky, M. J. & Kinman, T. D. Astrophys, J. Lett. 232, L151–154 (1979). Condon, J. J., Hicks, P. D. & Jauncey, D. L. Astr. J. 82, 692–700 (1977). Condon, J. J., Jauncey, D. L. & Wright, A. E. Astr. J. 83, 1036–1046 (1978).

- Neugebauer, G., Oke, J. B., Becklin, E. E. & Matthews, K. Astrophys. J. 230, 79-94 (1979). Hyland, A. R. & Schwartz, M. P. Proc. astr. Soc. Aust. 3, 137-140 (1977).
- Aller, H. D., Aller, M. F. & Hodge, P. E. Astr. J. 86, 325 (1981). Grasdalen, G. L. IAU Symp. 92, 269-276 (1980).
- Owen, F. N. & Mufson, S. L. Astr. J. 82, 776-780 (1977).

New family of homogeneous chemical oscillators: chlorite-iodate-substrate

Miklós Orbán*, Patrick De Kepper*, Irving R. Epstein & Kenneth Kustin

Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254, USA

Interest in oscillating chemical reactions arises because these systems serve as models of temporal organization in biological systems, as starting points for the development of spatial structure in initially homogeneous systems and as instructive examples of the wide variety of dynamic phenomena possible in chemical systems far from equilibrium. One obstacle to the development of a general theory of chemical oscillation has been the small number of fundamentally distinct homogeneous oscillators and the failure of efforts to design new oscillating reactions. We recently suggested a systematic approach to the construction of chemical oscillators and used it to produce a homogeneous oscillating reaction: the chlorite-iodate-arsenite oscillator1. We now report that the above system is the prototype of a family of homogeneous oscillators, all of whose members have the species chlorite and iodate in common, but which can utilize a wide variety of reducing agents or substrates in place of arsenite.

Figure 1 shows the time dependence of the potentials of an iodide-selective and a platinum redox electrode and of the absorbance at 460 nm when one-electron (ferrocyanide) and two-electron (thiosulphate) reductants react with NaClO2 and KIO₃ in the same experimental conditions in a continuous flow stirred tank reactor (CSTR). The CSTR maintains the system far from equilibrium, which is a necessary condition for sustained oscillation.

Table 1 gives the concentration range in which oscillations have been found for each of our substrates. Optimum concentrations, at whichparge amplitude oscillations are observed over a wide range of flow rates (80-2,000 s residence time), are also indicated. While the waveform and frequency of oscillation vary with the flow rate and with the nature and concentration of the substrate, relative changes in [I⁻] of 10⁴ or more in each cycle were obtained with all substrates. At the upper and lower ends of the substrate concentration range, oscillatory behaviour is observed only at relatively high or low flow rates, respectively. Above or below these concentration limits, only a single nonoscillatory steady state of high or low iodide and iodine concentrations can be observed. The transition from large amplitude

^{*} Permanent addresses: Institute of Inorganic and Analytical Chemistry, L. Eötvös University, H-1443, Budapest, Hungary (M.O.); Centre de Recherche Paul Pascal, Domaine Universitaire, 7705 Tacce Cedex, France (P. De K.).

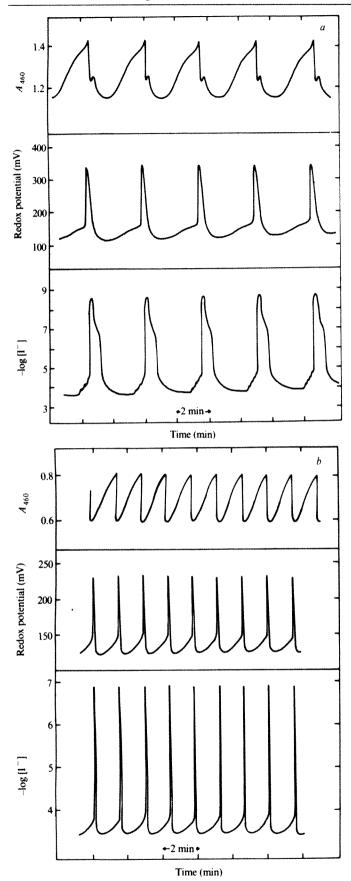


Fig. 1 a, Optical density at 460 nm, potential (compared with Hg/Hg₂SO₄ reference electrode) of platinum electrode, and [Γ] determined by iodide sensitive electrode in a CSTR with input composition (concentrations in the reactor after mixing, but before initiation of reaction), [NaClO₂]₀ = 0.002 M, [KlO₃]₀ = 0.01 M, [K₄(Fe(CN)₆)]₀ = 0.005 M. Temperature, 25 °C; pH, 2.06; residence time, 310 s. b, As in a but with ferrocyanide substrate replaced by thiosulphate, [Na₂S₂O₃]₀ = 0.005 M, and pH = 2.40.

oscillation to steady state as the substrate concentration is varied may occur either suddenly and directly or through a state of small amplitude oscillation about the steady state which is being approached.

Substrates which generate oscillations include both one- and two-electron reductants and cover a considerable range of redox potentials (see Table 1). Their common feature is apparently that they participate in a Landolt-like reaction².

$$(6/n) \operatorname{red} + IO_3^- = (6/n) \operatorname{ox} + I^-$$
 (1)

qere red and ox signify the reduced and oxidized form of the substrate, and n = 1 or 2 according to whether the substrate is a one or two electron reducing agent. Reaction (1) is autocatalytic in iodide³. The iodide produced then reacts with chlorite to yield iodine:

$$4I^{-} + CIO_{2}^{-} + 4H^{+} = 2I_{2} + CI^{-} + 2H_{2}O$$
 (2)

Reaction (2) is autocatalytic in I₂ and is inhibited by I (refs 4,5). The chlorite-iodide system gives rise to two different stable steady states in a CSTR⁶. The observed oscillation in the present reaction may then arise from the effect of the relatively slow substrate-generated feedback of iodide on the faster and intrinsically bistable chlorite-iodide subsystem. This view is based on a simple model of the relationship between oscillation and bistability due to Boissonade and De Kepper⁷.

Arsenite reacts less rapidly with iodate than do the other substrates in Table 1. Oscillation with arsenite as substrate must be initiated by the injection of a small amount of iodide into the reactor, while with the other substrates, oscillation begins spontaneously. These observations suggest that initiation of oscillations requires the production of a quantity of iodide above some threshold level.

Neither malonic acid nor iodide as substrate will produce oscillations in our conditions. Malonic acid reacts with iodate to form iodine, which slowly reacts with the excess substrate to yield iodomalonic acid and iodide. The stoichiometry of this process is such that only half as much I is produced with malonic acid as with the other substrates, and apparently an additional iodide flow is needed to produce oscillation.

The wide range of substrates examined allows us to infer several criteria for a substrate to generate oscillation in this system. The redox potential of the substrate, in the reaction conditions, should be less than that of the iodine-iodide couple (0.62 V(ref. 8)). For example, ferrous ion, hydroquinone and hydrogen peroxide, which react with iodate to yield I₂, have redox potentials of 0.77, 0.70 and 0.68 V respectively, and hence do not give oscillation.

The essential kinetic requirement seems to be an appropriate rate of iodide production either by the direct iodate—substrate reaction or by the subsequent reaction of iodine generated in the initial step with the remaining substrate. If the substrate produces iodide too slowly, then the Dushman reaction ($IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$) prevents iodide from accumulating to the point at which the autocatalytic reaction (2) with chlorite can generate a burst of I_2 to start the oscillation. Thus vanadium (III)

Table 1 Characteristics of substrates which give oscillation with $[IO_3^-] = 0.01 \text{ M}$, $[CIO_3] = 0.002 \text{ M}$

Substrate	Redox potential* (V)	Concentration range (M)	Optimal concentration (M)	pН
H,AsO,‡	0.56	0.0024-0.009	0.0075	2.06
Ascorbic acid	0.39†	0.010-0.020	0.010	2.238
Ka[Fe(CN)a]	0.35	0.0025-0.010	0.005	2.06
Na ₂ SO ₃	0.17	0.005-0.015	0.010	2.06
Na ₂ S ₂ O ₃	0.08	0.001-0.0125	0.005	2.40
CH ₂ O·HSO ₂ Na		0.0025-0.0075	0.005	2.06
Malonic Acid +KI¶	-morest	0.006-0.16	0.033	2.06

^{*} Ref. 8; † ref. 9.

[‡] Iodide initiation (~10⁻⁴ M) is necessary to start oscillation.

[§] No oscillation appears at pH = 2.06.

Sulphur precipitates at pH = 2.06.

[¶] Iodide concentration was kept at 0.0033 M.

has a suitable redox potential ($E^{\circ} = 0.34 \text{ V}$), but gives rise only to a stable steady state with low [I]. Other non-oscillatory substrates which we have tested, such as hydrazine and hydroxylamine, seem to yield iodide too rapidly, thereby inhibiting reaction (2) and forcing the system into a steady state of high iodide concentration. Stannous ion, which seems to satisfy both our redox potential and kinetic criteria, does not give oscillation because the formation of the slightly soluble SnI₂ places a relatively low limit on the iodide concentration that can be attained in the reactor. Substrates which, for kinetic reasons, do not give oscillations in the present conditions, may prove oscillatory in different conditions.

Further studies under way in this laboratory on the detailed mechanism of these systems will hopefully reveal more about the role of the substrate in the family of chlorite-iodate-substrate oscillators.

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- 1. De Kepper, P., Epstein, I. R. & Kustin, K. J. Am. chem. Soc. 103, 2133-2134 (1981).
- Bognár, J. & Sárosi, S. Analyt, chim. Acta 29, 406-411 (1963)
- Eggert, J. & Scharnow, B. Z. Electrochem. 27, 455-470 (1927 Kern, D. M. & Kim, C.-H. I. Am. chem. Soc. 87, 5309-5313 (1965).
- de Meeus, J. & Sigalla, J. J. chim. Phys. 63, 453-459 (1966).
- Dateo, C., Orbán, M., DeKepper, P. & Epstein, I. R. J. Am. chem. Soc. (in the press).
 Boissonade, J. & De Kepper, P. J. phys. Chem. 84, 501-506 (1980).
 Latimer, W. M. Oxidation Potentials 2nd edn (Prentice Hall, Englewood Cliffs, 1964).
- Clark, W. M. Oxidation-Reduction Potential of Organic Systems, 470 (Williams and Wilkins, Baltimore, 1960).

Crystal structure of tetrapropylammonium fluoride-silicalite

G. D. Price, J. J. Pluth, J. V. Smith & T. Araki

Department of the Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

J. M. Bennett

Union Carbide Corporation, Tarrytown, New York 10591, USA

The hydrophobic and organophilic nature of silicalite may prove commercially important for the removal of organic compounds from waste water. The framework linkage of silicalite¹ is topologically the same as that of synthetic zeolite ZSM-5 (ref. 2), a shape-selective catalyst³ capable of converting methanol into water and hydrocarbons useful in automobile engines4. The tetrapropylammonium (TPA) fluoride (F)containing precursor to fluoride-silicate⁵ crystallizes from a hydrothermal system containing silica, TPA+ and F- ions. Destruction of the organic cation during heating in air gives fluoride-silicalite, a polymorph of silica with some properties similar to those of silicalite^{1.5}. We show here how a TPAF complex in the precursor lies at the tetrahedral intersection of the 10-ring channels of a silica framework in a position consistent with a template scheme for crystallization⁶. There is not enough space to permit replacement of TPA by the tetra-nbutyl ammonium complex used in the synthesis of ZSM-11 (ref. 7) and its silica counterpart, silicalite-2 (ref. 8)

Crystals of the precursor are interpenetrant twins elongated along the c-axis $(180 \times 50 \times 50 \mu m)$. The twin boundaries are $\{(110)\}\$ and the twin law is 90° rotation about [001]. Singlecrystal X-ray and optical studies yielded monoclinic symmetry $(a, 20.04; b, 19.92; c, 13.39 \text{ Å}; \beta \sim 90^{\circ})$. Because of the nearequivalence of the a and b axial lengths, the twinning causes near-superposition of hkl and khl diffractions. The intensities of over 12,000 diffractions, each an hkl, khl pair, were measured over one quadrant of reciprocal space (Mo $K\alpha$ radiation) using a four-circle diffractometer. In spite of the optically monoclinic symmetry, the intensities are consistent with orthorhombic space group Pnma used for the refinement of silicalite⁹. The following structure description uses this space group and an

orthorhombic cell with the above cell dimensions. Averaged intensities for 1,645 unique diffractions with $F_{\rm obs} > 2\sigma$ allowed refinement of the silica framework to R = 0.10 with a special least-squares program TWXLLS. The combined intensity of pI(khl) + (1-p)I(hkl) for a pseudo-tetragonal twin was split into the individual intensities I(hkl) and I(khl) for the pseudoorthorhombic structure where the fractional volume p of one twin component was refined from all the intensities. A difference-Fourier synthesis revealed one organic complex at each of the four intersections of the 10-ring channels (Fig. 1), corresponding to a unit cell composition of 4 TPAF.96 SiO₂. Least-squares refinement yielded R = 0.07 and the atomic coordinates in Table 1. The coordinates of the silica framework are similar to those for silicalite9. Because of the restricted data set and some disorder of the TPAF complex, individual isotropic displacement factors were used for the framework atoms and a single displacement factor for the TPAF. No significant electron density was found in a difference-Fourier map based on the coordinates of Table 1.

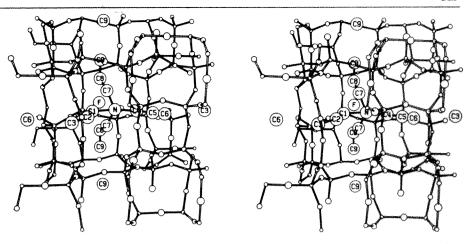
The silica framework has a mean Si-O bond length of 1.60 Å (σ 0.04) and a range of Si-O-Si angles of 136-178° about a

Table 1 Atomic coordinates for the fluoride silicalite precursor

Atom x y z Si(1) 0.1213 0.3255 0.9729 Si(2) 0.2741 0.3252 0.9712 Si(3) 0.0768 0.4410 0.8364 Si(4) 0.0712 0.3692 0.1828 Si(5) 0.3144 0.4403 0.8319 Si(6) 0.3080 0.3711 0.1878 Si(8) 0.3080 0.5296 0.1926 Si(9) 0.3127 0.6736 0.8214 Si(10) 0.2799 0.5600 0.9709 Si(11) 0.1227 0.5635 0.9716 Si(12) 0.0762 0.6712 0.8279 O(1) 0.1137 0.2500 0.9287 O(2) 0.1957 0.3464 0.9619 O(3) 0.0825 0.3711 0.8876 O(4) 0.0933 0.3374 0.0843 O(5) 0.2915 0.2500 0.9450 O(6) 0.3155 0.3704 0.8917	***************************************			
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C(9) 0.5023 0.5688 0.9842				
r 0.3034 0.7300 0.9300				
	F	0.3039	0.7300	0.9300

Standard deviations for Si, O and non-framework atoms are ~0.0006. 0.001 and 0.005 respectively.

Fig. 1 Stereoview of TPA-F complex and adjacent atoms from the silica framework. ORTEP plot with spheres at 20% probability level.



mean of 153.6° (σ 11.4). A negative correlation between Si-O bond lengths and secant (Si-O-Si) for the 4-coordinated silica polymorphs¹⁰ was interpreted in terms of overlap of molecular orbitals. The present structure shows a similar relationship with slope 0.13 (correlation coefficient 0.6), close to that predicted theoretically for a silica cluster in Fig. 8a of ref. 11.

The tetrahedral configuration of the four propyl limbs of the TPA ion and the close coordination of the fluoride to the central nitrogen at 2.52 Å and four carbons all at 2.21 Å is physically reasonable and resembles that in the crystal structure of TPAbromide¹². Two propyl limbs partly enwrap the fluorine, and a fifth carbon atom from a third limb lies at 2.75 Å to the fluorine atom. Because hydrogen bonds involving F span a range 13 of 2.3-2.8 Å which almost encompasses the above distances, the fluoride ion is probably held in place by hydrogen bonding.

The neat fit between the TPAF complex and the internal surface of the silica framework is consistent with a template mechanism for crystallization⁶. Furthermore, the end carbon atom of each propyl limb lies at 2.8-3.1 Å to the end carbon atom of the propyl limb from the next TPAF complex, and there would not be enough space for a butyl limb. Although there are no published atomic coordinates for the structures of ZSM-11 (ref. 7) or its silica counterpart, silicalite-2 (ref. 8) which can be synthesized from tetra-n-butylammonium-containing systems, models with plausible geometry show that the channel intersections are larger than for the ZSM-5 and silicalite type of linkage, and in particular are large enough for a butyl-bearing complex. Knowledge of the structural interactions between tetrahedral frameworks and occluded complexes should prove useful in designing conditions for crystallization of useful shapeselective catalysts.

Further data are being collected to increase the precision of the atomic coordinates. The cause of the monoclinic symmetry is also being investigated. The present study has indirect implications for various inorganic-organic complexes, including ones involving clays14 and biologically important compounds.

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- 1. Flanigen, E. M. et al. Nature 271, 512-516 (1978).
- Kokotailo, G. T., Lawton, S. L., Olson, D. H. & Meier, W. M. Nature 272, 437-438 (1978). Kokotailo, G. T. & Meier, W. M. Chem. Soc. spec. Publ. 33, 133-139 (1980).
- Meisel, S. L., McCullough, J. P., Lechthaler, C. H. & Weisz, P. B. Chem. Technol. 6, 86
- 5. Flanigen, E. M. & Patton, R. L. US Patent NM 4073865 (1978)
- Flanigen, E. M. & Patton, K. L. US ratent NM 4073603 (1976).
 Flanigen, E. M. Adv. Chem. Ser. 121, 119-139 (1973).
 Kokotailo, G. T., Chu, P., Lawton, S. L. & Meier, W. M. Nature 275, 119-120 (1978).
 Bibby, D. M., Milestone, N. B. & Aldridge, L. P. Nature 280, 664-665 (1979).
 Smith, J. V. Discuss. 5th int. Conf. on Zeolites (in the press).

- Meagher, E. P., Tossell, J. A. & Gibbs, G. V. Phys. Chem. Miner. 4, 11-21 (1979). Newton, M. D. & Gibbs, G. V. Phys. Chem. Miner. 6, 221-246 (1980). Zalkin, A. Acta Crystallogr. 10, 557-560 (1957).
- Durrant, P. J. & Durrant, B. Advanced Inorganic Chemistry (Wiley, New York, 1964) Theng, B. K. G. Formation and Properties of Clay-Polymer Complexes (Elsevier, Amster-

Dehydration-induced luminescence in clay minerals

L. M. Coyne, N. Lahav & J. G. Lawless

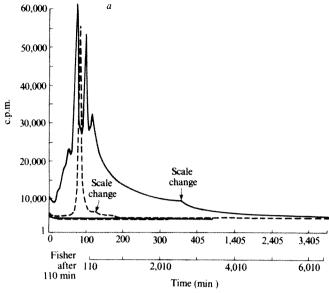
Ames Research Center, NASA, Moffett Field, California 94035, USA

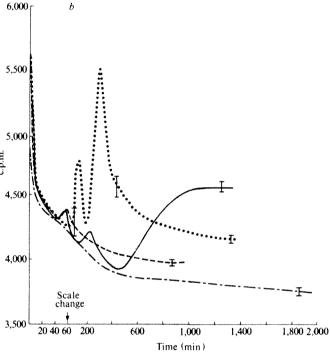
Reports of triboluminescent phenomena in organic crystalline materials1,2 prompted us to look for related processes in clay minerals. We found the reported extensive mechanical distortion produced on freezing and drying of montmorillonite particularly interesting because of our studies of condensation reactions in a wet/dry cycled reaction sequence4-7. We now report the discovery of an unusual luminescent process in several clay minerals and describe its characteristics.

We have found that blue to near UV photons are emitted when a thin layer of an aqueous suspension of certain clays is dried over a desiccant or by gentle heating to 85 °C (ref. 7). Figure 1a-c shows typical examples of three distinctive patterns of photon release as a function of time. The patterns are: (1) a delayed burst, (2) a delayed rise to a slowly decaying plateau and (3) a simple monotonic (exponential-like) decay from the time of introduction of the desiccant.

Kaolinites show an initial monotonically decaying emission of photons followed, some minutes to hours later, by the delayed burst. Two examples are shown in Fig. 1a, Fisher kaolinite and Mesa Alta rock, ground in a mortar and pestle a few days before sample preparation. A similar pattern of emission was observed from all other kaolinites examined; Peerless no. 2: kaolin no. 53 from Birch Pit, Macon, Georgia, and kaolin no. 17 from Lewistown, Missouri. The photon yield and relative proportion of monotonic to delayed emission varied from kaolinite to kaolinite. The time of onset of the burst is increased by a thicker film. The light is released when the average moisture content approaches 40% H₂O. The significance of this moisture content with respect to water content at the drying front is uncertain, but the average range is within the quoted range for the adsorption and long-range ordering of water by kaolin8. Multiple peaks are frequently observed, especially in the non-commercial kaolinites; the peaks seem to be largely, but not fully, explained as experimental artefacts resulting from non-uniformity in the thickness of the clay film and the size of the particles.

The amount of light released on dehydration is an approximately linear function of the amount of kaolin, up to a film thickness of $\sim 10 \,\mu m$ (as calculated from the dry weight of kaolin, the exposed surface area of the emitting film and its estimated density), after which self-absorption and scattering produce a gradual saturation⁷. Light release can be regenerated by repeated cycles of wetting and drying, up to at least four cycles, or until deterioration of the clay film prevents further trials.

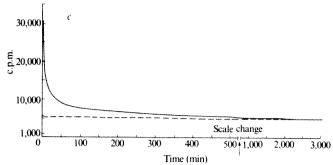




Emission from three halloysites is shown in Fig. 1b. Bedford shows weak emission and Wagon Wheel shows a pattern similar to that of the kaolinites, but with considerably reduced photon yield. Dragon Iron, however, gives a third characteristic mode of emission: the customary initial monotonic decay is followed by a relatively rapid rise to a slowly diminishing plateau. All three halloysites are colourless, relative to the 2:1-layer clays.

Of several 2:1-layered clays examined, none has shown a delayed peak. For example, montmorillonites, illites and a mica show stronger monotonic components of emission than kaolinites, but no delayed burst. This general form of emission is also characteristic of the attapulgites examined, an example of which is shown in Fig. 1c. These clays are more highly coloured than the kaolins; therefore, absence of the delayed burst may be attributable to reabsorption depending on the spectral properties of the different components of the emitted light. However, as the monotonic component is commonly enhanced relative to that in the kaolins, it is reasonable to assume that the differences also imply structurally dependent differences in the modes of emissive relaxation of the materials.

The relative proportion of light emitted as the delayed and as the monotonic components is a sensitive function of a variety of treatments and clay sources. The monotonic component is



Dehydration-induced luminescence of several clays. a, Kaolin no. 9 from Mesa Alta, New Mexico, Wards 48 W 0290 and background (Vial+Drierite) for same—upper timescale (note scale change after 300 min); ---, kaolin, Fisher lot no. 753828 and background (Vial + Drierite) for same—lower time scale after scale change at 110 min. b, -, Halloysite no. 13, Dragon Iron Mine, Eureka, Utah; ---, halloysite no. 29, Wagon Wheel Gap, Colorado; ..., halloysite no. 12 48 W 0120, Bedford (Huron), Indiana, North Gardner Mine; -Vial and desiccant background. (The curves for Bedford and Wagon Wheel halloysites have been displaced upwards slightly, for clarity of presentation. The return to hackground is actually within -, Attapulgite of unknown origin; ---, empty tube and tube+drying agent. Luminescence from a clay-water paste (1:1-1:3) was measured as a function of desiccation over CaSO₄ or silica gel. The photon output, integrated over the spectral sensitivity of the EMI 9635 QB Phototube (maximum sensitivity at 3,700 Å), was measured in a Packard 3320 Tri-Carb liquid scintillation counter in the anticoincidence mode with a 50-1,000 window on the discriminators and full gain. Photon output was displayed on a Davidson model 10565 B multichannel analyser. The paste was applied uniformly around the interior of Pyrex scintillation vials, which were scrupulously cleaned in boiling nitric acid and rinsed repeatedly with triply distilled water, air-dried and stored, with their caps, in the dark. Vials were wiped with a damp towel before counting to reduce spurious count-rate from static electricity. Common photon counting artefacts, such as luminescence from the vial, the vial cap or the desiccant, lack of temperature equilibration, and heat release from the wetted desiccant, are negligible after the first 10-30 min, as is clear from the accompanying backgrounds of empty vial and empty vial plus Drierite, and the following three controls. (1) The integrated count rate from the cooling of hot (70 °C) water to the temperature of the counter (10 °C) was 300 counts per g H₂O. (2) The integrated count rate from the addition of water to Drierite was 40 counts per mg H₂O. The clay samples typically contained 30-200 mg of clay and thus 60-400 mg H₂O. Typical peaks had an integrated count of 10⁶ counts. (3) No appreciable activity is seen from pastes contained in vials that have been painted black.

increased by orders of magnitude by prior γ -ray irradiation and grinding. In freshly ground samples, it is increased severalfold by a 10-min sonication of the clay/water paste immediately before application. Sonication may simply produce a paste of more uniform particle size by increased dispersion of the larger particles, rather than cause an electronic excitation, but the grinding effect is definitely of triboluminescent origin; this will be reported elsewhere.

An important feature of the delayed peak, with respect to its potential relationship to clay structure-reactivity correlations, is its dependence on the thermal history of the clay. Figure 2 shows the total photon output in the delayed peak after preheating to different temperatures. Heating to temperatures above 500 K temporarily destroys the luminescent effect. Samples of Fisher kaolin preheated to 650 and 1,000 K were remeasured after 6 months, with considerable restoration of the delayed peak in the 650 K sample, but none in the 1,000 K sample. Heating to 650 K for 1 h would not be expected to dehydrate kaolinite irreversibly, but similar heating to 1,000 K would probably result in considerable irreversible dehydration 10 . Preheating results in diminution of luminescence induced by both dehydration and grinding. Preliminary results suggest that destruction by preheating, and restoration by γ -ray irradiation, of the

capacity of the material to produce delayed luminescence may resemble similar destruction and restoration of centres active in electron spin resonance which have been identified in natural and doped synthetic kaolins11

Because of the low overall emissivity of the highly isomorphously substituted 2:1-layer clays and the intermediate emissivity of halloysites relative to kaolinites, we tentatively associate differences in the luminescent yield with those structural parameters that affect the relative magnitude of clay platelet-water and clay platelet-clay platelet attractions such as geometry of platelet stacking or isomorphous substitution. Verification of this hypothesis will rest on detailed study of the relationship between the rate of water loss and photon yield as a function of time in additional specially characterized and purified clays, as clays are notable scavengers, adsorbing and occluding a variety of organic and inorganic materials.

Similar measurements of luminescence have been made on several other naturally occurring, commercially available inorganic compounds such as: TiO2, a common contaminant of kaolin; Al₂O₃ and SiO₂, the chemical constituents of the layered silicates under investigation; hydroxyapatite, a substance with similar high positive-charge deficiency in the crystal lattice; and CaCO3, with its characteristic complex thermoluminescent behaviour 12-14, which we have shown to be more active than kaolin in producing glycine condensation in prebiotic simulation experiments7. These materials exhibited no delayed burst and considerable reduction in the monotonically decaying component of light emission. However, calcite, prepared for the measurements by grinding, shows the exponential-like behaviour. Solid material remaining after a 1-week digestion of Fisher kaolin in concentrated HCl showed multiple delayed photon-emission bursts of three-to six fold diminished overall intensity (not shown).

Much work remains in characterizing the spectroscopic properties of the effect-its wavelength dependence, the photon yield, the temperature dependence of the light output, molecu-

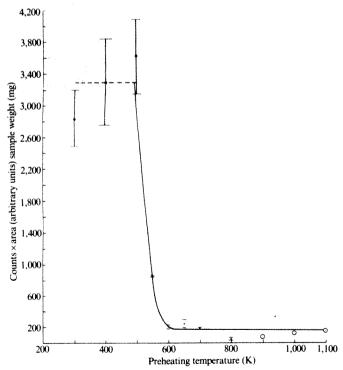


Fig. 2 Effects of preannealing on photon output from kaolin. Samples were heated by placing in a Thermolyne CPS-A8720 oven with a Dubuque III solid-state temperature controller, equilibrated to the nominal temperature, where they were held for ~ 1 h. Cooling was facilitated by partial opening of the oven door. Samples were removed when the temperature had dropped to below 500 K. The dashed line indicates uncertainty as to whether the apparent rise from room temperature to 500 K is real or an experimental uncertainty.

lar or atomic details of the trapping site or sites, mechanisms of trap population and energy release, and the relationship between monotonic and delayed emission.

Preliminary studies of the ability of preheated kaolin to produce peptide bonds under the fluctuating reaction protocol indicate an anti correlation between capacity to produce light and capacity to produce peptide bonds; luminescence may well represent wastage of chemically useful energy. Establishment of a relationship between stored electronic energy and the ability of clays to promote chemical reactions would be important to clay chemistry, not only with respect to catalysis in laboratory systems, but also to chemistry in present and past natural environments

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- Angelos, R., Zink J. & Hardy, G. J. chem. Educ. 56, 413-414 (1979). Hardy, G. E. et al. J. Am. chem. Soc. 99, 3552-3558 (1977).
- Cebula, D. J., Thomas, R. K., Middleton, S., Ottewill, R. H. & White, J. W. Clays Clay Miner. 27, 19-52 (1979).

- Miner. 41, 19-52 (1979).

 Lahav, N., White, D. & Chang, S. Science 201, 67-69 (1978).

 Lahav, N. & Chang, S. J. molec. Evol. 8, 357-380 (1976).

 Lawless, J. G. & Levi, N. J. molec. Evol. 13, 281-286 (1979).

 Coyne, L. M., Lawless, J. G., Lahav, N., Sutton, S. & Sweeney, M. in Origin of Life, 115-124 (Reidel, Dordrecht, 1981).
- Grim, R. E. Clay Minerology, 251-261 (McGraw-Hill, New York, 1968)
- Lahav, N., Coyne, L. M. & Lawless, J. G. Clays Clay Miner. (in the press).

 Grimshaw, R. W. The Chemistry and Physics of Clays 4th edn (Benn, London, 1971).
- Angel, B. R., Jones, J. P. E. & Hall, P. E. Clay Miner. 10, 247 (1974). Nishita, H. & Hamilton, M. Soil Sci 108, 1-10 (1969).
- Ferraresso, G. Am. Miner. 52, 1288-1926 (1967).
- Aitken, M. J., Fleming, S. J., Reid, J. & Tite, M. S. in Thermoluminescence of Geological Materials (ed. McDougall, D. J.) Ch. 3.5 (Academic, New York, 1968).

(100) Deformation twins in naturally deformed amphiboles

C. Biermann

Geologisch Instituut der Universiteit van Amsterdam, Nieuwe Prinsengracht 130, Amsterdam, The Netherlands

The occurrence of growth twins with (100) as a composition plane has been well established in clinoamphiboles, but no evidence has been presented that (100) twins may form by natural deformation. We now report that in naturally deformed hornblende crystals, selected from an amphibolite mylonite in the Central Scandinavian Caledonides, lamellar (100) deformation twins have been identified using optical and transmission electron microscopy (TEM). The lamellar twins are present within highly bent and kinked parts of the hornblende crystals. Selected area diffraction patterns show that twin and matrix domains are related by a 180° rotation about a^* . Additional defects associated with the (100) planes are unit dislocations with $\bar{b} = [001]$ and stacking faults bounded by partial dislocations. The identification of (100) mechanical twins in clinoamphiboles illustrates the similarity in mechanical twinning behaviour of clinoamphibole and the related clinopyroxene structure.

Deformation experiments on single crystals of clinoamphibole have been described elsewhere 1-7. The dominant mode of deformation in these experiments is mechanical twinning on $(\bar{1}01)$ in the C2/m setting of the unit cell. Natural occurrences of (101) deformation twins are rare, having only been reported from shock-loaded rocks^{8,9} and in extremely deformed amphibole gabbro from the Ivrea-Verbano Zone in the Alps⁶.

The absence of mechanical (100) twins has not been understood in terms of the crystal structure. In the related clinopyroxene structure two types of mechanical twins occur¹⁰. In experimentally deformed and shock-loaded rocks (001) twins form the analogue with $(\bar{1}01)$ twins in clinoamphibole 11.12. The

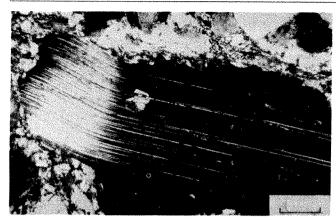


Fig. 1 Bending of a hornblende crystal is accompanied by numerous (100) deformation twins. Amphibolite mylonite Kittelfjäll. Crossed polarized light. Scale bar, 0.1 mm.

(001) plane in clinopyroxene (C2/c) is structurally related to the $(\bar{1}01)$ plane in clinoamphibole (C2/m). In naturally and experimentally deformed clinopyroxene thin lamellar (100) twins are common. There has been no proof that mechanical (100) twins are present in naturally deformed amphibole, although Kirby and Christie¹⁰ have suggested that (100) polysynthetic twins in natural amphiboles may be mechanical in origin. On the other hand, Dolinger and Blacic¹⁴ concluded that (100) [001] slip operates during kinking of hornblendes in experimentally deformed igneous hornblendite and they suggest that (100) [001] slip prevents the occurrence of (100) twinning as it is activated at a lower critical resolved shear stress.

The material described here was selected from a mylonite zone in the basal part of the Kittelfjäll amphibolite massif¹⁵ in the Central Scandinavian Caledonides. The mylonite zone separates the Kittelfjäll amphibolite from the underlying Kittelfjäll peridotite¹⁶. Both lithological units are part of the Seve Nappe, one of the major nappe structures in the Scandinavian Caledonides¹⁷. The mylonite zone formed during the late stages of the Caledonian deformation history at a lower greenschist facies metamorphic regime at intermediate pressures.

Several rock types are present within the mylonite zone, but amphibolites are dominant. In thin section the individual amphiboles are often fractured, bent or kinked, showing numerous lamellar features. The presence of lamellae within the individual crystals is consistently associated with the bent and kinked domains and their number increases towards the more intensely deformed areas (Fig. 1). Based on these observations it was concluded that the lamellae are related to deformation.

Using the optical microscope the crystallographic orientation of the lamellae was shown to be parallel with (100). In sections

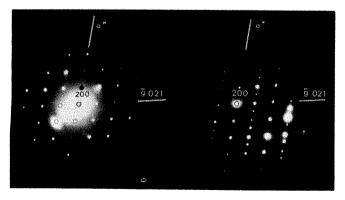


Fig. 2 Selected area diffraction patterns showing the simple pattern of an area from a matrix domain (left) and the complex pattern of an area across the twin interface (right). The extra spots originate from the spots in the left hand pattern by a 180° rotation about a^* . The trace of the twin interface in the corresponding TEM micrograph (not shown) is oriented perpendicular to a^* .

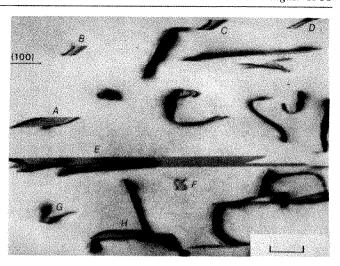


Fig. 3 Partial dislocations at the termination of stacking faults at (A-H). The stacking fault fringes are oriented parallel to the (100) composition planes of the twins. Scale bar, $0.1 \mu m$.

through the crystals parallel to the c-axis the trace of the lamellae extends parallel to the prismatic cleavage. In a few cases the lamellae have also been observed in sections perpendicular to the c-axis. The trace of the lamellae in these sections is parallel to the acute bisector of the dominant $\{110\}$ cleavage planes. When observed in the electron microscope the lamellae appear as long domains, $<20\mu m$ wide, and bounded by planar coherent interfaces. Chemical analyses on thin foils 18 , using an EDAX analytical system, showed no chemical differences between adjacent domains 13 . Selected area diffraction (SAD) showed the twin relation between the adjacent domains. SAD patterns across vertically aligned interfaces produced Laue zones, that contained extra spots, symmetrically distributed with respect to the h00 row of reflections (Fig. 2). These extra spots were not present in the electron diffraction patterns from areas selected from a single twin domain.

The $h\,00$ row of reflections in the complex pattern is common to the diffraction patterns of material at both sides of the interface. The extra spots are related to those of the diffraction pattern from a single twin by a 180° rotation about a^* . This systematic relationship persisted for all diffraction patterns in which the interface was vertical. Dark-field imaging, using twin and matrix reflections, confirmed the above interpretation. In all SAD patterns of areas with vertically aligned twin interfaces the $h\,00$ row of reflections was perpendicular to the trace of the twin interface in the corresponding TEM images, thus confirming the (100) orientation of the twins.

Additional defect structures observed in the samples are long segments of dislocations parallel with the trace of the (100) planes. Contrast experiments on these dislocations show invisibility for $\bar{g}=020$ and minimum contrast for $\bar{g}=200$. It is suggested that the Burgers vector of these dislocations is [001], confirming the slip system identified by Dolinger and Blacic¹⁴ using optical measurements and by Morrison-Smith¹⁹ using TEM. Stacking faults parallel with (100) are present in these samples and are bounded by partial dislocations (Fig. 3).

Mechanical ($\overline{101}$) twins have not been found in the Kittelfjäll amphibolite mylonites. Mechanical twinning on ($\overline{101}$) does not in general seem important in the natural deformation of clinoamphiboles. Various authors ^{14,19} have demonstrated (100) [001] slip, and the observations on the material described here confirm their conclusions.

The mechanism for the formation of (100) mechanical twins is considered to be the same as that described for clinopyroxene 10 . The essential difference between the clinopyroxene and clinoamphibole structure is the presence of double layers of SiO₄ tetrahedrons in the amphiboles. Consequently the b-parameter of the amphibole unit cell is approximately twice that of clinopyroxene, but there are no important differences in the

plane perpendicular to [010]. The geometry of (100) mechanical twinning in clinopyroxene requires the more or less rigid translation of tetrahedral layers by $\frac{1}{2}c$, resulting in an appropriate simple shear deformation of the octahedral layers in between. The composition plane in this model is therefore situated in the tetrahedral layer, which connects the sheared and unsheared octahedral layers. In addition, small shuffles are needed to obtain the exact twin geometry. The twinning shear can be propagated by the movement of partial dislocations with \bar{b} = $\frac{1}{2}[001]$, gliding in the octahedral layer¹⁰. The symmetry operator that is present in the tetrahedral layer of the C2/m clinoamphibole structure, is a diad screw axis parallel to b. The twinning mechanism described above will therefore result in a deformation twin with b-glide symmetry on (100).

The study of the deformation behaviour of crystalline silicate materials has become important since interest has concentrated more and more on fundamental mechanisms of deformation. The material from the Kittelfjäll amphibolite mylonites proves that (100) mechanical twinning is a possible mode of deformation in naturally deformed clinoamphiboles. identification of these twins confirms the similarity in twinning behaviour of clinoamphiboles and the related clinopyroxenes.

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- 1. Buck, P. & Paulitsch, P. Naturwissenschaften 56, 460 (1969)
- Buck, P. Contr. Miner. Petrol. 28, 62-71 (1970). Rooney, T. P. & Riecker, R. E. Trans. Am. geophys. Un. 50, 322 (1969).
- Rooney, T. P., Riecker, R. E. & Ross, M. Science 169, 173–175 (1970). Rooney, T. P., & Riecker, R. E. Envir. Res. Pap. 430, AFCRL-TR-0045 (1973).
- Rooney, T. P., Gavasci, A. T. & Riecker, R. E. Envir. Res. Pap. 484, AFCRL-TR-0361
- (1974), Rooney, T. P., Riecker, R. E. & Gavasci, A. T. Geology 3, 364-366 (1975). Chao, E. C. T. Science 156, 192-202 (1967). Borg, I. Am. Geophys. Un. Geophys. Mon. 16, 293-311 (1972). Kirby, S. H. & Christie, J. M. Phys. Chem. Miner. 1, 137-163 (1977). Raleigh, C. B. Science 150, 739-741 (1965). Hornemann, U. & Müller, W. F. N. Jb. Miner. Monatsh. 6, 247-256 (1971).

- Gittos, M. F., Lorimer, G. W. & Champness, P. E. in Electron Microscopy in Mineralogy (ed. Wenk, H. R.) 238–247 (Springer, Berlin, 1976).
- Dolinger, G. & Blacic, J. D. Earth planet. Sci. Lett. 26, 409-416 (1975). Biermann, C. thesis, Univ. Leiden (1979).

- Biermann, C. thesis, Univ. Leiden (1979).
 Calon, T. J. thesis, Univ. Leiden (1979).
 Williams, P. F. & Zwart, H. J. in Energetics of Geological Processes (eds Saxena, S. K. & Bhattacharji, S.) 170-187 (Springer, Berlin, 1977),
 Lorimer, G. W. & Cliff, G. in Electron Microscopy in Mineralogy (ed. Wenk, H. R.) 506-519
- (Springer, Berlin, 1976).
 19. Morrison-Smith, D. J. Am. Miner. 61, 272-280 (1976).

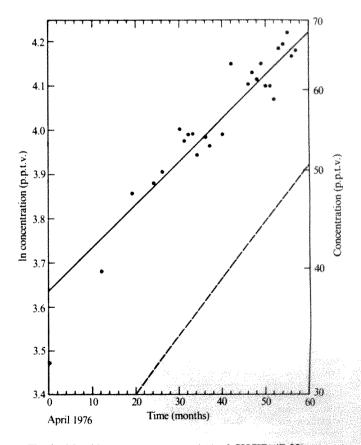
Increase of CHClF₂ in the Earth's atmosphere

M. A. K. Khalil & R. A. Rasmussen

Department of Environmental Science, Oregon Graduate Center, 19600 NW Walker Road, Beaverton, Oregon 97006, USA

Ambient air collected and stored in calibration tanks with inert internal surfaces was analysed by the electron capture-gas chromatography techniques (EC-GC) for measuring CHClF₂ (fluorocarbon-22 or F-22) at atmospheric concentrations of between 30 and 60 p.p.t.v. (10^{-12}) . Repeated measurements of F-22 in these tanks over the past 18 months have shown no significant changes in concentration, implying that the measurements reflect the atmospheric concentrations of F-22 when the air was collected. The resulting time series, based on 360 measurements made on 100 different samples, shows that CHClF₂ concentrations increased at an average exponential rate of 11.7% yr⁻¹ (90% confidence limits: 9.8%-13.2% yr⁻¹) between April 1976 and January 1981. Based on this time series, it is shown here that a constant 200×10^6 kg of F-22 in the Earth's atmosphere cannot be accounted for by the global anthropogenic emissions estimated by McCarthy et al. 1.2. This excess is ~26% of the estimated present global burden of F-22 in the entire atmosphere.

Since the early 1950s, emissions of the inert man-made fluorocarbons CCl₃F (F-11) and CCl₂F₂ (F-12) rose rapidly until 1974, when Molina and Rowland³ proposed that chlorine atoms from the dissociation of these gases could ultimately destroy enough of the Earth's stratospheric ozone layer to affect human health adversely by allowing more UV radiation to reach the Earth's surface. Subsequent theoretical studies also concluded that the large expected accumulations of these gases from continued anthropogenic emissions would lead to a global warming of the Earth's surface, which could cause climatic changes and affect agriculture^{4,5}. In the US these concerns led to restrictions on unessential uses of F-11 and F-12 and a search for substitutes. The National Academy of Sciences (NAS)6 suggested that F-22 would be safer for the ozone layer, because it contains only one chlorine atom and some of the F-22 is destroyed in the troposphere by reactions with hydroxyl radicals (HO). The NAS report also cautioned that "... if current trends in the rapidly increasing use of F-22 and methyl chloroform continued unabated, the release rates and atmospheric behaviour of these compounds will require careful attention". F-22 emissions have been increasing rapidly^{1,2}, but so far the total emissions and the atmospheric concentrations remain much less than those of CCl₃F, CCl₂F₂ and CH₃CCl₃. Continuing increases in the emissions of F-22 could, however, lead to a large accumulation in the Earth's atmosphere, partly because it reacts slowly with the hydroxyl radicals, resulting in an atmospheric lifetime of ~ 20 yr (ref 7.). It is believed that reaction with HO radicals is the major global sink of F-22. Furthermore, F-22 efficiently absorbs outgoing IR radiation at wavelengths of ~7.5, 9 and 12 µm (ref. 8) which are, as for CCl₃F and CCl₂F₂, in a region of the spectrum where natural atmospheric gases absorb little of the outgoing radiation. Thus, the general



Monthly average concentrations of CHCIF2 (F-22) over the US Pacific Northwest (~45 °N). . Average concentration line derived by nonparametric statistical methods. Concentrations expected on the basis of global emissions. The error margin for this line is quite large. It is conceivable that when the error estimates are better defined, the two lines will come closer together.

behaviour of F-22 in the Earth's environment is expected to be similar to that of F-11 and F-12, although the magnitudes of possible environmental perturbations may be less and have yet to be determined quantitatively.

The first measurements of F-22 in the atmosphere were made in 1979 by gas chromatography-mass spectrometry (GC-MS) and EC-GC techniques9. Ambient air collected periodically since early 1976, before it was possible to measure F-22 at the atmospheric concentration of 30-60 p.p.t.v, had been stored in tanks with inert internal surfaces.

Measurements to obtain a time series were made on about 100 different samples collected between April 1978 and January 1981. An in-situ air liquefaction method, which introduces no trace halocarbon contamination, was used to compress about 1,000 l of air at STP to 450 p.s.i.g. and store it in 35 l stainless steel tanks internally passivated by the SUMMA process. The water condensed in the tanks was drained and before analysis the air was further dried to less than 1% RH by a Nafion dryer. The results we report here are F-22 concentrations in dry air.

We believe that the measurements reflect the atmospheric concentrations of F-22 at the time of collection because since we first measured F-22 in the atmosphere more than a year ago, we have made repeated measurements of F-22 in each of our tanks and found no statistically significant systematic changes of concentrations. Random variabilities, however, typically <4% of the initial concentrations, were observed. In addition, repeated measurements over the past 3.5 yr have shown that CCl₃F, CCl₂F₂, CH₃CCl₃ and N₂O have all remained at constant concentrations in all the tanks used for this study, thus establishing the stability of long-lived trace gases in our tanks. These experiments also showed that the precision of analysis was good (estimated $\sigma = 2\%$ and 4% for two tanks studied intensively) whereas the absolute accuracy assigned to the primary standard is only $\pm 10\%$ (estimated σ) (see also ref. 7).

Figure 1 shows the atmospheric concentrations and the increase of F-22 in the atmosphere at 45 °N latitude in the US Pacific Northwest. The rate of increase, obtained by non-parametric statistical methods ¹⁰, is given in Table 1 and shown in Fig. 1. The statistical techniques used are less sensitive to a few extreme deviations than the classical methods, and the evaluation of the increase by $(1/C)dC/dt = \beta$, where C is the concentration and β the rate of increase, is not sensitive to errors of absolute accuracy.

Next, we estimated the concentrations to be expected from the estimated industrial release of F-22 since 19506.

Estimated rates of increase (% yr⁻¹) of CHClF₂ (F-22) in the Table 1 atmosphere and the estimates of global emissions

	Obse	rved*	
	$\hat{\boldsymbol{\beta}}$ (% yr ⁻¹)	(β_L, β_u)	$\beta > 0$?
Measurements			
(April 1976-January 1981)	11.7 (9.8	8, 13.2)	Yes $(\alpha < 0.001)$
	Expect	ted †	
	$\hat{oldsymbol{eta}}_1$	$\hat{oldsymbol{eta}}_2$	
Average			
(April 1976-January 1981)	11.4	15.9	
	Global er	missions‡	
a(10	0^6 kg yr^{-1}	b	r
January 1950-January 1960	0.12	0.33	0.983
January 1960-January 1968	2.92	0.19	0.998
January 1968-January 1978	16.2	0.153	0.998

^{*} β is assumed constant and given by (1/C) (dC/dt) where C is the concentration. $\hat{\beta}$ is the estimate of β obtained from nonparametric statistical methods based on the Theil test. (β_L , β_u) are the 90% confidence limits of $\hat{\beta}$.

Concentrations determined by a global mass balance were corrected for latitude variation of F-22 and for the amount in the stratosphere⁷ to arrive at the concentrations expected at 45 °N latitude¹¹, which are shown in Fig. 1. [The techniques are discussed in ref. 7. It was assumed that the lifetime of F-22 is 20 yr, and that the ratio of $C(F-22 \text{ at latitudes} > 45 ^{\circ}\text{N})$ to C(F-22 at)latitudes < 45 °S) ≈ 1.3 as determined by measurements made at several times at the south pole, Tasmania and Cape Meares. Oregon, as well as the measurements reported in ref. 9.] We found that the concentration expected on the basis of anthropogenic release estimates is less than that determined by atmospheric observations. We defined the difference as

$$\Delta(t) = C_{\text{obs}}(t) - C_{\text{theor}}(t) \tag{1}$$

The Δ at 45 °N was found to be approximately constant with an average value $\bar{\Delta} = 17$ p.p.t.v. (estimated $\sigma = 3$ p.p.t.v., estimated $\sigma/\sqrt{N}=0.6$ p.p.t.v.). Extrapolating to global scales this amounts to about 200×10^6 kg more F-22 in the Earth's atmosphere than can be accounted for by anthropogenic emissions, and this excess amount has remained nearly constant for the past 4 yr. The independent global F-22 measurements reported earlier9 showed the same excess in May 1979. The expected rate of increase since 1976, based on estimates of global emissions, is nearly 16% yr⁻¹, which is faster than the upper limit of the observed rate of increase ($\sim 13\% \text{ yr}^{-1}$). This result also suggests that the disagreement between observation and theory is not given by a proportionality constant as would be expected from errors in absolute calibration.

We believe that the most probable explanation for the observed excess is that past industrial releases of F-22 have been underestimated. According to the release estimates of McCarthy et al. $^{1.2}$, only $\sim 41\%$ of the F-22 produced has been released compared with ~85% release/production ratio for CCl₃F and CCl₂F₂, thus allowing for a greater margin of error in the release estimates. If, for instance, we assume that the release to production ratio is $\sim 50\%$ rather than 41%, the discrepancy between our observations and the expected burden disappears. As the published release estimates considered only the production of F-22 used in refrigeration and did not include uses of F-22 as a chemical intermediate for the production of compounds such as polytetrafluoroethylene (PTFE), the amount released may have been underestimated. Estimates of F-22 emissions from eastern Europe, the USSR and China¹¹ are also subject to large uncertainties. Alternative explanations include a possible conversion of CCl₂F₂ to CHClF₂ on sand, particle and other surfaces¹², natural emissions¹³, possible errors in absolute calibration or slow losses of F-22 in the calibration tanks as well as inadequate data on the latitudinal and height distributions of F-22. Work is now in progress to determine the causes of this

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- Jesson, J. P. in Proc. of the NATO Advanced Study Institute on Atmospheric Ozone (ed. Aikin, A. C.) 373-396 (US Department of Transportation, Washington DC, 1980).
 McCarthy, R. L., Bower, F. A. & Jesson, J. P. Atmos. Envir. 11, 491-497 (1977).
 Molina, M. J. & Rowland, F. S. Nature 249, 810-812 (1974).

- Ramanathan, V. Science 190, 50-52 (1975). Kellogg, W. Ambio 9, 216-221 (1980).
- Stratospheric Ozone Depletion by Halocarbons: Chemistry and Transport (National Academy of Sciences, Washington DC, 1979)
- Khalii, M. A. K. thesis, Oregon Graduate Center, Beaverton (1979).

 Thompson, B. Hazardous Gases and Vapors: Infrared Spectra and Physical Constants (Beckman Instruments, Tech. Rep. No. 595, Fullerton, California, 1974).
- 9. Rasmussen, R. A., Khalil, M. A. K., Penkett, S. A. & Prosser, N. J. D. Geophys. Res. Lett. 7, 809-812 (1980)
- 10. Hollander, M. & Wolfe, D. A. Nonparametric Statistical Methods (Wiley, New York, 1973).
- Rasmussen, R. A., Khalil, M. A. K. & Chang, J. S. Envir. Sci. Technol. (submitted).
 Ausnoss, P., Rebbert, R. E. & Glasgow, L. C. J. Res. natn. Bur. Stand. 82, 1 (1977); Pierotti, D., Rasmussen, L. E. & Rasmussen, R. A. Geophys. Res. Lett. 5, 1001-1004 (1978).
 Stoiber, R. E., Leggett, D. C., Jenkins, T. F., Murrman, R. P. & Rose, W. I. Bull. geol. Soc.
- Am. 82, 2299-2302 (1971).

[†] Expected average rates of increase. $\hat{\beta}_2$ is obtained by a global mass balance and the estimated global emissions. $\hat{\beta}_1$ is the obtained by assuming a constant background (17 p.p.t. at 45 °N) in addition to the concentrations expected from anthropogenic emissions.

[‡] Functional representation of global emissions based on the model: $S(10^6 \text{ kg yr}^{-1}) = a \text{ exp } (bt), b \text{ in yr}^{-1}, t \text{ in yr. } r \text{ is the correlation}$ coefficient of 1nS and t.

Cosmogenic ¹⁰Be concentrations in Antarctic ice during the past 30,000 years

G. M. Raisbeck*, F. Yiou*, M. Fruneau†, J. M. Loiseaux†, M. Lieuvin†, J. C. Ravel† & C. Lorius‡

* Laboratoire René Bernas, Centre de Spectrométrie Nucléaire et de Spectrométrie de Masse, 91406 Orsay, France

† Institut des Sciences Nucléaires, 53 avenue des Martyrs,

38026 Grenoble, France

‡ Laboratoire de Glaciologie et Géophysique de l'Environnement, 2, rue Très Cloîtres, 38031 Grenoble Cedex, France

We have previously discussed how measurements of the isotope ¹⁶Be (half life 1.5 Myr) in geophysical reservoirs can be used to probe variations in the production rate of cosmogenic nuclides^{1,2}. Such variations can be caused by changes in the primary cosmic ray intensity, the geomagnetic field intensity3, and solar activity (through the modulating influence of the solar wind)4. We report here the first significant measurements in our programme to determine the 10 Be concentration profile over the entire length of a 906-m Antarctic ice core. The results suggest an increased production of 10 Be during the Maunder minimum, a period of apparently low solar activity lasting from 1645 to 1715 . More suprisingly, we have also found a substantially increased ¹⁶Be concentration in snow deposited during the last ice age. While the interpretation of this latter effect is not yet clear, it will almost certainly have important implications for climatology studies. If production variations are indeed involved, there are also important implications for solar-terrestrial relationships and radiocarbon dating.

The measurements were carried out on samples from a 906-m ice core at Dome C (74° 39'S, 124° 10'E) in Antarctica6, and from a 180-m core taken a few metres away 1 yr later. We have previously reported some preliminary measurements from the 906-m core, using 10Be extracted from meltwater filters in the field2. However, additional studies using laboratory extracted ¹⁰Be from ice at the same depths showed that the laboratory extracted experiments often gave higher concentrations. We are not sure whether this is due to adsorption during recovery of the meltwater, or whether the small quantity of ion-exchange resin in the filters became saturated with impurities during the filtering process. All results reported here were made on ¹⁰Be extracted from ice in the laboratory. Some results from the upper 250 m of the cores have been reported elsewhere.

Samples consisted of 1-3 kg of ice, each representing at least 10 yr accumulation to minimize possible 11-yr solar cycle effects. The ice was melted in plastic containers, together with 1.5 mg of Be carrier. The Be was then recovered by passing the water through Dowex 50W-8X, 200-300-mesh ion-exchange columns. The Be was eluted with 1.5 M HCl, and then converted to BeO pellets, as described in ref. 1. The technique of measuring the ¹⁰Be/⁹Be ratio using the Grenoble cyclotron to determine the 10Be concentration in the ice, has been outlined elsewhere^{2,3} and a more detailed description is given in ref. 10.

The results from 33 samples are shown in Figs 1 and 2. The ages of the samples have been estimated from previous studies on these cores (ref. 11 and M. Pourchet, personal communication). Figure 1 shows results for samples corresponding to the past ~1,000 yr. Our objective was to see whether we could see any medium term (~ 100 yr) concentration variations, such as those observed for ^{14}C in tree rings 12 which are attributed to solar modulation effects. Although the errors are rather large, we do apparently see an enhanced 10Be concentration about the time of the Maunder minimum (given by Eddy⁵ as lasting from 1645 to 1715). There are not enough points to test whether or not there is evidence for earlier variations such as the Sporer and Wolf minima, and Medieval maximum¹².

Although the experimental uncertainties on the present measurements are quite large, the suggested enhancement during the Maunder minimum (~50%), gives some idea of the potential sensitivity of this procedure for detecting modulation effects. Thus, it is hoped that, not only will it be possible to investigate such variations much further back in time than with 14C tree-ring data, but also that one could establish whether the 11-yr solar cycle itself was operating during such times4.

Figure 2 shows results over the whole length (in metres of ice equivalent) of the available cores (for clarity, the 20 points of Fig. 1 have been averaged, and are shown by the open symbol). At depths of more than ~400 m, the ¹⁰Be concentration is 2-3 times larger than in the upper part. Figure 2 also shows a smoothed curve of the $^{18}{\rm O}/^{16}{\rm O}$ ratio (given as $\delta^{18}{\rm O}$) measured in the same core¹¹. This ratio is indicative of the temperature at which precipitation occurred, and the large change at ~400 m is believed to correspond to the end of the last ice age, ~10,000 yr ago¹¹. Thus the concentration of ¹⁰Be in precipitation in the Antarctic was considerably greater during the last ice age. There are at least three possible explanations for such a situation.

The first, and perhaps the most probable, is that the general rate of precipitation in the Antarctic was much less during the last ice age. Robin¹³, for example, suggests that precipitation in the Antarctic is limited by the saturation vapour pressure of H₂O in the atmosphere. He states that a 7 °C decrease in the average atmospheric temperature, which corresponds approximately to that estimated on the basis of 18O/16O data11, would be likely to "halve the accumulation rate of precipitation" 13. The adopted chronology of Lorius et al. 11, based on correlation of identified 18O/16O features with 14C dated sediments and the ¹⁸O/¹⁶O variation, assumed a 25% decrease in the precipitation rate beyond ~15,000 yr BP. To account for the ¹⁰Be results by this phenomenon, the decrease would have to be considerably greater.

A second explanation related to meteorology is that the circulation pattern in the atmosphere during the ice age led to a greater fraction of the global production of ¹⁰Be going into the Antarctic troposphere. This is possible because the largest part of ¹⁰Be (and other cosmogenic isotope) production is in the stratosphere14. How these isoptopes reach the Earth's surface depends on the details of stratospheric-tropospheric exchange processes. The features of such transfer which control cosmogenic deposition in Antarctica, are not well understood1

The third possibility for the increased 10Be concentration during the last ice age is that the explanation is the same as that for the increase during the Maunder minimum, that is, reduced solar activity, leading to increased 10Be production. If this were the case, one would be tempted to speculate on a causal relationship. The possible connection between reduced solar activity during the Maunder minimum and the 'Little Ice Age' has been discussed extensively 16-19.

Fortunately, there are ways of testing which of the above explanations for the increased ¹⁰Be concentration is correct. If lower precipitation is the answer, then clearly the ice at the bottom of the Dome C core is considerably older than the present estimate (~50,000 BP instead of ~30,000 BP). Some

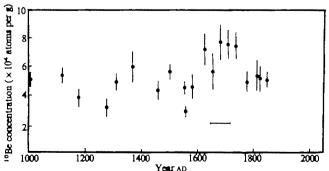


Fig. 1 ¹⁰Be concentration in Antarctic ice at Dome C. The solid line corresponds to the Maunder minimum (1645-1715, according to Eddy³).

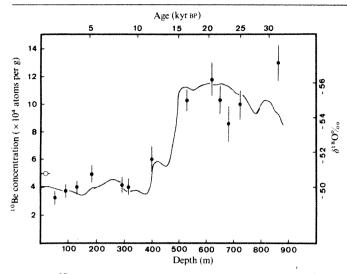


Fig. 2 $^{-10}$ Be concentration (data points, left-hand scale) and δ^{18} O (solid line, right-hand scale) in ice at Dome C. The 20 points from Fig. 1 have been averaged, and are given by the open symbol. The δ^{18} O data are from ref. 11.

evidence for a longer chronology at Dome C has been presented²⁰. Thus any direct dating of the ice at the bottom of the core would test this hypothesis. The use of the accelerator technique to ¹⁴C date the CO₂ trapped in the ice at the bottom of this core might soon be able to provide such a date.

If the explanation is changing atmospheric circulation, then the pre-Holocene deposition of ¹⁰Be in other geophysical reservoirs, such as marine and lake sediments, should not all show the same effect (meteorology can change the pattern of ¹⁰Be deposition, but cannot increase the total amount deposited). 10Be measurements in these other reservoirs, including an Arctic ice core, are in progress.

If the larger 10Be concentration does reflect increased production before 10,000 BP, then it should be seen worldwide (although not necessarily with the same amplitude). In this case, in addition to the climate implications mentioned earlier, there would be an obvious consequence for 14C dating, as an increased production of this isotope would also be implied. Indeed a combination of ¹⁰Be and ¹⁴C measurements in the same reservoir could be used to calibrate the ¹⁴C scale beyond the presently available tree-ring limit8.

Note that we have not considered the possibility that the increase in 10Be could be caused by a decrease in the geomagnetic field intensity. The argument against this is that at ~6,000 yr BP, when palaeomagnetic data suggest a geomagnetic intensity of approximately only half that of the present² the 10Be concentration is still much less than during the earlier period (although there is some hint in Fig. 2 of an increase at -6,000 yr BP). Thus, while such an effect may be present, it does not seem to be the major cause of the observed increase before 10,000 yr BP. The effect on ¹⁰Be deposition of a varying geomagnetic field is probably minimized near the geomagnetic poles4

While many more and precise measurements will be necessary before the implications of the present results can be appreciated. these initial data fully justify our previous enthusiasm^{2,4} regarding the potential of ¹⁰Be measurements in polar ice cores.

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- Raisbeck, G. M., Yiou, F., Fruneau, M. & Loiseaux, J. M. Science 202, 215-217 (1978).
- Raisbeck, G. M., Yiou, F., Fruneau, M., Lieuvin, M. Loiseaux, J. M. Nature 275, 731-733
- Raisbeck, G. M. et al. Geophys. Res. Lett. 6, 717-719 (1979).

- Raisbeck, G. M. & Yiou, F. in *Proc. Conf. Ancient Sun* (eds Pepin, R. O., Eddy, J. A. & Merrill, R. B.) 185-190 (Pergamon, Oxford, 1980).
 Eddy, J. A. *Science* 192, 1189-1202 (1976).
- Lorius, C. & Connou, D. Cour, CNRS 6, 17 (1978) Gilet, F. & Rado, C. Antarctic J. U.S. 14, 101 (1979)
- Raisbeck, G. M. & Yiou, F. Radiocarbon 22, 245-249 (1980)
- Raisbeck, G. M. & Yiou, F. in Proc. int. Conf. Sun and Climate Toulouse (CNES, Toulouse,
- in the press).

 10. Raisbeck, G. M. & Yiou, F. in Proc. 2nd int. Conf. on Low Level Counting, High Tatras, Czechoslovakia (in the press)
- Lorius, C., Merlivat, L., Jouzel, J. & Pourchet, M. Nature 280, 644-648 (1979) Stuiver, M. & Ouay, P. D. Science 207, 11-19 (1980).
- Robin, G. de Q. Phil. Trans. R. Soc. B280, 143-168 (1977) Lal, D. & Peters, B. Handb. Phys. 46, 551-612 (1967).
- Maenhaut, W., Zoller, W. H. & Coles, D. G. J. geophys. Res. 84, 3131-3137 (1979)
- 16. Herman, J. R. & Goldberg, R. A. Sun, Weather and Climate (NASA, Washington DC,
- 17. Eddy, J. A. Climate Change 1, 173-190 (1977)
- - Robock, A. Science **206**, 1402–1404 (19' Stuiver, M. Nature **286**, 868–871 (1980).
- Duval, P. & Lorius, C. Earth planet. Sci. Lett. 48, 59-64 (1980).
- 21. Barton, C. E., Merrill, R. T. & Barbetti, M. Phys. Earth planet. Inter. 20, 96-110 (1979).

Molecular carbon isotopic evidence for the origin of geothermal hydrocarbons

David J. Des Marais

Ames Research Center, NASA, Moffett Field, California 94035, USA

Jason H. Donchin, Nancy L. Nehring & Alfred H. Truesdell

US Geological Survey, Menlo Park, California 94035, USA

Previous interest in light hydrocarbons from geothermal systems has focused principally on the origin of the methane¹ and the estimation of subsurface temperatures from the carbon isotopic content of coexisting methane and carbon dioxide1-3. Higher molecular weight hydrocarbons were first reported in gases from Yellowstone National Park4, and have since been found to occur commonly in geothermal emanations in the western United States⁵. Isotopic measurements of individual geothermal hydrocarbons are now reported which help to explain the origin of these hydrocarbons. The thermal decomposition of sedimentary or groundwater organic matter is a principal source of hydrocarbons in four geothermal areas in western North America.

Steam from production (water-steam) separators at Cerro Prieto (Baja, California, Mexico) or from steam wells at The Geysers (California) was cooled in a stainless-steel condenser. Steam from fumaroles and hot springs at Steamboat Springs (Nevada) and Yellowstone (Wyoming) was sampled with an inverted funnel held underwater over the steam source. All samples were collected in evacuated 500-cm³ Pyrex flasks containing 100 cm³ of 4 M NaOH solution and having Viton O-ring valve seals. Carbon dioxide and hydrogen suphide readily dissolved in this solution, allowing only the less abundant gases, including the light hydrocarbons, to accumulate in the flask's headspace. This procedure produced negligible amounts of contaminating hydrocarbons. The geothermal hydrocarbons were separated and combusted, and the carbon dioxide product purified and measured using a combined gas chromatographcombustion system^{6,7}. Hydrocarbon identifications were confirmed by comparing the mass spectra obtained by gas chromatography-mass spectrometry with known standard spectra. The carbon isotopic analyses were performed using a modified Nuclide 6-60 RMS isotope mass spectrometer8. The entire procedure was tested using a mixture of hydrocarbons whose individual 13 C contents were known. Accurate δ^{13} C values were obtained which typically had a standard deviation of 0.3%.

The gases studied contained most of the possible saturated hydrocarbon isomers ranging in size from methane to the hexanes5. Benzene was always relatively abundant, but the

abundances of other unsaturated molecules were relatively low and highly variable between sites. The concentration ranges observed in dry gas for the four typically most abundant hydrocarbons are as follows: methane (25–7,000 parts per million (p.p.m.) by volume), ethane (1–200 p.p.m.), propane (10^{-2} –25 p.p.m.) and benzene (10^{-1} to 15 p.p.m.). The balance of abundant headspace gases included nitrogen, hydrogen, helium and argon.

Concerning the origin of the hydrocarbon mixtures, it is useful to evaluate whether the molecules have attained thermodynamic equilibrium with each other. In an equilibrium mixture at 500 K and 20 atm which has relative carbon, hydrogen and oxygen abundances similar to those in these geothermal fluids, methane should be at least 10⁵ times more abundant than all the larger hydrocarbons⁹. The methane to ethane ratios observed in these samples were always less than 600. The abundances of the other larger hydrocarbons were also greatly in excess of the abundances permitted at equilibrium. Therefore, the molecular patterns of these hydrocarbons reflect, at least in part, the kinetically controlled processes responsible for their production.

Two processes have been proposed for the thermal origin of light hydrocarbons in the Earth's crust. First, methane, which is derived either from storage deep in the Earth¹⁰ or synthesized from carbon dioxide and hydrogen¹, might react with itself to produce larger hydrocarbons^{10,11}. Alternatively, higher molecular weight organic matter from sediments or groundwater might thermally decompose to yield light hydrocarbons^{4,11-13}. The hydrocarbons could then escape thermodynamic equilibration, and hence destruction, by migrating to cooler zones in the Earth's crust. If equilibration is incomplete, the products of

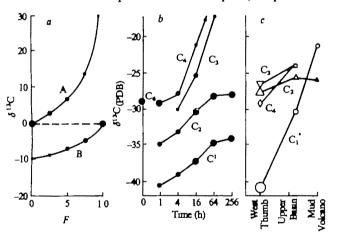


Fig. 1 Relationship between δ^{13} C values of hydrocarbon products and certain parameters which control the products' isotopic composition. Sizes of the data points depict qualitatively the relative molar abundances of the individual hydrocarbon species. a, δ^{13} C values of hypothetical reactant A and product B plotted against F, the fraction of A which has reacted. The curves for A and B have been calculated assuming that the ¹³C in A reacts at a rate which is 1% lower than that of ¹²C. The equations used in these calculations were obtained from ref. 14 b, Isotopic data from the thermal decomposition of hexane Approximately 8-um aliquots of hexane $(8^{13}C_{PDB} = -29)$ were added to 0 4-cm i.d. × 10-cm long Pyrex tubes with a 10-µl syringe. The tubes were evacuated and glass-scaled while immersed in -90 °C bath. The tubes were heated at 500 °C for the various times shown and their contents analysed. Notation adjacent to the curves denotes the compounds as follows: C_1 , methane; C_2 , ethane; C_3 , propane; C_4 , butane; C_9 became (the reactant). c, The δ ¹³ C_{PDS} values of hydrocarbons obtained from three localities in Yellowstone National Park, Wyoming, during October 1980. The δ ¹²C_{FDB} values are plotted against the same y-axis scale used for b. Data point symbols denote hydrocarbons as follows: O, methane (C_1) ; \triangle , ethane (C_2) ; ∇ , propene (C_3) ; \diamondsuit , butane (C_4) ; \square , morture of propene and butane. Sizes of symbols depact qualitatively the relative abundances of the hydrocarbons at the three localities. The abundances of hydrocarbons larger than ethane were insufficient at Mud Volcano to be measured isotopically Both the inferred subsurface temperature and the relative ³He enrichment in the gases increase (see text), whereas hydrocarbon concentrations decrease, from West Thumb to Upper Basin to Mud Volcano The $\delta^{13}C_{PDB}$ values of CO₂ in the West Thumb, Upper Basin and Mud Volcano samples were -0.2 ± 0.2 , -3.4 ± 0.1 and -2.6 ± 0.2 , respectively. For a given sample, $\delta^{13}C_{PDB} = [(^{12}C)^{12}C)_{PDB}, (^{13}C)^{12}C)_{PDB} = 1]]1,000$, where PDB is the PeeDee Belemmite standard

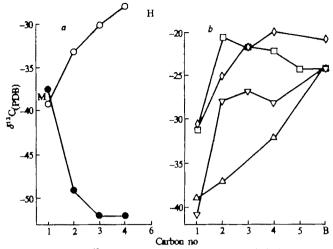


Fig. 2. Plots of δ ¹³C_{TDB} values of individual saturated light hydrocarbons against their carbon number. Numbers on x axis denote carbon number; for example, 1 denotes methane, 2 ethane, and so on. The letter B denotes bearens, a, Results of separate laboratory experiments where hydrocarbons were synthesized by thermal decomposition of hexane at 500 °C for 4 h (O) and a spark in a methane atmosphere (♠). The procedure for the hexane experiment was the same as that described for Fig. 1b. A 2-l Pyrex flask was filled with reagent grade methane (δ ¹³C_{TDB} = −38 6%) to a pressure of 200 mm Hg. The methane was subjected for 1 h to a 2-cm cod spark established between two tungsten electrodes in the flask. The products were extracted from the flask and analysed. The letters H and M identify the δ ¹³C values of the hexane and methane, respectively, at the beginning of the pyrolysis and spark discharge experiments. b, Isotopic abundance of hydrocarbons from four geothermal localities as follows: □, Cerro Prieto, well M-5, sampled January 1979; ⋄, The Geysers, 1979, △, Steamboat Springs, September 1979; ▽, Yellowstone National Park, West Thumb, October 1980

synthesis from methane should differ in their relative intermolecular ¹³C abundances from the products of organic decomposition. It is instructive to discuss next the reasons for such a difference.

Consider a kinetically controlled reaction where chemical compound A is converted to compound B and where the isotope ¹²C in these molecules reacts 1% faster than the ¹³C. The calculated carbon isotopic compositions of A and B are plotted against F in Fig. 1a, where \hat{F} represents the fraction of A (between zero and unity) which is converted to B. The first B which is produced is almost 10% depleted in ¹³C, relative to A. As the abundance of A decreases with increasing F, the ¹³C contents of both A and B must increase to preserve the isotopic mass balance¹⁴. When hexane is thermally decomposed in the laboratory at 500 °C, the 13C contents of the products (see Fig. 1b) change in a similar fashion to that depicted in Fig. 1a. As with the simple system involving A and B, this increase with time in the ¹³C contents of the reacting compounds is derived both from isotopic discrimination which accompanies compounds' production and destruction, and from the requirement that an isotopic mass balance be preserved. Note especially that, in the decomposition process, the lower molecular weight products have lower 13C contents, and that methane has the lowest 13C content of all.

Alternatively, if methane is the carbon source for the thermodynamically unequilibrated synthesis of larger hydrocarbons, these larger hydrocarbons will have lower ¹³C contents than the methane has. For example, this pattern is observed during the formation of hydrocarbons from methane in a spark discharge (Fig. 2a).

The methane in these geothermal samples was consistently more depleted in 13 C than were the associated larger hydrocarbons (Figs 1c, 2b). Thus, the thermal decomposition of organic matter to produce methane and other products is the most important source of these hydrocarbons. This conclusion is supported by our isotopic analyses of coal in drill cuttings from a well in the Cerro Prieto geothermal field. Such coal is a likely carbon source for the light hydrocarbons in the Cerro Prieto steam. The δ^{13} C value of the coal (-24.3) is very similar both to the values of the benzene (-24.0) and hexane (-23.6) in the

geothermal steam and to the values of the benzene (-24.6) and butane (-24.9) produced during vacuum pyrolysis of the coal in the laboratory for 16 h at 400 °C. Furthermore, methane produced from the coal during this laboratory pyrolysis had a δ^{13} C value of -30.0, very similar to the typical value (-31.4) observed for methane from several Cerro Prieto wells.

If the kinetically controlled geothermal decomposition of sedimentary or groundwater organic compounds produces the gaseous hydrocarbons larger than methane, an appreciable portion of the methane itself must be formed by the same process. If some of this methane is not isotopically equilibrated, as the high abundances of the larger hydrocarbons indicate, then such methane is not suitable for use in a geothermometric method which assumes that carbon isotopic equilibrium has been achieved between coexisting methane and carbon dioxide.

Even though thermodynamic equilibrium is not attained, the carbon isotopic contents of methane and carbon dioxide can give apparent equilibrium isotopic temperatures which are higher in hotter subsurface environments3. An example of this correlation can be found in gases from Yellowstone National Park (Fig. 1c). Maximum subsurface aquifer temperatures are inferred by other methods (ref. 15 and unpublished observations) to decrease from Mud Volcano (steam dominated) to Upper Basin (276 °C) to West Thumb (226 °C). The δ ¹³C values of methane and carbon dioxide (Fig. 1c and its legend) yield calculated isotopic temperatures that are highest at Mud Volcano (428 °C), lower at Upper Basin (267 °C) and lowest at West Thumb (142 °C). The ¹³C enrichments in methane with increasing temperature could result from kinetic isotope effects similar to those displayed in Fig. 1b. Note in Fig. 1b that the δ^{13} C of the methane increased with the extent of the hexane pyrolysis; in that experiment, greater pyrolysis was achieved by longer laboratory pyrolysis times. In the case of the Yellowstone and other gases, a more extensive pyrolysis of sediment or groundwater organic matter could similarly be achieved by higher subsurface temperatures. Consequently, the observed 13C enrichment of methane in the hotter subsurface localities might reflect simply a more extensive pyrolysis of sedimentary organic matter, rather than a thermodynamic equilibration between the carbon dioxide and all the methane which is present.

Alternatively, these Yellowstone gases might contain varying proportions of the methane of deep, unknown origins mixed with methane having a shallower subsurface source. The presence of an abundant unequilibrated shallow methane component is indicated by the occurrence of abundant larger hydrocarbons at West Thumb and Upper Basin. The abundances and ¹³C contents of these higher hydrocarbons (Fig. 1c) indicate that their associated methane has a relatively low 13C content and has an abundance which decreases, perhaps by a factor of 10 or more, from West Thumb to Upper Basin to Mud Volcano. Accordingly, the total methane content decreases by a factor of approximately eight from West Thumb to Upper Basin to Mud Volcano. Therefore, the relative proportions of 13Cenriched methane having a different and perhaps deeper origin are successively greater at Upper Basin and Mud Volcano. The presence of successively more concentrated deep components at Upper Basin and Mud Volcano is consistent with the successively greater enrichments of ³He observed at these localities (H. Craig, personal communication).

Additional evidence is being sought to establish more definitively the origin of geothermal methane, including that methane which emanates from the ocean floor 16. Nonetheless, it is now apparent that the ubiquitous presence of organic matter in subsurface continental environments has a key role in the production of geothermal hydrocarbons.

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- Craig, H. Geochim. cosmochim. Acta 6, 53-92 (1953).
- Hulston, J. R. & McCabe, W. J. Geochim. cosmochim. Acta 26, 399-410 (1962). Panichi, C., Ferrara, G. C. & Confiantini, R. Geothermics 5, 81-88 (1977).
- Gunter, B. D. & Musgrave, B. C. Geochim. cosmochim. Acta 35, 113-118 (1971).

- Nehring, N. L. & Truesdell, A. H. Geotherm. Res. Counc. Trans. 2, 483-486 (1978).
 Matthews, D. E. & Hayes, J. M. Analyt. Chem. 50, 1465-1473 (1978).

- Des Marais, D. J. Analys, J. M. Analys, Chem. 50, 1405–1406 (1978).

 Hayes, J. M., Des Marais, D. J., Peterson D. W., Schoeller, D. A. & Taylor, S. P. in
- Advances in Mass Spectrometry (ed. Daly, N. R.) 475-480 (Heyden, Philadelphia, 1977).
 Dayhoff, M. O., Lippincott, E. R., Eck, R. V. & Nagarajan, G. Thermodynamic Equilibrium in Prebiological Atmospheres of C, H, O, N, P, S and Cl (NASA SP-3040, Office of Technology Utilization, NASA, Washington DC, 1967).
- oter, S. Scient. Am. 242 (6), 130-137 (1980).
- Galimov, E. M. Izotopy Ugleroda v Neftegazovoy Geologii (Nedra, Moscow, 1973).
 Craig, H. in Nuclear Geology of Geothermal Areas (ed. Tongiorgi, E.) 17-54 (CNR Laboratorio Di Geologia Nucleare, Pisa, 1963).
- Laboratorio Di Geologia Nucleare, risa, 1903).

 13. Silverman, S. R. Proc. 8th Wild Petrol. Congr. 2, 47-54 (1971).

 14. Melander, L. & Saunders, W. M. Jr Reaction Rates of Isotopic Molecules (Wiley, New York,
- 15. Trusdell, A. H. & Fournier, R. O. Conditions in the Deeper Parts of the Hot Spring Systems of Yellowstone National Park, Wyoming (Open File Rep. 76-428, US Geological Survey, Menlo Park. 1976)
- 16. Welhan, J. EOS 61 (46), 0-120 (1980).

Lower Palaeozoic strata on the Pacific Plate of North America

R. Gordon Gastil & Richard H. Miller

Department of Geological Sciences, San Diego State University, San Diego, California 92182, USA

The portion of California (USA) and Baja California (Mexico) lying west of the San Andreas-Gulf of California plate boundary is part of the Pacific Plate, and consists of at least seven distinct lithological terranes (Fig. 1). The only pre-Carboniferous rocks previously identified in any of these terranes are the Precambrian gneiss and plutonic rocks of the San Gabriel Mountains in California (Fig. 1, terrane III). Previous reports of Palaeozoic strata west of the San Andreas Fault in southern California are either erroneous1 or unconfirmed (R. V. Sharp, personal communication). Fossils near El Volcan, Baja California, originally reported as possibly Palaeozoic2, are now recognized to be early Triassic3. Unidentified cup corals and crinoid-like specimens were reported in metamorphosed limestone of the Gabilan Range, central California (Fig. 1, terrane I)4. Unidentified brachiopods and crinoidal debris in the Sierra Pinta of Baja California were assigned to the Carboniferous (Fig. 1, terrane VII; ref. 5). Fossils of Pennsylvanian age were found in a block of limestone and quartzite in a mélange on Isla Cedros. We describe here the first Lower Palaeozoic rocks from the Pacific Plate; this carbonate-quartzite sequence contains Lower Ordovician conodonts and is exposed at San Marcos, 50-km south of the International Border between Tecate and Ensenada (Fig. 1).

The San Marcos section consists of carbonate, quartzite, bedded chert and subordinate slate, and is exposed in a tectonic envelope ~6 km long and 1 km wide. Preliminary mapping suggests that the rocks were thrust over sandstone-slate turbidite deposits presently considered to be of Triassic or Jurassic age (Fig. 1, terrane V). Following tectonic emplacement both the upper and lower plates were folded, weakly metamorphosed and intruded by Cretaceous granite and rhyolite/andesite dykes.

Table 1 Conodont taxa from sample VS3

Drepandus arcuatus Pander	NA	vc
Eoplacognathus? sp.	NA	vr
Prioniodus (Oepikodus) evae (Lindström)	NA	vc
Prioniodus (Periodon) flabellum (Lindström)	NA	c
Protopanderodus varicostatus Sweet & Bergström	NA	vc
Scolopodus cornutiformis Branson & Mehl	M	c
Walliserodus ethingonti? (Fåhraeus) Form taxa	NA	r
Cordylodus? sp.	M?	vr
Oistodiform element	?	c
Scandodiform element	?	r
Trichonodelliform element	?	r

NA, North Atlantic elements; M, mid-continent elements; vr, very rare; r, rare; c, common; vc, very common.

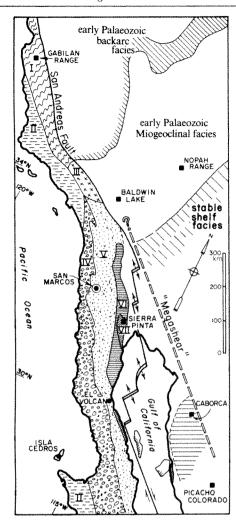


Fig. 1 Index map of seven pre-batholithic basement terranes of the Pacific Plate of western North America. I, Salinian block of highly metamorphosed carbonate-argillite of pre-Cretaceous age. II, Ophiolite, mélange and associated trench facies; Triassic to Cretaceous in age. III, Igneous rocks and gneiss of Precambrian age. IV, Island arc rocks of late Triassic to medial Cretaceous age. V, Flysch facies of early Triassic to late Jurassic age. VI, Amphibolite grade carbonate-quartzite rocks of unknown age. VII, Chertargillite-volcanic rocks of Carboniferous age. Structural features, geographic locations and boundaries of shelf, miogeoclinal and back-arc facies are indicated. Conodont-bearing rocks occur at San Marcos within terrane V, latitude 116°25′33″W, longitude 32°09′46″N. Samples were collected on a saddle of a north-west trending ridge at an elevation of ~750 metres, Hoja I11D82, Francisco Zarco quadrangle, Baja California, Mexico.

Lithological specimens ranging from reddish-brown weathered, dark-grey, fine- to medium-grained dolomitic limestone to very dark-grey, fine-grained dolomitic limestone were collected from limited exposures on brush-covered slopes. Six samples, weighing 535-1,025 g, were dissolved in 10-12% acetic acid. Fossils were recovered from three samples, but only one (VS3, 797 g) contained abundant conodonts (~250) and other fossils, including inarticulate brachiopod fragments, small gastropod steinkerns, pelmatozoan columnals and sponge? spicules. Only the conodonts are preserved well enough for more specific identification.

Most of the conodonts are broken, etched, compressed and have been thermally altered to a dark brown or black (CAI index of 5-6)⁷. Despite poor preservation seven multi-element taxa and four other morphological types were identified (Table 1, Fig. 2). With the exception of fragments tentatively identified as Eoplacognathus? sp., other species are characteristic of the Prioniodus evae/Oepikodus evae Biozone of early Ordovician (medial Arenigian) age as recognized in the British Isles^{8.9}. These taxa and Scolopodus cornutiformis suggest a correlation with early Ordovician Faunas D/E of late Canadian age as described for the US mid-continent¹⁰. Therefore the conodonts provide evidence of close correlation with European biozones and series, but provide only tentative correlation with North American mid-continent taxa.

These taxa are representative of two Ordovician biogeographic provinces. Six species, which represent most of the recovered specimens, are diagnostic of the North Atlantic province^{11,12}; two species are diagnostic of the North American mid-continent province (Table 1)^{11,12}. The diversity and abundance of the specimens indicate that these rocks formed in a North Atlantic province setting. In North America, rocks containing medial Arenigian conodonts of this province have been reported from the northern Appalachians, the Marathon region in Texas, and from the Cordilleran region of Canada and the United States¹². Medial Arenigian rocks outside North America that contain conodonts of this province have been reported from central, western and southern Europe, south-east Asia, Australia and Argentina^{9,11,12}. The distribution of these localities on Ordovician palaeogeographic maps 13 indicates that all occur at least 10-15° north or south of the Ordovician palaeoequator. Thus the conodont taxa, the abundance of specimens in the dissolved sample, and the lithology indicate the rocks were deposited on a continental shelf (miogeocline) in subtropical to temperate palaeoclimates.

These Lower Palaeozoic miogeoclinal rocks are isolated from other known outcrops of carbonate rocks, and lithological relationships with other sections are very tentative. The San Marcos exposures are 70 km south-west of strongly metamorphosed quartzite-carbonate rocks of unknown age found in the desert

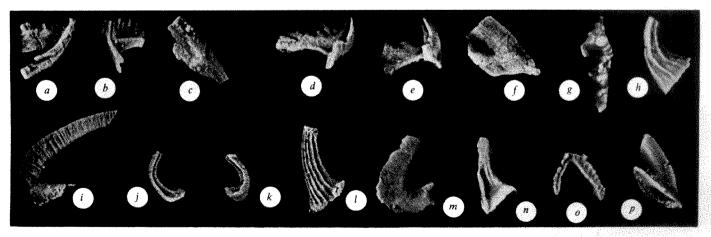


Fig. 2 Conodonts from sample VS3. All specimens are from SDSU location number 3236, Allison Center, Department of Geological Sciences, Specimens i. j. k. l., m, and p are magnified by ~22 diameters, all others by ~35 diameters. a, b, c, Prioniodus (Oepikodus) evae (Lindström), oepikodiform, prioniodiform and oistodiform elements; d, e, f, Prioniodus (Periodon) flabellum (Lindström), ramiform, ramiform and oistodiform elements; g, Eoplacognathus? sp.; h, Walliserodus ethingtoni? (Fåhraeus); i, Drepanodus arcuatus Pander, j, k, Protopanderodus varicostatus Sweet & Bergström; l, Scolopodus cornutiformis Branson & Mehl; m, Cordylodus? sp.; m, scandodiform element; o, trichonodelliform element; p, oistodiform element.

ranges west of the plate boundary (Fig. 1, terrane VI). On the east side of the plate boundary, in northwestern Sonora near Caborca (Fig. 1), is a relatively unmetamorphosed sequence of miogeoclinal rocks of late Precambrian and Cambrian ages^{14,15} that was correlated with the miogeoclinal succession of the Nopah Range in the southern Great Basin of California 16. No Ordovician rocks were recognized in the Caborca section, but Upper Ordovician strata of deep water facies occur 200 km to the south at Picacho Colorado (Fig. 1)^{17,18}. Another possibly related section of carbonate-quartzite rocks, near Baldwin Lake in the San Bernardino Mountains, California (Fig. 1), was also correlated with Cambrian and older rocks in the Nopah Range^{19,20}.

Despite difficulties in establishing lithological relationships, this well documented section of Ordovician miogeoclinal rocks west of the plate boundary is important in formulating interpretations of Palaeozoic and younger tectonic history of the southwestern part of the Cordilleran geocline. Geologists have long speculated about the possible truncation and/or displacement of geocline rocks in areas that now represent the Mojave desert, Baja California, and northern Sonora. Anderson and Silver²¹ suggested that some of these rocks, such as those represented by the Caborca sequence, were transported southeastwards some 700 km along a left-lateral 'megashear', perhaps in medial Jurassic time. Others²² have suggested that displacement of the miogeoclinal rocks was to the north-west. Whether or not the Caborca rocks were once adjacent to the southern Great Basin/Mojave Desert, the carbonate-quartzite terrane of the desert ranges (Fig. 1, terrane VI) is now separated from the western edge of the Caborca terrane by more than 300 km across the northern Gulf of California rhombochasm. The sense of motion and distance agree with independent evidence for plate boundary separation across the Gulf^{23,24} Palaeomagnetic studies, however, suggest that northern Baja California was much further south relative to the rest of North America at the time of Cretaceous granitic intrusion^{25,26} Resolution of these palaeogeographic arguments is not yet possible. The Lower Ordovician carbonate-quartzite rocks of San Marcos, Baja California must be taken into account in reconstruction of displaced terranes along the western margin of North America during the past 100 Myr.

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Received 7 May; accepted 30 June 1981.

1. Schwartz, H. P. Geol. Soc. Am. Spec. Pap. 100 (1969).

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Schwartz, H. P. Leoli Soc. Am. Spec. Pap. 100 (1909).
Gastil, R. G. et al. Geol. Soc. Am. Mem. 140 (1975).
Gastil, R. G. et al. Geol. Soc. Am. Abstr. Prog. 13, 57 (1981).
Bowen, D. E. & Gray, C. H. Jr California Division of Mines, Spec. Rep. 56 (1959).
McEldowny, R. C. thesis, San Diego State Univ. (1970).
      Kilmer, F. H. in Baja California Geology (eds Abbott, P. L. & Gastil, R. G.) 11-28 (San
          Diego State University, 1979).
      Epstein, A. G. et al. U.S. geol. Surv. prof. Pap. 995 (1977).
Lindström, M. Geol. Soc. Am. Mem. 127, 21-61 (1977).
Löfgren, A. Fossils and Strata 13, 1-129 (1978).
      Ethington, R. L. & Clark, D. L. Geol. Soc. Am. Mem. 127, 63-82 (1971)
11. Bergström, S. M. in Atlas of Paleobiogeography (ed. Hallam, A.) 47-58 (Elsevier, Amster-
12. Lindström, M. in The Ordovician System (ed. Bassett, M. G.) 501-522 (University of Wales
          Press, Cardiff, 1976).
13. Scotese, C. R. et al. J. Geol. 87, 217-277 (1979).

    Cooper, G. A. & Arellano, A. R. V. Smithsonian Misc. Coll. 119, 1-183 (1952).
    Longoria, J. F. & Gonzales, M. A. Bol. Dept Geol. Univ. Sonora 2, 106-149 (1979).
    Eells, J. L. thesis, San Diego State Univ. (1972).

    King, R. E. Bull. geo. Soc. Am. 50, 1625-1727 (1939).
    Noll, J. H. Geol. Soc. Am. Abstr. Prog. 13, 99 (1981).

     Tyler, D. L. thesis, Rice Univ. (1975).

    Cameron, C. S. Geol. Soc. Am. Abst. Prog. 12, 100 (1980).

    Anderson, T. H. & Silver, L. T. Geol. Soc. Am. Abstr. Prog. 13, 47 (1981).
    Saleeby, J. in The Geotectonic Development of California (ed. Ernst, W. G.) 132-181

(Prentice Hall, New Jersey, 1981).
23. Gastil, R. G. et al. Bull. Am. Ass. petrol. Geol. 57, 746-747 (1973)
```

24. Gastil, R. G. et al. in The Geotectonic Development of California (ed. Ernst, W. G.) 284-305

(Prentice Hall, New Jersey, 1981).

25. Beck, M. E. Jr & Plumley, P. W. Bull. geol. Soc. Am. 90, 792-794 (1979).

26. Erskine, B. G. & Marshall, C. M. A. EOS 61, 948 (1980)

Musth in the African elephant, Loxodonta africana

Joyce H. Poole* & Cynthia J. Moss†

*Sub-Department of Animal Behaviour, University of Cambridge, High Street, Madingley, Cambridge CB3 8AA, UK †African Wildlife Leadership Foundation, PO Box 48177, Nairobi, Kenva

The phenomenon of musth in male Asian elephants, Elephas maximus, has long been recognized1. Musth, which has been likened to rutting behaviour in ungulates2, refers to a set of physical and behavioural characteristics displayed periodically by adult male elephants. The most obvious manifestations are a sharp rise in aggressive behaviour, copious secretions from and enlargement of the temporal glands, and the continuous discharge of urine3. It has been speculated that a similar phenomenon occurs in males of the African genus, Loxodonta africana, but most workers have concluded that it does not exist⁴⁻⁷. Here we show that musth does occur in the African elephant and that its manifestations are similar to those in the Asian elephant.

Musth, well documented in the domestic and wild Asian elephant^{2,3,8,9}, is confined to post-pubertal males, is accompanied by high testosterone levels8, and usually lasts for 2-3 months, with a range of several weeks to 9 months³. Individual males tend to come into musth annually or biannually, but unlike the seasonal rutting of most ungulates, must periods are unsynchronized and occur throughout the year, with usually only one male in musth at a time⁹. In wild populations males in musth show a positive association with female herds9.

The most important indicator of musth in the Asian elephant is the onset of secretion from the temporal glands, which, with rare exceptions, occurs only in males in this condition^{2,3}. In the African genus, however, temporal gland secretions of short duration often occur in both immature and mature elephants of both sexes. Previous studies have shown that there is no relationship between such secretions and sexual activity^{4,6}. It is perhaps for this reason that many workers have concluded that musth does not occur in the African elephant.

The discussion of musth has been further confused by the incorrect use of the term, which in Africa is often applied to temporal gland secretions alone, rather than to the behavioural and physiological syndrome originally intended in Asia. Thus males, females and juveniles are often referred to as 'having musth' when they are observed with temporal gland secretions. To avoid such confusion we use the term 'musth' in its original sense and the term 'temporin' as used by Sikes7 to refer to secretions from the temporal glands.

We have made continuous observations on male African elephants since September 1975, as part of a long-term study of the elephant population in Amboseli National Park, Kenya. The Park covers an area of 390 km² and consists of semi-arid wooded, bushed and open grassland interspersed with a series of permanent swamps. The surrounding area, making up an ecosystem of ~3,500 km², is semi-arid savannah in which water availability is highly seasonal¹⁰. This ecosystem is inhabited by a free-ranging population of elephants numbering 580 individuals, of which ~ 155 are adult males. All adults of both sexes and most juveniles are known individually.

During the 5 years of data collection, we observed a pattern of characteristics displayed by the older males (those estimated as being > 30 years) in the population. We first noticed individuals with continuously dripping urine, which was accompanied by a strong odour and greenish coloration to the proximal part of the penis and the distal part of the sheath. We referred to this as the 'green penis syndrome' or 'GP'. GP in bulls was recorded whenever it was observed together with other accompanying characteristics, which included a pronounced enlargement of and continuous and copious secretions from the temporal

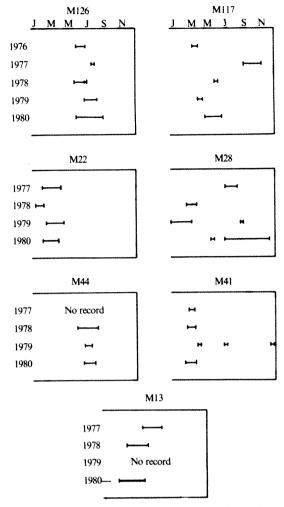


Fig. 1 The minimum duration and degree of periodicity of musth in seven males for the years 1976-1980. The vertical lines indicate the first and last days that the bull was observed to be in musth.

glands, an increase in aggression, and a positive association with female herds. The occurrence of these characteristics, their frequency, duration and association are examined here for seven large bulls in the population for which we have at least 3 years of data.

The coincidence of copious temporin and GP was pronounced in the seven bulls ($\chi^2 = 84.58$, d.f. = 1, P < 0.001). There was also a positive correlation between the occurrence of the secretion of both temporin and urine (GP) and the amount of aggressive behaviour shown by these bulls. For each of the seven bulls, we examined the rate of aggressive interactions during secreting and non-secreting periods and found a significant increase in aggression among all bulls who were secreting (Wilcoxon matched-pairs test: T = 0, n = 7, P < 0.01). In addition there was a positive correlation between GP and association with female herds. When the bulls were seen with females, GP was observed in 93% of the sightings (n = 123), whereas when they were in groups of bulls alone, GP was observed in only 11% of the sightings (n = 83). GP is thus strongly correlated with the association of males with females ($\chi^2 = 141.29$, d.f. = 2, P <

An examination of the records of frequency and duration of the described characteristics in the seven bulls reveals an individual periodicity which is variable (Fig. 1). Five of the bulls exhibited the characteristics once each year, one twice (M28), and another three times (M41). For some males (M126, M22, M44 and M13), the onset of the phenomenon is fairly consistent from one year to the next, whereas for others (M117, M28 and M41) there is no clear pattern. For all bulls observed exhibiting the characteristics at some point in the study (n = 18), the range of duration was from 1 to 103 days.

The similarity between the physical and behavioural characteristics shown periodically by African male elephants and those described as musth for Asian males leads us to conclude that musth does occur in the African genus. Several factors have probably contributed to the fact that musth has been overlooked in the African elephant. In wild populations the presence of temporin in both sexes confused the issue and without long-term records on individually known males musth was not detected. Among captive African males, there are few individuals > 30 years old, because large adults are difficult to handle, thus most would not be old enough to come into musth.

Recent evidence from our study reveals that bulls in musth become sexually more active and achieve a higher dominance rank than at other times, and that musth males are significantly more often in consort relations with oestrous females than are non-musth males. Thus musth may play a part in an individual's reproductive success. Further investigations by J.H.P. are examining the behavioural and ecological factors that determine the age and rank at which males initially come into musth, the timing and duration of musth periods, and how these considerations relate to reproductive success.

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Received 15 December 1980; accepted 12 May 1981.

- 1. Sanderson, G. P. Thirteen Years Among the Wild Beasts of India (W. H. Allan, London,
- Eisenberg, J. F., McKay, G. M. & Jainudeen, M. R. Behaviour 38, 193-225 (1971).
- Eisenberg, J. F., McKay, G. M. & Januacen, M. R., Behaussa 36, 133-225 (1971). Jainudeen, M. R., McKay, G. M. & Eisenberg, J. F. Mammalia 36, 247-261 (1972). Hanks, J. J. S. Afr. Wildi. Mgmt Ass. 3, 31-39 (1973). Buss, I. O., Rasmussen, L. E. & Smuts, G. L. Mammalia 40, 437-451 (1976).
- Short, R. V., Mann, T. & Hay, M. F. J. Reprod. Fert. 13, 517-536 (1967).
 Sikes, S. K. The Natural History of the African Elephant (Weidenfeld and Nicolson, London.
- Jainudeen, M. R., Katongole, C. B. & Short, R. V. J. Reprod. Fert. 29, 99-103 (1972).
- 9. Kurt, F. IUCN N.S. Publ. 24(2), 618-634 (1974). 10. Western, D. E. Afr. Wildl. J. 13, 265-286 (1975)

Formation of ACh receptor clusters induced by positively charged latex beads

H. Benjamin Peng, Ping-Chin Cheng & Paul W. Luther

Department of Anatomy, University of Illinois College of Medicine, PO Box 6998, Chicago, Illinois 60680, USA

An early event in the formation of neuromuscular junctions in tissue cultures of neurones and muscle cells is an accumulation of acetylcholine receptors (AChRs) in the postsynaptic membrane 1-5. As well as direct neuronal contact, AChR cluster formation can also be induced by certain soluble factors extracted or released from neurones⁶⁻⁸, by isolated basal lamina material9 or by a piece of degenerating nerve10. These experiments suggest that certain 'trophic factors' released by the neurones or the neuronal cell surface itself may cause the receptor clustering. On the other hand, stable AChR clusters are also present in pure muscle cultures without neuronal influence 1,10-13, which indicates that an interaction between the muscle and an exogenous cue existing on the substrate or in the medium may be sufficient to trigger the mechanism for the clustering of AChRs in the muscle. Here we report the effect of charged latex beads on the formation of AChR clusters in cultured muscle cells. Our results clearly indicate that AChR clusters form specifically at the contacts with polylysine-coated beads. Furthermore, these beads suppressed clusters that formed in the non-contact area before the introduction of the heads.

Table 1 Association of AChR clusters with beads

	Days of		Α	ChR clu	sters (%)*		
	bead-muscle		Top/	edge	Bot	tom	No. of clusters	No. of cells
Experiment	co-culture	Beads	+		+	****	scored	scored
\boldsymbol{A}	2	Polylysine	96	3	0	1	283	22
		Uncoated	12	64	0	24	175	22
		Polycarboxylate	6	73	0	21	188	22
В	1	Polylysine	87	11	0	2	188	20
		Uncoated	8	66	0	26	135	14
B	3	Polylysine	91	9	0	0	286	20
		Uncoated	9	73	0	18	225	20
\boldsymbol{B}	6	Polylysine	94	4	2	0	176	21
		Uncoated	4	60	0	36	109	13

Experiments A and B were from two separate batches of muscle cultures. To minimize any influence on the formation of AChR clusters by an interaction between neighbouring muscle cells or by factor(s) released by the cells, we plated the cells at extremely low density: 20-30 cells per 18×18 mm cover-glass on average. Usually all clusters located on each cell, including its top, edge and bottom, and all cells in each culture were scored. Polylysine beads were prepared from uncoated polystyrene latex beads. Polycarboxylate beads were obtained directly from Polysciences (surface charge, 0.46 mequiv. COO⁻ per g polymer).

* AChR clusters located on the top or the edge of the cell, or on the bottom of the cell as judged by the focusing level of the microscope. Each cluster, after fluorescence observation, was examined with phase-contrast optics to assess its association with beads by simply turning on the transmitted light illuminator without moving the specimen. +, Cluster associated with beads; -, cluster not associated with beads.

Muscle cells isolated from Xenopus laevis embryos were cultured on coverslips in Steinberg's solution: 60 mM NaCl, 0.7 mM KCl, 0.4 mM Ca(NO₃)₂, 0.8 mM MgSO₄, 10 mM HEPES, pH 7.4, supplemented with 10% L-15 (Leibovitz) medium and 1% fetal bovine serum^{1,14}. Positively charged beads were made by incubating 1-µm polystyrene latex beads with 1 mg ml⁻¹ poly-L-lysine (molecular weight (MW) 1,000-4,000; Sigma), dissolved either in distilled water or in phosphate-buffered saline (PBS), overnight at 4 °C, then washing thoroughly with distilled water or PBS. Uncoated beads were used as control. Negatively charged 0.9-µm polycarboxylated beads (Polysciences) were also used. Beads of each type were suspended in culture medium and applied to separate muscle cultures. After incubation for 10-30 min, excess beads were washed off and the cultures transferred to fresh medium. All three kinds of beads brought into contact with the cells adhered strongly to them, even when subjected to repeated washing, as shown in Fig. 1. At the stage required, the cultures were labelled with tetramethyl rhodamine-conjugated α-bungarotoxin (R-BTX), which binds specifically to AChRs 1,15,16. After washing and fixing with 95% ethanol at -20 °C, the culture was mounted on a slide with a mixture of polyvinyl alcohol and glycerol and examined using a Leitz Orthoplan microscope equipped with an epifluorescence illuminator. The location of fluorescent R-BTX-bound AChR clusters was correlated with the phasecontrast image of the cell and the beads by switching between epifluorescence and phase-contrast optics. Fluorescence of AChR clusters was completely suppressed when the cultures were first incubated with native α -BTX then with R-BTX.

In 1-week control cultures (without beads), an average of eight AChR clusters (0.5 µm to >10 µm) per cell were observed. Most of these (60-80%) were observed on the top or along the edge of the cell but 20-40% were also observed on the bottom of the cell. A class of large clusters similar to the one shown in Fig. 2a was observed in $\sim 50\%$ of the cells. These large clusters consisted of many smaller AChR aggregates and were found predominantly on the bottom of the cell, in contact with the substrate. In cultures treated for 1-2 days with uncoated or polycarboxylated beads, the distribution of AChR clusters was similar to that of the control. There was no significant association between the beads and the AChR clusters (Fig. 2a-d, Table 1A). About 45% of these cultured cells had large bottomlocated clusters (see Fig. 2a). However, in cultures treated with polylysine-coated beads for 1-2 days, almost all AChR clusters observed by R-BTX fluorescence were associated with single beads or small aggregates of beads which seemed to be in contact with the muscle cell. The results of one set of experiments are given in Table 1A and representative examples shown in Fig. 2e-h. It is obvious from Table 1A and B that almost all AChR

clusters in polylysine bead-treated cultures are located on the top or the edge of the cell where the beads come into contact with the cell. The absence of AChR clusters on the bottom of the cell corresponds well with the absence of beads in that area, from which they are excluded by the narrow gap between the cell and the substrate. This is in contrast to the control cultures and those treated with uncoated or polycarboxylated beads which usually show a substantial number of bottom clusters, particularly the large type illustrated in Fig. 2a. This indicates that the polylysine-coated beads not only induce the formation of new AChR clusters at bead-muscle contacts but also suppress the pre-existing clusters in non-contact areas.

In these experiments we used a dilute bead concentration to reduce the possibility of random association of beads with existing receptor clusters. As the size and shape of the muscle cells are heterogeneous, the number of bead-muscle contacts per cell also shows a wide variation. However, from Fig. 3 it is clear that the number of bead-muscle contacts is positively correlated with the number of associated AChR clusters, which further supports the notion that the beads induce formation of new clusters. There were $\sim 30\%$ apparent bead-muscle contacts not associated with receptor clusters. Clusters formed around single beads were discrete and closely apposed to the beads (Fig. 2e-h) whereas those formed under an aggregate of beads were usually composed of several discrete sub-clusters (Figs 2g-h and 4a-b).

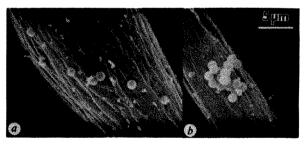


Fig. 1 Scanning electron micrograph of the association of latex beads with muscle cells. Poly-L-lysine-coated beads were used in this experiment. Two days after the bead-muscle co-culture, the cells were fixed using a mixture of 3% glutaraldehyde and 4% tannic acid, followed by post-fixation with 1% OsO₄. The preparation was then dehydrated in a graduated ethanol series, critical-point dried through CO₂, coated with palladium-platinum on a sputter coater and viewed with a JEOL 35C scanning electron microscope at an accelerating voltage of 25 kV. The attachment of single beads (a) or small aggregates of beads (b) to muscle cells can be clearly seen. This bead-muscle association is obviously strong enough to withstand the various medium changes during specimen preparation.

Fig. 2 Relationship between the AChR clusters and the bead-muscle contacts. The cells were treated with latex beads for various time periods and the location of AChR clusters assayed by labelling with R-BTX and fluorescence microscopy after fixation with cold 95% ethanol. A Leitz Orthoplan microscope equipped with a ×40 phase-contrast objective (n.a. 1.3) and epifluorescence illuminator with filter cube N2.1 was used. a, c, e and g are fluorescence micrographs; b, d, f and h the corresponding phasecontrast images. a, b, Cells were treated with polycarboxylated beads for 2 days. In a, the focus was on the bottom of the cell in contact with the substrate. In this area, the beads are excluded. A large AChR cluster, composed of many smaller subunits, is seen. This type of cluster was observed in about 50% of the cultures treated with polycarboxylated beads or uncoated beads as well as in control cultures without beads; b focuses on the centre of the cell. c, d, Cells were treated with uncoated beads for 2 days; c focuses on the AChR clusters on the top of the cell where the beads are also located (d). It is clear that the AChR clusters do not bear any

particular relationship to the beads. e-h, Cells were treated with poly-L-lysine-coated beads for 1 day (e, f) and 2 days (g, h). In e, most of the AChR clusters located on the top and the edge of the cell are found precisely at the bead-muscle contacts. The numbers denote the correspondence in location between the fluorescence (e) and phase-contrast (f) images obtained using identical focus. Beads which fall on the glass substrate are not associated with fluorescence. In g and h, a larger number of bead-muscle contacts is also associated with a larger number of bead-associated AChR clusters. Both single beads and small aggregates of beads (nos 5, 8-10) can cause AChR cluster formation.

Clusters formed shortly after the beads came into contact with the muscle cell; even after 6 h of bead-muscle co-culture, we detected 65% of the clusters already associated with beads. Table 1B summarizes the results of a time study using polylysine-coated and uncoated beads. It shows that almost a full effect of the polylysine-coated beads was evident after only 1 day of bead-muscle co-culture. The receptor clusters formed at

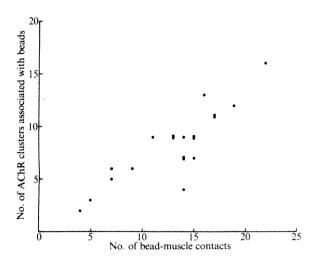


Fig. 3 Correlation between the number of AChR clusters associated with polylysine-coated beads and the number of beadmuscle contacts on individual cells. Each of the 20 cells scored is represented by a black circle. The number of bead-associated AChR clusters increases approximately linearly with the number of bead-muscle contacts.

the contacts seemed to be stable for at least the 6 days of this study. No R-BTX fluorescence was ever detected when beads came into contact with the culture substrate (Fig. 2e-h) or non-muscle cells (Fig. 4c-d).

These results indicate that the effect of polylysine-coated beads is very similar to that of nerve innervation. During the formation of a neuromuscular synapse in vitro, the nerve induces AChR cluster formation at the innervated site and suppresses pre-existing clusters in non-innervated areas². Our data indicate that a localized interaction between the muscle surface and polylysine-coated beads can trigger the mechanism for the formation of AChR clusters. This process may be mediated by the positive charge on the surface of the beads, a chemical interaction between the polylysine molecules and the cell surface, or even certain factor(s) from the culture medium adsorbed onto the positively charged beads. Further experiments are being done to investigate these possibilities.

We have repeated the above experiments using larger $(6 \mu m)$ polylysine-coated beads, which make larger contacts with the muscle cells and the resultant AChR clusters are also larger, but each cluster is still located within the confines of the contact area. This further indicates that a local cell-bead interaction triggers the clustering of AChRs. Although latex beads are known to be phagocytosed by certain cultured cells, for example, macrophages¹⁷, recent electron microscopic (EM) studies (H.B.P., P.-C.C. and P.W.L., in preparation) give no evidence of this in cultured muscle cells. Also, in our scanning electron micrographs (Fig. 1), beads were always seen on the cell surface and no intermediate stages of phagocytosis were observed. We have found that the bead-induced AChR clusters are accompanied by membrane-associated cytoplasmic densities in thin sections and clusters of large intramembranous particles in freeze-fracture replicas, which are also characteristic of spontaneously formed AChR clusters'. Closely related to our

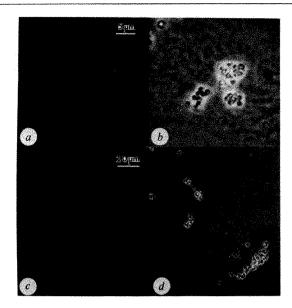


Fig. 4 a, b, AChR clusters formed at the bead-muscle contacts shown at a higher magnification than in Fig. 2. The small subunits in a cluster associated with multiple beads are obvious. c, d, The contact area between the bead and the non-muscle cell is not associated with any R-BTX fluorescence. a, c, R-BTX fluorescent images; b, d, phase-contrast images. These two cells were from the same culture and thus received the same treatment.

observation is the finding18 that neurites in cell cultures of rat cerebellum can form apparent presynaptic elements, including dense material and accumulation of synaptic vesicles, at their contacts with positively charged Sepharose beads. Thus a surface interaction between the neurite and the target cell may trigger the formation and registration of both the pre- and postsynaptic specialization during the initial stages of synaptogenesis.

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- Anderson, M. L. Coben, M. W. & Zorychta, E. I. Physiol. Lond. 268, 731-756 (1977).
- Anderson, M. J., Cohen, M. W. & Zolyenia, E. J. Physiot., Lond. 206, 1711 Frank, E. & Fischbach, G. D. J. Cell Biol. 83, 143-158 (1979).
 Peng, H. B., Nakajima, Y. & Bridgman, P. C. Brain Res. 196, 11-31 (1980).
 Cohen, M. W. & Weldon, P. R. J. Cell Biol. 86, 388-401 (1980).
- Kidokoro, Y., Anderson, M. J. & Gruener, R. Devi Biol. 78, 464-483 (1980). Podleski, T. R. et al. Proc. natn. Acad. Sci. U.S.A. 75, 2035-2039 (1978).
- Christian, C. N. et al. Proc. natn. Acad. Sci. U.S.A. 75, 4011-4015 (1978)
- Jessell, T. M., Siegel, R. E. & Fischbach, G. D. Proc. natn. Acad. Sci. U.S.A. 76, 5397-5401
- Rubin, L. L., Gordon, A. S. & McMahan, U. J. Soc. Neurosci. Abstr. 6, 330 (1980). Jones, R. & Vrbova, G. J. Physiol., Lond. 236, 517-538 (1974).
- 11. Sytkowski, A. J., Vogel, Z. & Nirenberg, M. W. Proc. natn. Acad. Sci. U.S.A. 70, 270-274 (1973).
- Fischbach, G. D. & Cohen, S. A. Devl Biol. 31, 147-162 (1973).
 Bekoff, A. & Betz, W. J. Science 193, 915-917 (1976).
- Peng, H. B. & Nakajima, Y. Proc. natn. Acad. Sci. U.S.A. 75, 500-504 (1978).
 Lee, C. Y. A. Rev. Pharmac. 12, 265-286 (1972).

- Ect, C. 1. A. ext. Finantial. 12, 203–200 (1972). Ravdin, P. & Axelrod, D. Analyt. Biochem. 80, 585–592 (1977). Walter, R. J., Berlin, R. D., Pfeiffer, J. R. & Oliver, J. M. J. Cell Biol. 86, 199–211 (1980).
- 18. Burry, R. W. Brain Res. 184, 85-98 (1980)

Creation of direction selectivity in adult strobe-reared cats

Tatiana Pasternak*, J. Anthony Movshon† & William H. Merigan‡

*Center for Visual Science and ‡Department of Ophthalmology, University of Rochester, Rochester, New York 14627, USA †Department of Psychology, New York University, New York 10003, USA

Animals raised in a stroboscopically illuminated environment have deficits in several visual functions, including visuo-motor integration¹, discrimination learning² and spatial contrast sensitivity3. Moreover, recordings from the visual pathways of strobe-reared animals show severe functional abnormalities, including greatly reduced selectivity for orientation and for directional motion in neurones of the visual cortex and superior colliculus⁴⁻⁹. Subsequent normal visual experience improves cortical orientation selectivity, but does not alter the neural deficit in direction selectivity6.7. As the motion-analysing capacities of strobe-reared animals have not been studied, we examined the ability of strobe-reared cats to discriminate stationary from moving patterns. We report here that the cats detected motion in the direction for which they had originally been trained much better than motion in other directions. In recordings from striate cortex in these animals, orientation and direction-selective neurones were encountered with a frequency much higher than that seen in strobe-reared cats not trained in motion discrimination, and comparable with that in normal cats. Moreover, the distribution of the preferred directions of these neurones was sharply biased towards the direction first seen in training. We conclude that there exists an extended period of cortical plasticity in strobe-reared animals, which, in contrast to that previously reported6, includes plasticity of direction selectivity.

We carried out behavioural tests on five cats; two were reared from birth to the age of at least 14 months in a room illuminated for 12 h each day with a stroboscopic flash of 3 µs duration at a rate of 0.67 Hz, and two were raised normally. One was raised in an environment intermittently illuminated with a flash of light 750 ms in duration at 0.67 Hz; the luminance of this flash was adjusted so that normal adult cats could resolve fine detail in this illumination as well as they could in the stroboscopic illumination. Behavioural testing began no less than 4 months after the animals were removed from their rearing environment.

The cats were trained to discriminate stationary from moving random-dot patterns using a forced-choice procedure based on that developed by Berkley¹⁰; our modification of this method has been described in detail elsewhere¹¹. The animals were trained to indicate which stimulus moved by pressing with their noses on one of two transparent panels through which they viewed the stimuli. Each stimulus consisted of a sheet of 400 bright dots, each 0.5° in diameter, on a dark background; the sheet subtended 22° at a viewing distance of 30 cm. Most of the energy in the pattern was concentrated at spatial frequencies below 1c/deg, which are visible to strobe-reared cats¹

During the initial training, the stimulus always moved to the right at 44° s⁻¹; after 12–30 days, with 200 trials per day, all cats satisfactorily discriminated between this stimulus and the stationary one. Strobe-reared cats were no slower in acquiring this discrimination than control cats. We next measured the lowest detectable speed using the method of constant stimuli. In each session five speeds were chosen to bracket threshold, and were presented randomly in blocks of five trials. Threshold was taken as the point at which resulting psychometric functions produced 75% correct performance.

The motion thresholds of the control cats improved over several months of testing from an initial value between 8 and $11^{\circ} \, s^{-1}$ to an asymptotic value between 0.9 and $1.5^{\circ} \, s^{-1}$. The initial thresholds of the strobe-reared cats were high in comparison (between 17 and 25° s⁻¹); after prolonged testing (~8 months during which the animals received ~18 h of exposure to moving dots) the thresholds stabilized near 3° s⁻¹, 2-3 times higher than those of control cats.

In all tests described above, rightward motion was used. We next examined the transfer of this training to other directions.

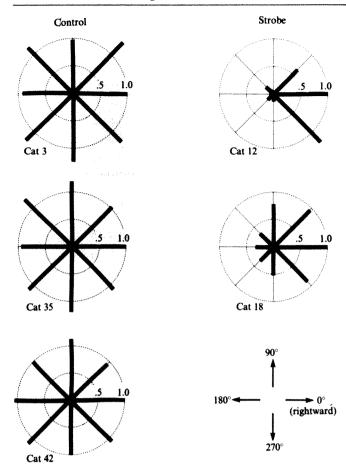


Fig. 1 Polar diagrams showing the speed sensitivity of control and strobe-reared cats to motion in eight directions. Each bar plots the inverse of the speed required for 75% correct performance on a two-choice discrimination between a stationary and a moving random-dot field. The bars in each case are normalized so that sensitivity to rightward motion is given a value of 1. Data are shown for two normal cats (3 and 35), one cat raised in an intermittently illuminated environment (42, see text) and two cats raised in a stroboscopically illuminated environment (12 and 18). Their actual thresholds for rightward motion were 0.9, 1.2, 2.0, 2.0 and 4.4° s⁻¹, respectively. A repeated measures analysis of variance showed that none of the control animals was significantly better at motion discrimination in any particular direction (P > 0.05). However, both strobe cats were significantly more sensitive to motion within 45° of rightward than they were to other directions (P < 0.05).

On each day, the threshold for one direction was tested. Over several days, an irregular sequence of eight directions, evenly spaced around 360°, was presented. Figure 1 shows the sensitivity of each of the five cats to motion as a function of the direction tested. In each polar diagram, the length of bar represents the inverse of the speed threshold for a particular direction of motion; the values are normalized with respect to the cat's sensitivity to rightward motion, which is given a value of 1. The control cats had a similar sensitivity in all test directions, whereas the strobe-reared animals were much more sensitive to rightward motion than they were to motion in directions more than 45° from this, the initial direction of training. The high thresholds for directions for which no training had been given remained stable over 7 months of further training, in which stimuli moved upwards, downwards and to the left, and did not improve as the initial high thresholds for rightward motion had done earlier. Note also that this deficit did not represent a simple failure to generalize from one direction to another, as the strobe-reared cats were capable of discriminating motion in all directions, yielding stable psychometric functions of normal slope; it was simply that when tested with rightward motion, they required only low stimulus speeds compared with those required for detection of motion in other directions.

The peculiar sensitivity of these strobe-reared cats to rightward motion led us to examine the orientation and direction selectivity of striate cortical units, using methods described elsewhere¹². Control data were obtained from six other adult strobe-reared cats that had received comparable periods of normal visual experience but had not been trained to discriminate motion, and from seven normally reared adult cats. Animals were prepared for electrophysiology using barbiturate anaesthesia (Pentothal); they were then paralysed with Flaxedil and artificially ventilated with 80% N₂O in O₂ and CO₂. Their corneas were covered with contact lenses containing 4-mm artificial pupils, and supplementary lenses were used to focus the eyes on a screen 72 cm distant. Single cortical units were isolated using tungsten microelectrodes, and their activity amplified and displayed. We studied the properties of 88 and 87 units in the two strobe-reared animals, in each case drawing our samples equally from long medially directed penetrations in the two hemispheres. For comparison, we made recordings of 183 units from normal cats. All units were histologically verified to lie in area 17.

Receptive fields were mapped on a tangent screen using bars, edges and spots of light, and classified according to the scheme of Hubel and Wiesel¹³ as modified by Blakemore and Van Sluyters¹⁴. No quantitative response measures were used but we paid careful attention to the orientation and direction selectivity of each neurone. Cells were classified as orientation selective (OS), orientation biased (OB), or not oriented (NO) using criteria described elsewhere¹⁴. Cells were direction selective (DS) if they responded markedly better to one of the two directions of motion of an optimally oriented stimulus, direction biased (DB) if they responded discriminably better to one direction than the other, and not directional (ND) if there was no difference in the responses to the two directions.

The data from the two cats trained to discriminate motion were generally similar to those from normal cats, and differed markedly from those of other strobe-reared animals studied by us and others^{4-7,15}. Table 1 lists the proportions of OS and OB neurones in the three groups of cats, and the proportions of those in each of the three classes of direction sensitivity. While strobe-reared cats given a period of normal visual experience showed abnormally low proportions of orientation- and direction-sensitive neurones (62% and 35% respectively), both motion-trained animals had roughly normal proportions of selective cells: in our sample, 93% of neurones were classified as OS or OB; of these 65% were either DS or DB. By comparison, 99% of neurones in normal cats were orientation sensitive, and 74% of these were DS or DB.

While cells preferring all orientations were direction selective in the motion-trained cats, there was a marked bias in the

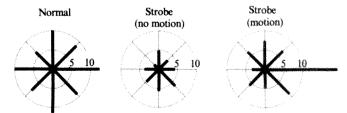


Fig. 2 Polar histograms showing the distributions of direction preference for cortical neurones showing direction sensitivity in three groups of cats: normal; strobe-reared but not motion-trained; and strobe-reared and trained in motion discrimination (cats 12 and 18 from Fig. 1). The histograms show the percentage of the total population of cortical neurones in each group that preferred directions in the ranges indicated. The results are based on totals of 192 cells from seven normal cats, 183 from six strobe-reared cats not trained in motion discrimination, and 175 cells from two strobe-reared cats trained in motion discrimination using rightward motion. The distributions of direction preference were similar in the two motion-trained cats: 21% of all cells in cat 18 and 17.5% in cat 12 preferred rightward motion, whereas only 5% of cells in cat 18 and 2% in cat 12 responded preferentially to leftward

Table 1 Proportion of orientation- and direction-sensitive neurones

		All	cells	OS and OB cells		
Experimental group	Cells	% OS	%ОВ	%DS	%DB	%ND
Normal cats	192	90	9	42	32	26
Strobe cats (not motion- trained)	183	40	22	12	23	65
Strobe cats (motion- trained)	175	91	2	32	33	35

distribution of direction selectivity. This is shown in Fig. 2, which gives the direction preferences of cortical units from the three groups of cats. In the two motion-trained cats, 59 units had preferred orientations within 22.5° of vertical: of these 40 (68%) showed a direction preference, with 34 of the 40 (85%) preferring movement to the right and only six (15%) preferring movement to the left. In these cats, 89 cells were both direction selective and preferred orientations within 67.5° of vertical; 62 of these (70%) preferred the direction having a rightward component; only 27 (30%) preferred motion with a leftward component. By comparison, among the 70 direction-sensitive cells preferring orientations within 67.5° of horizontal, 36 (51%) preferred motion with an upward component and 34 (49%) preferred motion with a downward component. Neither the direction-sensitive neurones from normal cats nor the few such neurones from strobe-reared cats not motion-trained showed any significant anisotropy in the distributions of their preferred directions.

Thus in two adult strobe-reared cats trained extensively to discriminate motion, we found both recovery of motion detection performance and recovery of cortical direction selectivity. This confirms previous suggestions^{6,16,17} that in strobe- and dark-reared cats it is possible to demonstrate a period of cortical plasticity that extends beyond the traditional 'sensitive period' for cortical development. Our results are unusual in that they suggest that this extended plasticity, which does not normally include direction selectivity, may do so if animals are preferentially exposed to moving stimuli.

Also interesting is our finding of a bias towards the initially trained direction in both the psychophysical motion sensitivity and the directional preferences of cortical neurones in these cats. Cortical direction selectivity can be biased in young kittens exposed to a restricted range of directions of motion 18-20, but these changes have only been shown to occur during a restricted period in early life that ends well before the period of sensitivity to the effects of monocular occlusion^{21,22}. Our data show that a qualitatively and quantitatively similar effect may be seen in adult strobe-reared cats exposed to a motion-biased environment. The magnitude of the effects is similar to those reported elsewhere for young animals, which is surprising in view of the fact that our animals received 4 months of normal vision before training and received 12 h per day of concurrent unbiased visual stimulation. In our experiments the cats were required to attend to the moving stimuli to obtain behavioural reward, which may have rendered those stimuli in some way more potent modifiers of cortical function.

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- Hein, A., Gower, B. C. & Diamond, R. M. J. comp. physiol. Psychol. 73, 188-192 (1970).
- Chalupa, L. M. & Rhoades, R. W. Expl Neurol. 61, 442-454 (1978).
 Pasternak, T. & Merigan, W. H. Nature 280, 313-314 (1979).
 Cynader, M., Berman, N. & Hein, A. Proc. natn. Acad. Sci. U.S.A. 70, 1353-1354 (1973).
 Olson, C. & Pettigrew, J. D. Brain Res. 70, 189-204 (1974).
 Cynader, M. Berreno, N. & Hein, A. Frod Paris, Res. 2130, 155 (1975).
- Cynader, M., Berman, N. & Hein, A. Expl Brain Res. 25, 139-156 (1976).
- Cynader, M. & Chernenko, G. Science 193, 504-505 (1976).

- 8. Orban, G., Kennedy, M., Maes, H. & Amblard, B. Archs ital. Biol. 116, 413-419 (1978).
- 9. Flandrin, J. H., Kennedy, H. & Amblard, B. Brain Res. 101, 576-581 (1976).
 10. Berkley, M. A. in Animal Psychophysics (ed. Stebbins, W.) 231-247 (Appleton-Century-
- Crofts, New York, 1970).

 Pasternak, T. & Merigan, W. H. J. comp. physiol. Psychol. 94, 943-952 (1980).

 Movshon, J. A. J. Physiol., Lond. 261, 125-174 (1976).

 Hubel, D. H. & Wiesel, T. N. J. Physiol., Lond. 160, 106-154 (1962).

- Blakemore, C. & van Sluyters, R. C. J. Physiol., Lond. 248, 663-716 (1975). Pasternak, T. & Movshon, J. A. Invest. ophthalmol. vis. Sci. Suppl. 225 (1980).
- Cynader, M. & Mitchell, D. E. J. Neurophysiol. 43, 1026–1040 (1980). Timney, B., Mitchell, D. E. & Cynader, M. J. Neurophysiol. 43, 1041–1054 (1980).
- Cynader, M., Berman, N. & Hein, A. Expl Brain Res. 22, 267-280 (1975). Tretter, F., Cynader, M. & Singer, W. Brain Res. 84, 143-149 (1975).
- Daw, N. W. & Wyatt, H. J. J. Physiol., Lond. 257, 155-170 (1976).
- Berman, N. & Daw, N. W. J. Physiol., Lond. 265, 249-259 (1977).
- Daw, N. W., Berman, N. & Ariel, M. Science 199, 565-567 (1978)

Neuronal precursor cells in the chick neural tube express neurofilament proteins

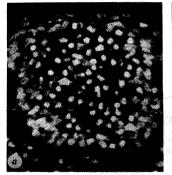
S. J. Tapscott, G. S. Bennett & H. Holtzer

Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Biochemical and immunological differences have been demonstrated in intermediate-sized (or 10-nm) filaments (IFs) from a variety of cell types (for reviews see refs 1-4). Neurofilaments, the IFs of neurones (NIF), are composed of three proteins that differ from those of non-neuronal IFs 5-12. Yet, in the 2-day-old chick neural tube, virtually all the replicating neuroepithelial cells, the precursor population to spinal cord neurones and glia. contain IF proteins that are immunologically and biochemically identical to the major IF protein present in chick fibroblasts, FIF protein (vimentin¹³, decamin¹⁴); they contain neither NIF proteins nor the IF proteins characteristic of astrocytes (glial fibrillarv acidic protein 15,16), muscle cells (desmin 17, skeletin 18) and epithelial cells (prekeratin 19,20)21. As the neuronal progeny of these cells mature, they synthesize NIF proteins and cease expression of FIF protein 10,21. In this study, we have used antisera against different IF proteins to show that, at a time when neurones are withdrawing from the cell cycle, NIF proteins are present in a small percentage of replicating neuroepithelial cells, probably appearing during the terminal cell cycle of the neuronal precursor; and that for a brief period the postmitotic neuroblast expresses both NIF and FIF proteins.

Our findings are based on immunofluorescent localization of IF proteins using previously characterized antisera against FIF protein^{22,23}, the 180,000-molecular weight (MW)^{10,21} and 70,000-MW²¹ NIF proteins, and IF proteins characteristic of astrocytes²¹ and muscle cells^{23,24}. Cryostat sections were taken primarily from the brachial region of the embryonic chick neural tube, although more rostral regions of the nervous system were also observed. Two stages of embryonic development were examined: Hamburger and Hamilton's²⁵ stage 13 (48-52 h of incubation), before significant neuronal birth, and stage 18 (3 days of incubation), when many neurones are withdrawing from the cell cycle ^{26,27}. Both stage 13 and 18 embryos were treated with demecolcine for 4 h before they were killed, as described elsewhere21

Before neuronal birth, the cells of the neural tube form a pseudostratified columnar epithelium—the neuroepithelium. They are a population of replicating cells that contain the precursors to neurones, glial cells and ependymal cells. During mitosis the cells lose their basal attachment to the external limiting membrane, assume a round shape adjacent to the neurocoel and undergo cytokinesis (see ref. 28). Treatment of the embyro with demecolcine arrests mitotic cells in metaphase and causes the IFs to aggregate and form a cable, or ring, around the cell's condensed chromatin. The IF in interphase cells also aggregate into cables in the presence of demecolcine, but these cells do not lose their bipolar shape and the IF cables are predominantly located in the basal processes. In this manner, treatment with demecolcine facilitates detection of small



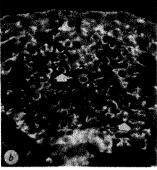


Fig. 1 Two exposures of a single microscopic field in a transverse section through the neural tube of a demecolcine-treated stage 13 embryo after staining with a, the fluorescent chromosome stain Bisbenzamid-H (Hoechst, H3325), and b, antiserum to FIF protein, localized by indirect immunofluorescence with a rhodamine-conjugated secondary antibody. Tissue preparation and immunofluorescence were performed as previously described²¹. Appropriate microscope filters allow separate visualization of the bisbenzimide and rhodamine. The metaphase cells appear as brightly staining areas of condensed chromatin (arrows in a). Associated with each of the metaphase cells is a ring labelled with anti-FIF (corresponding arrows in b). ×140.

amounts of IF proteins by immunofluorescence, and also allows the unambiguous association of IF aggregates with a specific metaphase cell. As we reported previously²¹, virtually all the metaphase cells in the stage 13 neural tube have FIF cables (Fig. 1), as determined by a fluorescent chromosome stain combined with immunofluorescent labelling using antiserum to FIF protein. None of these cables bind antibodies against the 70,000- or 180,000-NIF proteins, or the IF proteins characteristic of astrocytes or muscle cells.

In the stage 18 neural tube, as in stage 13, almost all (\sim 98%) the metaphase cells contain FIF cables. Unlike stage 13, however, some cells contain cables that bind antibodies to the 70,000- and 180,000-MW NIF proteins. Interphase nuclei positioned in the developing mantle layer have NIF-positive processes, presumably representing axonal formation and NIF synthesis in postmitotic neuroblasts. In addition, we were surprised to find that a small percentage (\sim 0.75%) of the metaphase cells surrounding the neurocoel also have NIF-positive rings (Fig. 2) and must therefore represent cells that have initiated synthesis of NIF before terminal mitosis. Furthermore, outside the neural tube, a population of cells in the vicinity of the otic vesicle is remarkable in that it has a high percentage of metaphase cells with NIF-positive rings (Fig. 3).

Double labelling of sections using antibodies against FIF and NIF shows that metaphase cells with NIF cables also contain FIF, although not always in the same distribution (Fig. 4a, b). Similarly, NIF-positive cables associated with interphase nuclei in the ventricular zone also have FIF (Fig. 4c, d). While some neuronal processes in the marginal layer of the stage 18 neural tube stain with both anti-NIF and anti-FIF, others bind only anti-NIF. At later stages all neuronal processes have no FIF and contain only NIF²¹.

These findings indicate a definite pattern of IF protein expression during neurogenesis in the spinal cord: (1) replicating neuroepithelial cells contain only FIF protein until the terminal cell cycle; (2) immediately before the final division the neuronal precursor cell begins to synthesize NIF proteins; (3) the postmitotic neuroblast expresses both NIF and FIF for a short period; and (4) with neuronal maturation, FIF expression ceases and only NIF proteins are detectable ^{10,21}.

The absence of NIF at embryonic stages before neuronal birth and the *de novo* appearance of NIF in replicating cells at a stage when neurones are withdrawing from the cell cycle indicates that the neuronal precursor cell initiates NIF synthesis during its terminal cell cycle. In this regard, molecular expression of neuronal differentiation occurs at least by the final S/G2 period of the precursor cell. This is consistent with the appearance of

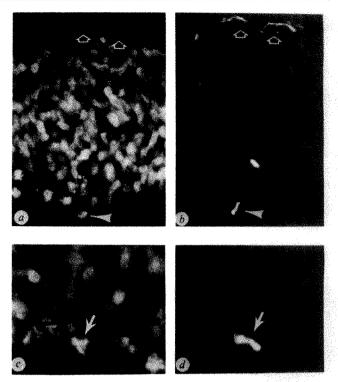


Fig. 2 Each pair of photographs shows one microscopic field of a section from a demecolcine-treated stage 18 neural tube stained with bisbenzimide (a, c) and antibodies to the 70,000-MW NIF protein (b, d). a, b, Interphase nuclei in the developing mantle1 layer (arrows) have associated NIF-containing processes. A metaphase cell (arrowhead) adjacent to the neurocoel has an NIF-positive ring lying perpendicular to the plane of section. NIF proteins are also associated with an interphase cell further from the neurocoel. $\times 280$. c, d, Arrow indicates a metaphase cell adjacent to the neurocoel with an associated NIF-positive ring. Note that many metaphase cells do not have NIF-positive rings. $\times 840$.

argentophilic filaments in replicating cells of the embryonic chick retina²⁹. In the central nervous system, in contrast to NIF proteins, other 'markers' of neuronal differentiation appear only in postmitotic neurones. G_{M1} ganglioside³⁰, neurone-specific enolase³¹ and adrenergic characteristics³² are not found in replicating neuronal precursors. However, replicating precursor cells of sympathetic neurones exhibit several characteristics of adrenergic neurones (that is, formaldehyde-induced fluorescence, dense-core vesicles and noradrenaline uptake)^{32,33}, suggesting that neuronal precursors derived from neural crest might express neuronal properties for one or more generations before the terminal cell cycle. In this regard, the high percentage of replicating cells with NIF cables in the vicinity of the otic vesicle may indicate that some neuronal precursor populations

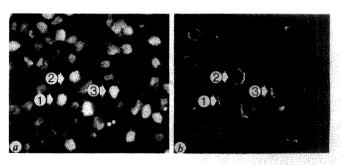


Fig. 3 A transverse section through a region postero-lateral to the otic vesicle from a demecolcine-treated stage 18 embryo stained with (a) bisbenzimide and (b) antibodies to the 70,000-MW NIF protein. A high percentage of metaphase cells in this region have associated NIF-positive rings. The numbered mitotic cells in a correspond to the numbered rings in b. ×380.

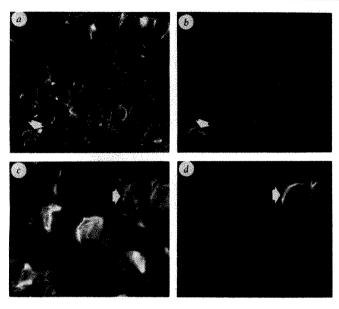


Fig. 4 Photographs of a demecolcine-treated stage 18 neural tube that has been double labelled with antibodies to FIF protein (a, c)and antibodies to either the 180,000-MW NIF protein (b) or the 70,000-MW NIF protein (d). a, b, Although most cells contain FIF, one (arrow) apparently has two cables, one mixed FIF and NIF cable and one pure FIF cable. × 560. c, d, Again, most cells contain FIF, but one cell migrating through the ventricular zone contains both NIF and FIF (arrow). ×840.

synthesize NIF for several generations. Alternatively, these cells may represent a neuronal population synchronously undergoing their terminal mitosis. Although the origin and fate of these cells remains to be determined, it is possible that they are derived from neural crest.

Finally, it is worth stressing that the early appearance of NIF reported here may represent a developmental sequence restricted to only certain types of neurones and may not apply to all neurones of the nervous system. Preliminary results indicate that for certain neuronal populations NIF expression follows, rather than precedes, the terminal mitosis. Further studies on NIF expression in different types of neurones are needed to understand the role of these proteins in development.

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- 1. Holtzer, H. et al. in International Cell Biology, 1980-1981 (ed. Schweiger, H. G.) 293-305 (Springer, Berlin, 1981).

 2. Holtzer, H., Fellini, S., Rubinstein, N., Chi, J. & Strahs, K. in Cell Motility (eds Goldman,
- R., Pollard, T. & Rosenbaum, J.) 823-839 (Cold Spring Harbor Laboratory, New York,
- 3. Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. & Yerna, M.-J. A. Rev. Physiol. 41,
- Lazarides, E. Naure 283, 249-256 (1980). Hoffman, P. N. & Lasek, R. J. J. Cell Biol. 66, 351-366 (1975).
- 6. Liem, R. K. H., Yen, S-H., Salomon, G. D. & Shelanski, M. L. J. Cell Biol. 79, 637-645

- Schlaepfer, W. W. & Freeman, L. A. J. Cell Biol. 78, 653-662 (1978).
 Runge, M. S., Detrich, H. W. III & Williams, R. C. Jr Biochemistry 18, 1689-1697 (1979).
 Czosnek, H., Soifer, D. & Wisniewski, H. M. J. Cell Biol. 85, 726-734 (1980).
 Bennett, G. S., Tapscott, S. J., Kleinbart, F. A., Antin, P. B. & Holtzer, H. Science 212, 567-569 (1981).
- Yen, S-H. & Fields, K. L. J. Cell Biol. 88, 115-126 (1981).
- Willard, M. & Simon, C. J. Cell Biol. 89, 198-205 (1981).
 Franke, W. W., Schmid, E., Osborn, M. & Weber, K. Proc. natn. Acad. Sci. U.S.A. 75, 5034-5038 (1978)
- Zackroff, R. V. & Goldman, R. D. Proc. natn. Acad. Sci. U.S.A. 76, 6226-6230 (1979).
- Eng, L. F., Vanderhaegen, J. J., Bignami, A. & Gerstl, B. Brain Res. 28, 351-354 (1971).
 Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. Y. Brain Res. 43, 429-435 (1973).
- Lazarides, E. & Hubbard, B. D. Proc. nam. Acad. Sci. U.S.A. 73, 4344-4348 (1976) Small, J. V. & Sobieszek, A. J. Cell Sci. 23, 243-268 (1977).
- 19. Franke, W. W., Weber, K., Osborn, M., Schmid, E. & Freudenstein, C. Expl Cell Res. 116, 429-445 (1978).
- Sun, T. T. & Green, H. Cell 14, 469-476 (1978).
 Tapscott, S. J., Bennett, G. S., Toyama, Y., Kleinbart, F. & Holtzer, H. Devl Biol. 85 (in the
- 22. Bennett, G. S. et al. Proc. natn. Acad. Sci. U.S.A. 75, 4364-4368 (1978)
- Bennett, G. S., Fellini, S. A. & Holtzer, H. Differentiation 12, 71-82 (1978).
 Fellini, S. A., Bennett, G. S., Toyama, Y. & Holtzer, H. Differentiation 12, 59-70 (1978).

- 25. Hamburger, V. & Hamilton, H. L. J. Morph. 88, 49-92 (1951)
- Langman, J. & Haden, C. C. J. comp. Neurol. 138, 419-432 (1970).
 Hollyday, M. & Hamburger, V. Brain Res. 132, 197-208 (1977).
 Jacobson, M. Developmental Neurobiology (Plenum, New York, 1978).
 Sechrist, J. W. Am. J. Anat. 124, 117-134 (1969).
 Willigner, M. & Schachner, M. Devl Biol. 74, 101-117 (1980).

- Schmechel, D. E., Brightman, M. W. & Marangos, P. J. Brain Res. 190, 195-214 (1980). 32. Rothman, T. P. et al. Proc. natn. Acad. Sci. U.S.A. 77, 6221-6225 (1980)
- 33. Rothman, T. P., Gershon, M. D. & Holtzer, H. Devl Biol. 65, 322-341 (1978).

A second messenger required for nerve growth factor biological activity?

Rolf Heumann, Martin Schwab & Hans Thoenen

Abteilung Neurochemie, Max-Planck-Institut für Psychiatrie, D-8033 Martinsried, FRG

After binding to specific membrane receptors of target neurones and responsive phaeochromocytoma cells, nerve growth factor (NGF) is internalized and accumulated in the perikaryon within membrane-confined compartments (H. Rohrer et al., unpublished observation, and refs 1-5). Although quantitative electron microscopic autoradiography and ultrahistochemical studies gave no evidence for further movement of NGF^{1,2,4,5}, it was claimed on the basis of light microscopy of PC12 cells (ref. 6 and P.C. Marchisio, personal communication) that some of the NGF taken up by the cell reaches the free cytoplasm and subsequently the nucleus where it would exert a physiological effect. We report here that the direct introduction of NGF into the free cytoplasm of phaeochromocytoma cells by fusion with NGFloaded erythrocyte ghosts does not induce fibre outgrowth, in contrast to the normal response of these cells seen when NGF is added to the culture medium. Correspondingly, NGF antibodies introduced into the cytoplasm do not prevent fibre outgrowth evoked by NGF added to the culture medium. Thus, the binding of NGF to its receptor must result in the production of a second messenger either from the cell surface or after internalization from an intracellular compartment^{7.8}.

Guinea pig erythrocyte ghosts loaded with NGF or NGF antibodies (see Fig. 2 legend) served as vehicles for injecting these molecules into the cytoplasm of NGF target cells through cell fusion. The target cells were a line of PC12 phaeochromocytoma cells which reacted rapidly to NGF with an increase in choline acetyltransferase and induction of fibre outgrowth which occurred in 50-70% of single cells within 48 h. In addition to NGF or NGF antibodies, the ghosts were loaded simultaneously with two different marker proteins: fluorescein isothiocyanate-conjugated bovine serum albumin (FITC-BSA), serving as a marker to follow directly the fusion procedure by fluorescence microscopy; and horseradish peroxidase (HRP), to quantify the number of injected cells in fixed preparations. The loading efficiency of the ghosts, that is, the equilibration between the concentration in the loading solution (for details, see Fig. 2 legend) and the interior of the ghosts, depends on the molecular weight (MW) of the protein to be loaded. Accordingly, the 'loading efficiency' for ¹²⁵I-labelled NGF (MW 26,000) was 100%, whereas that for purified anti-NGF IgG antibodies (MW 150,000) was only 30%. The biological activity of NGF or NGF antibodies in the ghosts was tested by their ability either to induce fibre outgrowth or antagonize NGF-induced fibre outgrowth, respectively in PC12 cells. On the average, one loaded ghost contained about 44,000 molecules of NGF or a quantity of NGF antibodies able to neutralize about 26,000 molecules of NGF. Fusion of ghosts with target cells was performed in sparsely seeded monolayer cultures of the latter. In principle, we followed the fusion procedure of Schlegel et al. 10 with the modifications specified in Fig. 2 legend. The low temperature (4 °C) used reduced general cell damage during the 5-min exposure to polyethylene glycol (PEG). After fusion, the cells were kept in regular tissue culture conditions at 37 °C. Ghosts which had not fused detached from the cells during the next 6-12 h.

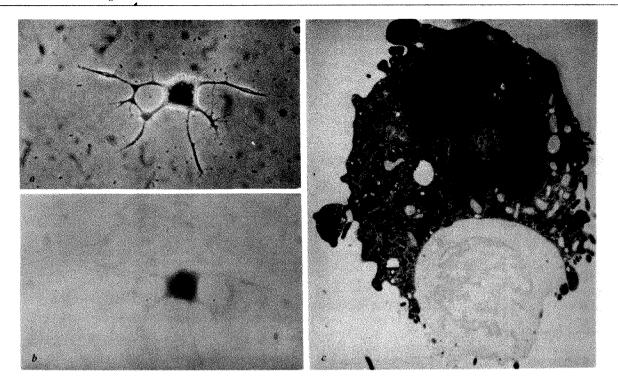
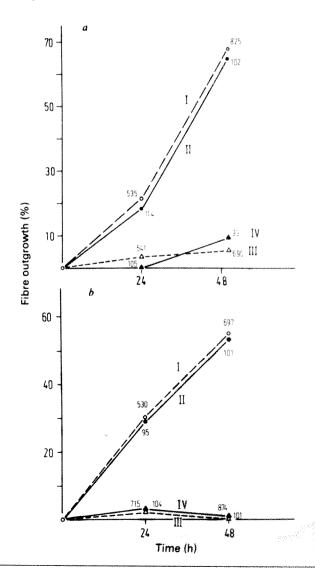


Fig. 1 Phase contrast (a), bright field (b) (same cell) and electron microscopic (c) photographs of fast-responding PC12 cells injected with HRP, FITC-BSA and NGF. The injection procedure is described in detail in Fig. 2 legend. The cells were grown in the presence of 50 ng ml⁻¹ NGF. The HRP reaction product¹⁸ is evenly distributed over the whole cell cytoplasm as well as the nucleus. The equal distribution of HRP in the free cytoplasm and the nuclear chromatin becomes impressively apparent in c which depicts neighbouring injected (top) and non-injected (bottom) cells. a, b, ×220; c, ×5,055.

Fig. 2 Effect of the injection of mouse NGF (a) or NGF antibodies (b) into PC12 cells. The loading procedure of the ghosts was essentially that of Yamaizumi et al.9. a, Guinea pig erythrocytes were equilibrated in 0.5 ml of 'reverse phosphate-buffered saline (PBS)' ('loading solution') containing 10 µg NGF, 0.3 mg HRP and 10 mg FITC-BSA (FITC/BSA = 2.4 in terms of molecules). The resulting ghosts were washed four times in Ca/Mg-free PBS (PBS-CMF: 8 g l $^{-1}$ NaCl, 0.4 g l $^{-1}$ KCl, 300 mg l $^{-1}$ KH $_2$ PO $_4$, 45 mg l $^{-1}$ Na $_2$ HPO $_4$ +7H $_2$ O, 1 g l $^{-1}$ glucose, 20 g l $^{-1}$ sucrose) and were finally resuspended at 10% (v/v) in the same buffer. The number of NGF molecules per ghost was determined by adding tracer quantities of ¹²⁵I-NGF (prepared by the lactoperoxidase method 19) to the loading solution. For electron-microscopic autoradiography 296 μ Ci of 125 I-NGF (178 \times 10 3 c.p.m. per ng) was used. The calculation of the loading efficiency was based on an average volume of $90~\mu m^3$ for guinea pig erythrocytes²⁰. Four days before the fusion experiments 30,000 'fast-reacting PC12' cells were plated on 35-mm polyornithine-coated dishes and cells were then incubated in Dulbecco's minimal essential medium (DMEM) containing 10% heat-inactivated horse serum and 5% heat-inactivated fetal calf serum. Cells were washed first with PBS-CMF, then with PBS-CMF containing 130 µg ml⁻¹ phytohaemagglutinin type V(Sigma), and finally $50 \mu l$ of ghost suspension (2%, v/v) corresponding to 1.2×10^7 ghosts were added to the cultures. After 4–5 min at $37 \, ^{\circ}$ C another $25 \mu l$ (0.6×10^7 ghosts) of the suspension were added and the cultures kept for 3–5 min at $37 \, ^{\circ}$ C. Plates were then washed carefully with 1 ml PBS-CMF and placed in a refrigerator (4 °C) for 2-3 min. Thereafter, 100 µl of ice-cold PEG 4000 (Roth, 45% (w/v) in PBS-CMF was added and cells were left at 4°C for 5 min. PEG treatment was terminated by addition of 1 ml DMEM (containing Ca2+, Mg2+). After 15-45 min at 37 °C the medium was changed to normal medium with serum. After 8-12 h (taken as time 0) the medium was changed and cells incubated in the presence of either 50 ng ml⁻¹ of NGF (curves I, II) or NGF antibodies (curves III, IV). The amount of NGF antibodies used was sufficient to neutralize all the NGF added previously to the cultures in loaded ghosts. At the times indicated cultures were fixed (2.5% glutaraldehyde, 20 min) and stained for HRP (30 min 0.03% diaminobenzidine, 2 h 0.03% diaminobenzidine plus 0.01% $\,\mathrm{H_2O_2})^{18}$. Randomly chosen regions of the plate were scored for fibre outgrowth (positive score for fibres ≥20 µm). The percentage of all the single cells carrying fibres was counted under phase contrast (curves I, III). Subsequently, the injected HRP-positive cells were evaluated under bright-field optics (curves II, IV). The number of cells counted per point are indicated and the injection frequencies can be directly derived from them. b, The same methods as described for a were used with the following modifications. Erythrocytes were loaded in 720 µl of a solution containing 504 μg affinity-purified NGF antibodies²¹ (neutralizing 40 μg NGF), 0.4 mg HRP and 10 mg FITC-BSA. At time 0 those plates which were subsequently incubated with 50 ng ml⁻¹ of NGF (curves I, II) were washed with NGFcontaining medium. Residual NGF antibody activity could be neutralized by this procedure. The rest of the plates received normal medium (curves III, IV). Injected cells (curves II, IV) and total cells (curves I, III) were evaluated as described for a.



The quantity of 125I-NGF present in the injected cells revealed that an average of six ghosts had fused with each cell. This high multiplicity is probably because the ghosts tended to aggregate with each other and so fusion took place between one PC12 cell and an aggregate of fused ghosts. The radioactivity in ¹²⁵I-NGF-injected cells decreased gradually over 2 days to ~30% of the initial value. About 50% of the radioactivity remaining after 2 days represented native NGF, as judged from its migration in SDS-polyacrylamide gel electrophoresis using a 15% gel. Thus, the quantity of native NGF present in a single injected cell amounts to an average of ~44,000 molecules 2 days after the injection. In comparison, when PC12 cells are incubated for 24 h with 100 ng ml⁻¹ of ¹²⁵I-NGF, the quantity of radioactivity accumulated (not corrected for possible degradation products) corresponds to ~15,000 molecules per cell—onethird of the amount of NGF injected. As shown in Fig. 1, the injected HRP is evenly distributed throughout the cytoplasm and nucleus of the cell. Electron microscopic autoradiography performed on the same sections indicated that the 125 I-labelled NGF, too, was distributed throughout the cell cytoplasm and also reached the nucleus.

The fusion procedure did not influence the NGF-mediated fibre outgrowth, that is, the frequency and extent of fibre outgrowth from injected cells in response to NGF added to the medium is the same as that of non-injected cells (Fig. 2). In those experiments where the response of the NGF-injected cells (in the absence of NGF in the culture medium) was studied, we supplied the medium with NGF antibodies to ensure that any NGF originating from leaking ghosts would not reach cellsurface NGF receptors. As demonstrated in Fig. 2a, the NGFinjected and non-injected cells behaved identically: fibre outgrowth occurred only if NGF was added to the culture medium. Conversely, if NGF antibodies were injected into the cytoplasm these antibodies did not interfere with fibre outgrowth produced by NGF in the culture medium (Fig. 2b). Preliminary results indicate that injected NGF is also unable to support the survival of neurones dissociated from 8-day old embryonic chick dorsal root ganglia or PC12 cells in serum-free medium (in serum-free medium PC12 cells need NGF for survival¹¹), whereas injected anti-NGF antibodies do not antagonize the ability of NGF in the culture medium to support the survival of these cells.

We conclude that even if an undetectably small proportion of NGF leaves the membrane-bound compartments in which it is always localized after receptor-mediated internalization, it would be unable to initiate the characteristic cellular responses to NGF-fibre outgrowth and cell survival. Thus the enhancement of polymerization of microtubules and microfilaments, demonstrated in the test tube in response to high concentrations of NGF¹²⁻¹⁴, must have no physiological significance in the context of fibre outgrowth, and the binding of NGF to chromatin isolated from chick dorsal root ganglia¹⁵ cannot be responsible for fibre outgrowth or for survival. The biological effects of NGF must be mediated by an interaction with specific cell-surface receptors and the generation of (a) second messenger(s) rather than by a direct action of intact NGF in the free cytoplasm or the nucleus.

The nature of the second messenger and its site of production (cell surface or cell interior after internalization) are unknown. In adrenergic neurones NGF binds to receptors or nerve terminals and is subsequently transported within vesicles and cisternae to the cell body. There, fusion of the transport vesicles with other intracellular membrane systems and eventually with lysosomes can be observed^{2,5}. It cannot be excluded that a cleavage product of NGF is transferred into the cytoplasm as is the case for diphtheria toxin where a fragment of the original toxin molecule is transferred across the membrane into the free cytoplasm where it exerts its blocking action on ribosomal protein synthesis 16,17. It is also possible that the NGF-receptor complex is transferred into the cytoplasm, although no such mechanism involving a polypeptide hormone and its receptor has previously been described. If NGF should be cleaved or bound to its receptor before transfer into the cytoplasm, then our results show that the resulting secondary product(s) of NGF or NGF-receptor complexes are not recognized by the antibodies raised against native NGF.

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- Schwab, M. & Thoenen, H. Brain Res. 122, 459-474 (1977).
- Schwab, M. Brain Res. 130, 190-196 (1977). Levi, A., Schechter, Y., Neufeld, E. J. & Schlessinger, J. Proc. natn. Acad. Sci. U.S.A. 77, 3469-3473 (1980)
- Claude, Ph., Dunis, D. A. & Hawrot, E. J. Cell Biol. 83, 632 (1979)
- Schwab, M., Suda, K. & Thoenen, H. J. Cell Biol. 72, 798-810 (1979). Marchisio, P. C., Naldini, L. & Calissano, P. Proc. natn. Acad. Sci. U.S.A. 77, 1656-1660
- Thoenen, H. & Barde, Y.-A. Physiol. Rev. 60, 1284-1335 (1980).
- Thoenen, H., Schäfer, Th., Heumann, R. & Schwab, M. in Hormones and Cell Regulation Vol. 5 (eds Dumont, J. E. & Nunez, J.) 15-34 (Elsevier, Amsterdam, 1981).
- Yamaizumi, M., Uchida, T., Mekada, E. & Okada, Y. Cell 18, 1009–1014 (1979).
 Schlegel, R. A. & Mercer, W. E. in Introduction of Macromolecules into Viable Mammalian
- Cells (eds Baserga, R., Croce, C. & Rovera, G.) 145-155 (Liss, New York, 1980). 11. Greene, L. A. J. Cell Biol. 78, 747-755 (1978).

- Calissano, P. & Cozzari, C. Proc. natn. Acad. Sci. U.S.A. 71, 2131–2135 (1974).
 Levi, A., Cimino, M., Mercanti, D., Chen, J. S. & Calissano, P. Biochim. biophys. Acta 399,
- 14. Calissano, P., Monaco, G., Castellani, L., Mercanti, D. & Levi, A. Proc. natn. Acad. Sci. U.S.A. 75, 2210-2214 (1978).

 Andres, R. Y., Jeng, J. & Bradshaw, R. A. Proc. natn. Acad. Sci. U.S.A. 74, 2785-2789
- 16. Pappenheimer, A. M. Jr & Gill, D. M. Science 182, 353-358 (1973)
- Collier, J. R. Bact. Rev. 39, 54-85 (1975).

 Mazurkiewicz, J. E. & Nakane, P. K. J. Histochem, Cytochem, 20, 969-974 (1972).
- Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, E. M. J. biol. Chem. 254, 5972-5982 (1979).
- 20. Valet, G., Hofmann, H. & Ruhenstroth-Bauer, G. J. Histochem. Cytochem. 24, 231-246
- 21. Stockel, K., Gagnon, C., Guroff, G. & Thoenen, H. J. Neurochem. 26, 1207-1211 (1976).

Reversal of transplantation immunity by liver grafting

Naoshi Kamada, H.ff.S. Davies & Bruce Roser

Department of Immunology, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK

The transplantation of organs between individuals of a species normally has two main consequences: (1) the tissue is rejected unless the individuals are matched for identity of transplantation antigens, especially those encoded by the major histocompatibility complex (MHC), and (2) the recipient is sensitized to the transplantation antigens of the donor so that second-set grafts are rejected in a more violent manner1. These rules of transplantation are not obeyed by liver grafts. Orthotopic transplants of liver are never rejected by many strains of rat even though the MHC barrier is crossed². We have recently shown that instead of sensitizing the recipient, these enduring liver grafts induce a state of donor-specific unresponsiveness in which subsequent grafts of other organs, such as skin, are accepted permanently. It is possible that many strains simply cannot mount a sufficiently vigorous destructive immune response to outstrip the liver's great capacity to repair immune damage and that the systemic unresponsiveness observed is due to diversion of alloreactive lymphocytes with donor specificity into the liver allograft. Manipulations which increase the vigour of the immune response might therefore tip the balance in favour of liver graft rejection and away from induction of unresponsiveness. We have previously measured the degree to which the immune response can be increased by previous exposure to MHC antigens in a quantitative adoptive transfer system3. In the strain combination DA (MHC haplotype RT-1*) grafted to PVG (RT-1°), the lymphocytes of immunized animals are 1,000 times more potent than those of non-immune animals in procuring graft destruction when transferred to irradiated hosts. Unexpectedly, we have now found that liver grafts transplanted into previously immunized rats not only fail to reject but convert the state of heightened reactivity to donor grafts characteristic of immune recipients into one of non-reactivity characteristic of tolerant animals.

Table 1 Induction of tolerance to MHC antigens by liver transplantation is systemic

Days after DA	Days of skin graft survival (no. of rats)						
liver grafts	DA	AO					
0	10, 12, 13, 14(2), 18	8, 9, 12					
5	13, 14, 15, 22, 100 (2)	11, 12 (2)					
15	23* > 100(5)	13 (2), 14					
45	65*, 88*, >100 (4)	13 (2), 15					

PVG rats received an orthotopic transplant of liver from DA donors on day 0 and then an orthotopic skin graft from both DA and AO (third party) donors on the day indicated. By day 15 some recipients were systemically tolerant of the DA alloantigens, as indicated by permanent survival of the indicator DA skin graft in perfect cosmetic condition. By day 45 all animals were tolerant. The specificity of this tolerance was absolute, as indicated by rejection of all third party (AO, RT1^u) skin grafts.

* These animals died with a perfect DA skin graft in place.

The technique for orthotopic liver transplantation used in these experiments is reported in detail elsewhere⁴. Surgical technique is not a significant variable in this series, as indicated by a 95% permanent survival rate of the past 72 DA to PVG liver transplants performed here (Fig. 1). Although DA livers transplanted to PVG recipients are never rejected, skin grafts are rejected in 8 days, heart grafts in 7-8 days and kidney grafts in 9-10 days. The reason for the prolonged survival of the liver in the rat and in other species⁵ is still being studied in this and in other laboratories, but it is not due to a failure of the recipients to mount an immune response against liver tissue. Indeed, the degree of early mononuclear cell infiltration, blast transformation and liver cell necrosis, as assessed histologically, is almost as great in PVG recipients which do not reject as in BN recipients which do (N.K., Wight and B.R., in preparation). The response in PVG rats is ephemeral, only minimal histological changes being detectable several months after grafting, whereas the response in BN rats is progressive.

While grafting with skin, heart or kidney provokes vigorous immunity, grafting with liver rapidly induces a state of systemic unresponsiveness (Table 1). Thus, within 15 days of liver transplantation, recipient rats become specifically unresponsive to subsequent skin grafts from the DA strain while retaining the capacity to reject third party (AO, RT1") strain grafts. This state of unresponsiveness is associated with the presence of specific blocking material in the circulation and deletion of the clones of cells required for DA graft rejection from the peripheral lymphocyte pool, but there is no evidence of cell-mediated suppression (data not shown).

Because we had previously established that immunization to antigens of the MHC in this strain combination led to a large

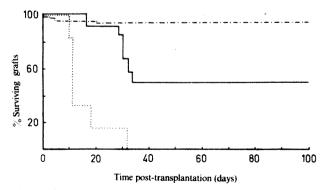


Fig. 1 Survival of liver grafted rats. Apart from three early technical failures⁵, 69 normal PVG rats grafted with DA livers (---) survived indefinitely while BN rats grafted with DA livers (...) all died within 32 days. When PVG rats were sensitized by rejection of DA skin grafts 28 days before liver transplantation (---), 50% behaved as high responder animals, rejecting their liver grafts within 34 days, while 50% failed to reject and survived indefinitely.

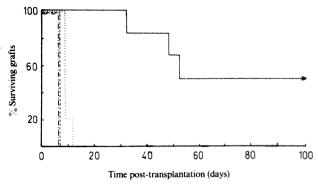


Fig. 2 Immune status of liver grafted rats. PVG rats, immunized to DA antigens, which had survived subsequent grafting with DA livers, were challenged with another DA skin graft 28 days after liver transplantation (——). All rats showed very prolonged survival of the second-set DA skin graft and 50% of animals retained the skin graft indefinitely. Where no liver graft intervened between the two DA skin grafts (-···) or where the liver graft was syngeneic with the recipient PVG (----), the second skin graft was rapidly rejected in second-set tempo. Substitution of third party (AO) antigens at the immunizing and challenge stages (····) showed that the DA liver graft was not nonspecifically immunosuppressive: the second AO skin grafts, placed after DA liver transplantation on PVG rats immunized by prior skin grafts, were all promptly rejected.

increase in the efficiency of heart graft rejection as measured in a quantitative adoptive transfer assay3, we felt confident that immunization would convert the non-rejector PVG to a rejector strain of equivalent potency to BN. Surprisingly, 50% of 12 previously immunized animals survived indefinitely after subsequent DA liver transplantation (Fig. 1). In those six animals which died after liver grafting, histological examination revealed a severe destructive immune response characterized by heavy mononuclear cell infiltrates and widespread hepatocellular necrosis. In the six animals which survived long-term, the livers showed minimal signs of tissue destruction and no significant cellular infiltrate (a picture similar to that seen longterm in normal rats in receipt of DA liver grafts). It was therefore of interest to determine whether, like normal nonimmune graft recipients, the immune animals had also become systemically tolerant to DA MHC antigens during the recovery phase. This proved to be the case. Subsequent skin grafts applied to such animals failed to reject in 50% of cases, for over 100 days (Fig. 2). The antigen specificity of this phenomenon was shown in two ways. One group of immunized animals received a syngeneic (PVG) liver graft instead of a DA graft. This has no suppressive effect on the second-set rejection of DA skin (Fig. 2). Another group of PVG rats was immunized against third party AO (RT1^u) antigens instead of DA (RT1^a) antigens before receiving a DA liver graft. In this group the fully allogeneic DA liver graft did not induce cross-tolerance to AO antigens, as indicated by rejection of all subsequent AO indicator skin grafts.

Using easily enhanced rat strains, Bowen et al. demonstrated a slight weakening of immunity against F, hybrid kidney grafts by temporary parabiosis of hyperimmune animals to animals bearing enhanced kidney grafts. Kawamura et al.8, again using F₁ hybrid kidney grafts, demonstrated that second-set grafts of identical type often have prolonged survival especially if the recipient animal was unable to reject the first graft promptly. However, they could only demonstrate this phenomenon where exposure to the first graft had not led to detectable sensitization of the recipient9. We believe this to be the first observation of specific conversion of a state of transplantation immunity against a full haplotype MHC difference to a state of tolerance. It had previously proved impossible both clinically and experimentally to erase memory of previous exposure to antigens of the MHC even with such potent immunosuppressive agents as cyclosporin A¹⁰. The facility with which liver transplantation achieves this result in a high proportion of animals undermines

the simple quantitative explanation of liver graft survival outlined in the introduction and obviously deserves further study. Of particular interest will be the analysis of the efficacy of liver tissue in the form of an intact organ or as dispersed cells in modifying the alloreactive repertoire of normal and immune animals.

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- Billingham, R. E., Brent, L. & Medawar, P. B. Proc. R. Soc. B143, 58-60 (1954).
- Kamada, N., Brons, G. & Davies, H.ff.S. Transplantation 29, 429-431 (1980).
 Hall, B. M., Dorsch, S. E. & Roser, B. Transplantation 26, 357-359 (1978); J. exp. Med. 148, 878-890, 891-903 (1978).
- Kamada, N. & Calne, R. Y. Transplantation 28, 47-50 (1979).
- Calne, R. Y. et al. Nature 233, 472-476 (1969).
- Kamada, N., Davies, H.ff.S. & Roser, B. Transplant. Proc. 13, 837-841 (1981). Bowen, J. E. et al. Transplantation 18, 322-327 (1974).
- Kawamura, H., Mullen, Y. & Hildermann, W. H. Transplantation 30, 302-307 (1980). Kawamura, H. et al. Transplant. Proc. 13, 114-116 (1981).
- Homan, W. P. et al. Transplantation 29, 361-366 (1980).

Immune (γ) interferon produced by a human T-lymphoblast cell line

Ilana Nathan, Jerome E. Groopman, Shirley G. Quan, Noelle Bersch & David W. Golde*

Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024, USA

(IFNs) are glycoproteins with antiviral. Interferons immunomodulatory and antiproliferative properties. They are classified on the basis of their acid stability, antigenic properties, stimuli for production and cellular origin. IFN- α and IFN- β are acid-stable and derived from leukocytes and fibroblasts. IFN-y (immune, type II) is acid-labile and generally derived from T lymphocytes. Elaboration of IFN-y occurs either after sensitized T lymphocytes are exposed to specific antigen or on induction of T lymphocytes with mitogens^{1,2}. IFN- α and IFN- β have been extensively characterized and much is known about their biochemical properties and gene structure³⁻¹¹. In contrast, there is relatively little information available regarding the physical and biological properties of human IFN-y (refs 1, 2, 12-17). Particular interest in IFN-y derives from in vitro and in vivo studies which indicate that IFN-y has greater antiproliferative effects on neoplastic cells than IFN- α and IFN- β (refs 18-21). We report here the production and characteristics of IFN-γ elaborated by a unique human T-lymphoblast cell line.

The paucity of data regarding IFN-y is largely related to difficulties in obtaining large quantities of purified material. In our laboratory, a human T-lymphocyte cell line (Mo) has been derived from the spleen of a patient with a T-cell variant of hairy-cell leukaemia that produces IFN-y and other lymphokines. The Mo cell line has been described in detail elsewhere 22-26.

The Mo cells constitutively produce low levels of interferon, both in the presence and absence of serum, and its production is not enhanced by exposing the cells to Sendai virus or polyinosinic polycytidylic acid. We tried to induce IFN-γ production using T-cell mitogens as well as chemical agents (Table 1). Induction with phytohaemagglutinin (PHA) markedly increased the production of IFN-y. As the ranges in Table 1 demonstrate, there was considerable variability in the titres elaborated in each induction regimen. Interferon production by Mo cells in culture was maximal after 3 to 4 days of incubation with PHA, thus the conditioned media used in all experiments were collected after 4 days of induction.

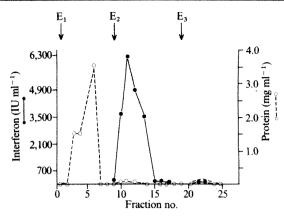


Fig. 1 Chromatography of Mo IFN-y on Con A-Sepharose. Conditioned medium containing 2% FCS induced with 3% PHA. 1 μM dexamethasone and 0.5 mM sodium butyrate was dialysed for 16 h at 4 °C against 100 volumes of PBS, pH 7.4 (E₁). The Con A-Sepharose (Pharmacia) column (0.9×5 cm) was equilibrated with PBS, and 5 ml of dialysed conditioned medium applied. The column was washed with PBS then developed with 0.2 Mα-Dmethyl mannoside in PBS (E₂) at a flow rate of 2 ml h⁻¹. The column was developed with 0.2 M \alpha-D-methyl mannoside and 20% ethylene glycol in PBS (E₃). Protein was determined using a dye binding procedure (Bio-Rad). Solid line represents interferon activity and broken line, protein concentration.

It has been shown 15,17,27,28 that chemical agents known to affect cell differentiation in vitro may enhance IFN production. The phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) proved to be a potent enhancing agent of IFN-y production by the Mo cells. Levels of up to 43,400 international units (IU) per ml per 10^6 cells were achieved in the presence of 2%fetal calf serum (FCS), 3% PHA and TPA (10 ng ml⁻¹). However in serum free conditions, PHA induction alone gave maximal interferon yield with specific activities of $\sim 2 \times 10^4$ IU per mg protein. Concanavalin A (Con A; 20-40 µg ml⁻¹) also induced IFN-y production but its effect was substantially less than that observed for PHA (data not shown).

Mo interferon proved to be entirely of the γ type by the criteria of acid lability, stimulus of induction, kinetics of production and failure of neutralization by antisera to leukocyte interferon (data not shown). The antiviral activity in Mo-conditioned medium was totally inactivated on reduction to pH 2 with 0.1 M HCl or 0.5 M citrate buffer. Also, Mo IFN-y is heatlabile, losing >98% activity when incubated at 56 °C for 1 h or on boiling for 1 min (Table 2). This observation is consistent with previous reports which indicate that mitogen-induced IFN-y is heat-labile 12,14. We examined the effect of various denaturing agents on the IFN-y and found that it was almost completely inactivated with 8 M urea, 6 M guanidine hydrochloride, 0.1% SDS and 0.01% zwitterionic detergent. However, note that there was retention of activity after incubation with 10 mM β-mercaptoethanol (Table 2), suggesting that, while disulphide linkages may be present in the molecule, they are not critical for antiviral activity. The activity of Mo IFN-y is completely destroyed by pronase (Table 2).

The chromatographic behaviour of Mo IFN-y on Con A-Sepharose is shown in Fig. 1. The bulk of the protein applied to the column passed through unretarded and this material contained only negligible amounts of interferon activity. The bound interferon was recovered by elution with 0.2 M α-Dmethyl mannoside in phosphate-buffered saline (PBS; pH 7.4) giving a purification of ~ 20 -fold to a specific activity of $6-8 \times$ 10⁴ IU per mg protein; 96% of the interferon activity loaded was recovered in the α -D-methyl mannoside eluate. Only 0.5% of the applied activity was recovered after elution with 20% ethylene glycol, 0.2 M α -D-methyl mannoside in PBS. These results suggest that almost all the Mo IFN- γ is glycosylated. It has previously been reported²⁹ that human IFN-y induced in

^{*} To whom reprint requests should be addressed

Table 1 Induction of IFN-y from Mo cells

	IFN titre (IV per ml per 10 ⁶ cells)		
	Mean	Range	
Serum-free medium			
Uninduced	35	5-50	
PHA	1,900	200-3,200	
$PHA + TPA (10 \text{ ng ml}^{-1})$	1,100	800-1,200	
Medium + serum (2% FCS)			
Uninduced	66	50-75	
PHA	5,700	900-9,400	
PHA + dexamethasone $(1 \mu M)$	4,350	2,700-6,000	
PHA + butyrate (0.5 mM)	4,100	900-7,900	
PHA + butyrate (0.5 mM) +			
dexamethasone (1 µM)	5,150	3,500-7,900	
$PHA + TPA (10 \text{ ng ml}^{-1})$	16,900	7,871–43,400	

The Mo cells were cultured in α medium (Flow) containing 20% FCS (screened lot). Interferon production, unless otherwise stated, was achieved by washing the cells three times with α medium and then culturing 10^6 cells ml⁻¹ for 4 days in α medium alone (serum-free) or in α medium containing 2% FCS. Inducers used were: phytohaemagglutinin (PHA; HA 15 reagent grade, Wellcome), sodium butyrate (Pfaltz & Bauer), dexamethasone (Sigma) and TPA (PL Biochemicals); these were added to the culture at the concentrations shown. PHA was present at 2% and 3% in serum-free and serum-containing cultures, respectively. The antiviral activity of interferon was determined by quantitation of the cytopathic effect of murine encephalomy-ocarditis virus on the foreskin fibroblast cell line CCL54 trisomic for chromosome 21 (ref. 30). All interferon units are expressed with reference to the NIH human leukocyte interferon standard (G-023-901-527). Titres are the results of four separate experiments.

leukocytes by PHA shows chromatographic heterogeneity on Con A-Sepharose, presumably resulting from varying degrees of glycosylation. In contrast, the Mo IFN-γ appears more homogeneous with respect to its glycosylation.

The hydrophobic nature of human IFN- γ has been described in previous studies¹²⁻¹⁴. Crude Mo-conditioned medium, induced in the presence of 2% FCS with 3% PHA, 0.5 mM butyrate and 1 μ M dexamethasone, was applied to a controlled-pore glass (CPG) 350-B mesh size 120/200 column (Electronucleonics). All the interferon activity was absorbed onto the column while 98% of the protein was voided. Elution with 1 M NaCl, 20% ethylene glycol in 0.2 M phosphate buffer (pH 7.4) recovered 2% of the loaded protein and almost all the interferon activity, giving a \sim 50-fold purification and a specific activity of \sim 10⁵ IU per mg protein.

Reported molecular weights (MWs) for IFN-γ have ranged from 20,000 to 80,000— these discrepancies may be related to molecular heterogeneity and binding to other proteins. Gel-

Table 2 Characterization of Mo IFN-y

Treatment	Residual interferon activity (% of control)
Urea (8 M)	4
Guanidine HCl (6 M)	0.7
β-Mercaptoethanol (10 mM)	100
SDS (0.1%)	0
Zwitterionic detergent (0.01%)	9
56 °C (1 h)	1.6
100 °C (1 min)	1.8
Pronase (0.1 mg ml ⁻¹)	< 0.1
pH 2	0

Conditioned medium containing 2% FCS obtained from Mo cells cultured with 3% PHA and 10 ng ml $^{-1}$ TPA was used. All treatments except heat and pronase inactivation were performed at 37 °C for 1 h. The material was then dialysed against 100 volumes of PBS, with two exchanges. Pronase (Sigma) was incubated with serum-free conditioned medium from cells induced with 2% PHA alone at 37 °C for 1 h. Control incubations with 2% FCS contained 2,000 IU ml $^{-1}$ interferon activity whereas the serum-free material had 3,200 IU ml $^{-1}$.

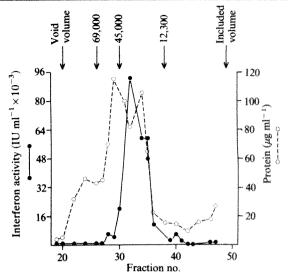


Fig. 2 Gel filtration of Mo IFN-γ. Serum-containing conditioned medium from Mo cells induced with 3% PHA and TPA (10 ng ml⁻¹) was partially purified on CPG beads and then applied to an Ultrogel AcA 44 (LKB) column (0.9×40 cm) equilibrated with 20% ethylene glycol, 1 M NaCl in 0.02 M phosphate buffer, pH 7.4. Column was developed with the same buffer at a flow rate of 2.2 ml h⁻¹. Fractions of 0.75 ml were collected. Molecular weight standards were: blue dextran (void volume), bovine serum albumin (MW 69,000), ovalbumin (MW 45,000), cytochrome c (MW 12,300) and 4-methylumbelliferone (included volume). Solid line represents interferon activity, and broken line, protein concentration.

filtration chromatography of serum-containing conditioned medium was performed using an Ultrogel AcA 44 column. An initial peak of interferon was obtained with the PBS elution at \sim 70,000 MW. The specific activity of this material was ~10⁴ IU per mg protein. Two smaller peaks of interferon activity were obtained with 1 M NaCl elution and then with 1 M NaCl, 20% ethylene glycol. The secondary peaks may represent species of interferon exhibiting ionic or hydrophobic interaction with the Ultrogel beads. To examine further the size characteristics of the Mo IFN-y, we applied material partially purified on CPG beads to a similar Ultrogel AcA 44 column (Fig. 2). To avoid ionic or hydrophobic interactions and focus solely on its gel-filtration properties, the column was developed using 1 M NaCl, 20% ethylene glycol in 0.02 M phosphate buffer (pH 7.4). A single peak of activity was obtained with an apparent molecular weight of ~40,000 and a specific activity of 1.2×10^6 IU per mg protein. It is possible that the high-molecular weight species observed when crude conditioned medium was applied directly to the gel-filtration column represented binding of Mo IFN-y to carrier proteins present in serum.

Mo IFN- γ is a hydrophobic glycoprotein which is elaborated in high titre on induction with T-cell mitogens and various chemical agents. It is clear from its physicochemical characteristics that the Mo IFN- γ is distinct from the erythroid-potentiating activity, colony-stimulating activity and neutrophil migration-inhibitory factor also produced by this cell line $^{22-26}$. As the Mo cell line is a homogeneous population of T lymphoblasts, the results suggest that IFN- γ may be induced by lectins without the need for cooperation by other cell types².

The Mo cell line provides a unique source of human IFN- γ , and should allow the large-scale production of IFN- γ for purification and clinical trials. It may also be of use in isolating the human gene(s) for IFN- γ .

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- Stewart II, W. E. The Interferon System (Springer, Vienna, 1979).
- Epstein, L. B. in Biology of the Lymphokines (eds Cohen, S. & Oppenheim, J. J.) 443-514 (Academic, New York, 1979.).
- Rubinstein, M. et al. Proc. natn. Acad. Sci. U.S.A. 76, 640-644 (1979)
 Zoon, K. C. et al. Science 207, 527-528 (1980).
- Knight, E. Jr, Hunkapiller, M. W., Korant, B. D., Hardy, R. W. F. & Hood, L. E. Science 207, 525-526 (1980).
- Dervnek, R. et al. Nature 285, 542-546 (1980).
- Betyinki, K. et al. Nucleic Acids Res. 8, 1913–1931 (1980).
 Nagata, S., Mantei, N. & Weissmann, C. Nature 287, 401–408 (1980).
 Goeddel, D. V. et al. Nature 290, 20–26 (1981).

- Taniguchi, T. et al. Nature 285, 547-550 (1980).
 Allen, G. & Fantes, K. H. Nature 287, 408-411 (1980).
- Langford, M. P., Georgiades, J. A., Stanton, G. J., Dianzani, F. & Johnson, H. M. Infect. Immunity 26, 36-41 (1979).
- 13. Wiranowska-Stewart, M., Lin, L. S., Braude, I. A. & Stewart, W. E. II Molec. Immun. 17, 625-633 (1980).
- de Ley, M. et al. Eur. J. Immun. 10, 877-883 (1980).

 Vilček, J., Sulea, I. T., Volvovitz, F. & Yip, Y. K. in Biochemical Characterization of Lymphokines (ed. de Weck, A. L.) 323-329 (Academic, New York, 1980).

 Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. Nature 291, 79-81 (1981).
- Yip, Y. K., Pang, R. H. L., Urban, C. & Vilček, J. Proc. natn. Acad. Sci. U.S.A. 78, 1601–1605 (1981).
- Bloom, B. R. Nature 284, 593-596 (1980).
 Krim, M. Blood 55, 875-884 (1980).

- Rubin, B. Y. & Gupta, S. L. Proc. natn. Acad. Sci. U.S.A. 77, 5928-5932 (1980). Glasgow, L. A., Crane, J. L. Jr & Kern, E. R. J. natn. Cancer Inst. 60, 659-666 (1978).
- Golde, D. W., Quan, S. G. & Cline, M. J. Blood 52, 1068-1072 (1978).
 Saxon, A., Stevens, R. H. & Golde, D. W. Ann. intern. Med. 88, 323-326 (1978).
- 24. Golde, D. W., Bersch, N., Quan, S. G. & Lusis, A. J. Proc. natn. Acad Sci. U.S.A. 77,
- Collet, D. W., Bersch, N., Quan, S. O. & Lusis, A. J. Proc. nam. Acad Sci. C. S.A. 11, 593-596 (1980).
 Lusis, A. J., Quon, D. H. & Golde, D. W. Blood 57, 13-21 (1981).
 Weisbart, R. H., Golde, D. W., Spolter, L., Eggena, P. & Rinderknecht, H. Clin. Immun.
- Immunopath. 14, 441-448 (1979).

 Adolf, G. R. & Swetly, P. in In Vivo and In Vitro Erythropoiesis: The Friend System (ed.
- Adolf, G. R. & Swetty, P. in In Vivo and In Vitro Erythropoiesis: The Friend System Rossi, G. B.) 577-584 (Elsevier, Amsterdam, 1980).

 Adolf, G. R. & Swetty P. Nature 282, 736-738 (1979).

 Mizrahi, A. et al. J. biol. Chem. 253, 7612-7615 (1978).

 Bryson, Y. J. & Kronenberg, L. H. Antimicrob. Ag. Chemother. 11, 299-306 (1977).

Establishment of a human T-cell hybrid line with suppressive activity

Catherine Grillot-Courvalin & Jean-Claude Brouet

Laboratory of Immunochemistry and Immunopathology, INSERM U 108, Research Institute of Blood Diseases and Laboratory of Oncology and Immunohematology of CNRS, Hôpital Saint-Louis, 75475 Paris Cedex 10, France

Roland Berger & Alain Bernheim

Laboratory of Cytogenetics, Hôpital Saint-Louis, 75475 Paris Cedex 10, France

The construction of murine B hybridomas producing homogeneous specific antibodies in almost unlimited quantities has led to major progress in immunology1. Recently, human B hybrid lines secreting antibody of defined specificities have also been established^{2,3}. Similarly, murine T hybrids have been obtained which may produce specific immunoregulatory factors such as antigen-specific suppressor factors 4-6, T-cell replacing factor7 or allogeneic effect factor8. The availability of functional T hybrids in man should allow a better understanding of human lymphocyte interaction and regulation. We describe here the fusion of a permanent human T-cell (KE37) with peripheral blood T lymphocytes to produce a human T hybridoma line which has a stable hypotetraploid karyotype and continuously produces a factor which suppresses pokeweed mitogen (PWM)induced B-cell differentiation.

The permanent T-cell line used for hybridization was KE37, which has been derived from a human acute lymphocytic leukaemia (provided by H. G. Kunkel and S. M. Fu). A 5bromodeoxyuridine (BUdR)-resistant variant of this line was selected after mutagenesis to obtain a line deficient in thymidine kinase and therefore sensitive to the hypoxanthine-aminopterine-thymidine (HAT) medium⁹. This variant line was then subcloned by limiting dilution. We fused one of these clones, D1R11, with peripheral blood lymphocytes (PBL) from a patient who presented with a variable agammaglobulinaemia and a moderate blood T-cell hyperlymphocytosis (5,000 mm⁻³); most of these T lymphocytes expressed Ia antigen and showed a

Table 1 Cell-surface phenotype of parental and hybrid cells (expressed as percentage of positive cells)

	Surface Ig	E rosettes	Т3	T4	Т5	Т6	Т8	T10	T11	Ia
DE	0	20	73	15	50	0	50	NT	NT	60
D1R11	0	10	0	0	0	100	10	50	4	0
$DE \times D1R11$	0	70	0	0	0	100	100	100	100	0

10×10⁶ cells from a subclone (D1R11) of the thymidine kinasedeficient variant of KE37 T-cell line were fused with 10×10^6 peripheral T cells from donor DE. For the fusion the parental cells were washed twice with Dulbecco's minimal essential medium (DMEM) without fetal calf serum (FCS), the last washing being performed in the presence of 5% dimethyl sulphoxide (DMSO). The pellet was then resuspended in 0.5 ml of 33% (w/v) polyethylene glycol (PEG) (molecular weight (MW) 4,000; Sigma), left for 3 h at 37 °C then centrifuged for 3 h at 200g. The supernatant was carefully discarded, the cells gently resuspended in DMEM plus 20% FCS then placed in 24-well Costar microlitre plates at a concentration of 1×10^6 cells per well, with a feeder of rat fibroblasts $(0.1 \times 10^6$ cells per well). Selective HAT medium (aminopterin, 4×10^{-7} M; thymidine, 1.6×10^{-5} M; hypoxanthine, $1.1 \times$ ⁴M) was added on days 2, 4, 8 and 10 after fusion, then replaced by HT medium for 6 days. Surface markers were studied on parental cells and on a given hybrid clone (DE×D1R11). Surface immunoglobulin (Ig) was detected with rhodamine-conjugated F(ab')₂ fragments of rabbit IgG antibody to human immunoglobulin. Monoclonal antibodies to Ia and T-cell antigens (designated T3, T4, T5, T6, T8 and T10) were detected by immunofluorescence using a second layer of fluoresceinconjugated IgG from a goat anti-mouse immunoglobulin serum (Nordic).

suppressor/cytotoxic antigenic phenotype (Table 1) as defined by the series of monoclonal OKT antibodies developed by Reinherz and Schlossman (reviewed in ref. 10; provided by G. Goldstein from Ortho Research Laboratory)

The fusion protocol used is described in Table 1 legend. In this, as well as in other experiments which used a variety of normal or stimulated T cells fused with either KE37 subclones or two other T-cell lines (JM and CEM), we found that the yield of hybrid clones was low; in this fusion 1 well out of 24 contained hybrids and their growth was delayed by 3-5 weeks. Subcloning the parental KE37 variant possibly improved the efficiency of fusion but it seems that human T-cell hybrids require special culture conditions during their initial growth phase. The hybrid nature of the clone (DE×D1R11), the functional properties of which are reported here, was documented by cytogenetic analysis and HLA phenotyping. The cells showed a stable hypotetraploid karyotype after 15 days to 6 months of continuous culture. Moreover, the presence of two morphologically distinct Y chromosomes, each from one parental cell, was shown using the quinacrine fluorescence technique (QFD) (Fig. 1). Although HLA typing of these hybrid cells was difficult because of their susceptibility to complement, they expressed definitely the HLA-A₂ antigen from DE, which was absent from the parental line, together with HLA antigens from KE37.

The hybrid line was studied for expression of several T-cell markers. The percentage of E rosette-forming cells was low in the D1R11 clone and varied from 25 to 75% in the hybrid line. However, when observed by immunofluorescence using a monoclonal antibody (OKT₁₁) to the E rosette-receptor, all DE×D1R11 cells were positive whereas <5% of cells of the parental cell line were stained. The staining by indirect immunofluorescence of DE×D1R11 cells using a rabbit antiserum to peripheral T cells¹¹ was much stronger than that of D1R11 cells. Additional studies were done using monoclonal antibodies to T cells (Table 2). Note that T6 (a common thymocyte antigen), which was absent on DE but present on D1R11 cells, was found on all hybrid cells. The T8 antigen, which was present on only a small fraction of D1R11 cells but found on most normal parental T cells, was expressed on all the hybrid cells. In contrast to T8, T5 antigen was absent from the hybrids although found together with T8 on DE lymphocytes (T5 and T8 define the suppressor/cytotoxic T-cell phenotype). T3 antigen was not detected on the hybrids although it is always associated with T5 and T8 on normal PBL as well as on the DE cells which were used in the fusion. Similarly, Ia antigens were not detectable by immunofluorescence on hybrids despite their expression on most DE T cells. Whether these findings reflect merely a lack of sensitivity of the assay, a chromosomal loss or possibly a complex regulation of T-cell antigen expression in hybrid cells is unknown.

Most interestingly, the hybrid line DE×D1R11 showed functional properties. As the normal cells used for fusion were obtained from a patient with agammaglobulinaemia and with a high number of circulating lymphocytes having the phenotype of activated suppressor/cytotoxic T cells (Ia, T5, T8 antigens) we determined whether the hybrids might produce a soluble factor with suppressive activity on polyclonal immunoglobulin production. As shown in Table 2, the supernatant of the hybrid line, and not that of D1R11, suppressed the generation of cytoplasmic immunoglobulin (cIg)-positive cells from normal PBL stimulated by PWM. The suppression ranged from 50 to 75% (mean 65.5%) at day 6 or 7. When tested with monospecific antisera to the various immunoglobulin chains, the isotypic distribution of cIg cells was not modified. The suppressive effect was observed when the supernatant was added at a final concentration of 10% and was maximum at a concentration of 20%. Note that this factor was produced without any triggering of the hybrid cells.

As the stimulation of murine T hybrids by lectins may enhance the production of soluble mediators, we also tested supernatants obtained in the presence of concanavalin A (10 µg ml⁻¹). In nine such experiments (data not shown) the suppression observed with DE×D1R11 supernatant compared with that of D1R11 supernatant ranged from 55 to 90% (mean 70%), which does not differ from the above results.

Experiments now in progress will attempt to characterize the biochemical nature of this factor as well as to determine its target cell. Until now, human suppressive factors have been shown to act either on T cells or at the B-cell level 12-14. No interferon was detectable in our supernatants and the hybrid cells did not carry Fc receptors for IgG, which are believed to modulate B-cell differentiation 15,16. Results of preliminary experiments indicate that the factor does not prevent PWM-induced mitogenesis; it has to be added in the first 36 h of culture to suppress PWMinduced B-cell differentiation, and it has no effect on B-cell

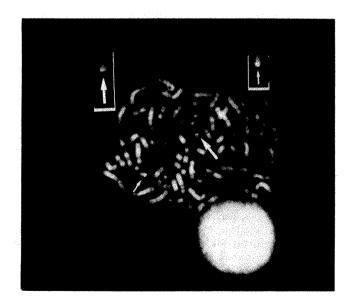


Fig. 1 Hypotetraploid (78 chromosomes) metaphase of DE× D1R11 hybrid clone. QFD staining shows two different Y chromosomes: one long chromosome from the T-cell line (main picture small arrow) and the second, small one originating from DE cells (main picture, large arrow). Inset right, DE×D1R11 long Y chromosome; inset left, DE × D1R11 small Y chromosome.

Table 2 Effects of supernatants from T-cell line (D1R11) or hybrid clone (DE × D1R11) on PWM-induced B-cell differentiation

	Exp	ot 1	Exp	nt 2	Exp	ot 3	Ex	pt 4
	a	b	а	b	a	b	a	b
PBL with PWM	16		13		6		9	
PBL+PWM+D1R11 supernatant	17	0	11.5	11	6	0	9.6	0
PBL+PWM+DE×D1R11 supernatant	7.5	52	4	71	1.5	75	3.3	64

Peripheral blood mononuclear cells from four normal donors were cultured for 7 days in the presence of 50 µg ml⁻¹ PWM with or without added supernatants (final dilution 20%) from the T-cell line D1R11 or DE×D1R11 hybrids. These supernatants were collected after 18 h culture of 2 × 106 cells ml-1 in DMEM plus 10% FCS. a, Percentage of cells with cytoplasmic immunoglobulin (cIg) estimated for at least 1,000 cells on day 7 of culture, detected by immunofluorescence of fixed cells treated with fluorescein-conjugated goat IgG antibody to human immunoglobulin. The number of cells and viability were similar in each case. b. Per cent suppression of the expected number of clg cells per

differentiation induced by Nocardia, a relatively T-independent polyclonal activator of B lymphocytes.

We have shown here that construction of hybrids with activated human T cells of homogeneous phenotype is feasible; availability of such functional T hybrids will be valuable in the production and characterization of human T-cell factors and possibly also T-cell receptors.

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- Köhler, G. & Milstein, C. Nature 256, 495 (1975). Olsson, L. & Kaplan, H. S. Proc. natn. Acad. Sci. U.S.A. 77, 5429 (1980)
- Croce, C. M., Linnenbach, A., Hall, W., Steplewski, Z. & Koprowski, H. Nature 288, 488
- Kontiainen S. et al. Nature 274, 477 (1978)
- Rollidanell, S. et al. Nature 274, 477 (1976).
 Taniguchi, M. & Miller, J. F. A. P. J. exp. Med. 148, 373 (1978).
 Taussig, M. J., Corvalan, J. R. F., Binns, R. M., Roser, B. & Holliman, A. Eur. J. Immun. 9,
- Takatsu, K., Tanaka, K., Tominaga, A., Kumahara, Y. & Hamaoka, T. J. Immun. 125, 2646
- (1980).
 Katz, D., Bechtold, T. & Altman, A. J. exp. Med. 152, 956 (1980).
 Littlefield, J. W. Science 145, 709 (1964).

- Littleheld, J. W. Science 145, 709 (1964).

 Reinherz, E. L. & Schlossman, S. F. Cell 19, 821 (1980).

 Brouet, J. C. & Chevalier, A. J. Immun. 122, 260 (1979).

 Fleisher, T. A., Greene, W. C., Blaese, R. M. & Waldmann, T. A. J. Immun. (in the press).

 Wolf, R. L., Whitsed, H., Rosen, F. S. & Merler, E. Cell. Immun. 36, 231 (1978).
- Saxon, A. & Stevens, R. H. Clin. Immun. Immunopath. 10, 427 (1978). Broder, B. R. & Merigan, T. C. J. Immun. 113, 1319 (1974).
- Lethibichthuy, Samarut, C., Brochier, J., Fridman, W. H. & Revillard, J. P. Eur. J. Immun 10, 894 (1980).

Switch region of immunoglobulin C_{μ} gene is composed of simple tandem repetitive sequences

Toshio Nikaido, Sumiko Nakai & Tasuku Honjo

Department of Genetics, Osaka University Medical School, Kita-ku, Osaka 530, Japan

Immunoglobulin heavy (H) chain genes comprise a family of variable region (V) genes and several constant region (C) genes which are classified, in mouse, into five major classes: μ , γ , α , δ and ϵ . During differentiation of a given B lymphocyte, a specific V_H gene is first expressed as a part of the μ-chain and at a later stage the expressed H chain switches the C region from μ to γ or α without alteration of the V_H region sequence 1-3. This phenomenon, called immunoglobulin class switch, involves a unique recombination event that takes place at the region 5' to each CH gene during B-lymphocyte differentiation 4-6. The

regions responsible for the class switch (or S-S) recombination are defined as switch (S) regions4. Recent structural analyses, which have revealed that S regions comprise tandem repetition of short unit sequences⁷⁻¹⁰, have allowed us to define the S region on a structural basis. The nucleotide sequences of S regions vary among different classes of CH gene, inevitably raising the possibility that the S_µ sequence contains separate subsets of sequences, each of which may pair with the S region of a different class. To test this possibility it is necessary to characterize the whole S, region. However, because the µ-gene clones isolated by other groups^{5,11,12} have lost a major portion (~3 kilobases [kb]) of the central core of the S_{μ} region, only a small part of the S_{μ} region sequence has been determined^{8,10,12,13}. We have now characterized the complete S_{μ} region by nucleotide sequence determination and restriction enzyme cleavage, and have found that it comprises simple tandem repetition of two kinds of 5-base pairs (bp) unit sequences, GAGCT and GGGGT. The nucleotide sequence of the S_{μ}^{-} region shares short common sequences with all the other S-region sequences. The results clearly exclude the abovementioned possibility and support the proposal^{7,9,10,13} that S-S recombination is mediated by repetitive homologous short sequences.

The 13-kb EcoRI fragment coding for the C region of the immunoglobulin μ -chain was previously cloned from newborn BALB/c mouse DNA using λ gtWES· λ B as vector (ref. 4). The recombinant phage and the insert were referred to as λ gtWES·IgH701 and IgH701, respectively. We have shown by Southern blot hybridization⁴ that IgH701 is indistinguishable from the germ-line C_{μ} -gene fragment. The 13-kb fragment was isolated by agarose gel electrophoresis and the fragments produced by HindIII digestion were subcloned into the HindIII

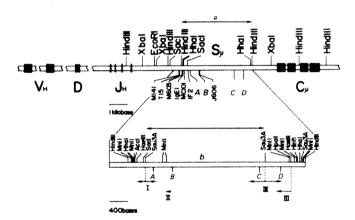


Fig. 1 Restriction endonuclease cleavage map of the S_u region and strategy for sequencing. The 3.7-kb HindIII fragment (Hind-A) which contained the S_{μ} region was subcloned in pBR322 and purified according to the method of Clewell and Helinski² Restriction cleavage sites were determined by combined cleavages of two or more enzymes. Restriction enzyme cleavage sites (EcoRI, HindIII, SacI, HhaI, XbaI and AccI) of IgH701 and the location of J_H segments were determined previously 4,12,14. Sources of restriction enzymes have also been described previously 26,27. The Hind A fragment was isolated by 5% polyacrylamide gel electrophoresis after digestion of IgH701 with HindIII, cleaved with other restriction enzymes and used as substrates of phosphorylation. The restriction sites used for sequencing are indicated in the enlarged map shown below. Ranges and directions of the sequences read are indicated by horizontal arrows I, II and III. Cloned DNA was displayed with the direction of transcription from left to right. M141(MOPC141)^{12,13}, T15(TEPC15)⁸, M603(MOPC603)⁸, IgE1²⁸, MC101^{8,9}, IF-2¹⁰ and J606 (T.N., unpublished data) indicate recombination sites of expressed CH genes in the respective myelomas and hybridoma. A, B, C and D indicate deletion sites in bacteria (see text). Closed boxes indicate coding regions. Horizontal bars a and b show Hind A and 2.6-kb Sau3A fragments, respectively.

Table 1 Nucleotide sequences of repeat units of S regions

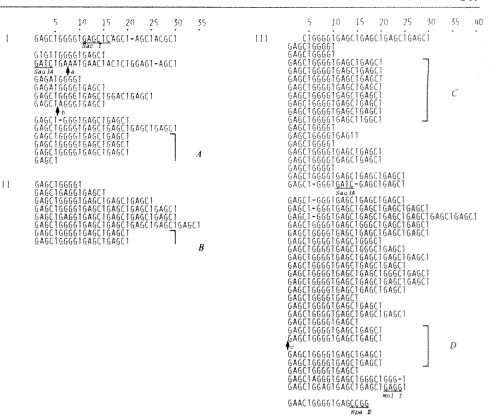
S region	Unit sequences
Sµ	GAGCTGAGCTGGGGTGAGCT
s ₁₁	${\tt GPPTCCAGGCTGAGCAGCTACAGGGGAGCTGGGGYAPP\underline{TGGG}APTPTPG}$
S _{72b}	$\tt GGGACCAG^T_ACCTAGCAGCTP\underline{TGGGGGAGCTGGGGA^A_TGG\underline{TPGG}APTPTGA$
S 7 3	$\texttt{PGNACC}_{T}^{A}\texttt{GPNTPAGCAPYYACAGGG}\underline{\texttt{GAGC}}_{A}^{T}\texttt{GGGG}\underline{\texttt{A}}^{A}\texttt{PGG}\underline{\texttt{TGGGA}}\texttt{GTATPP}$
Sα	ATGAGCTGGGATGAGCTGAGCTAGGCTGGAATAGGCTGGGCTGGGCTGGT
	GTGAGCTGGGTTAGGCTGAGCTGGGA
Common sequences	GAGCTG, TGGGG

Common sequences are underlined. Sources of unit sequences of the S_{μ} , S_{γ} and S_{α} are this paper, ref. 7 and refs 8 and 9, respectively. P, purine; Y, pyrimidine; N, any nucleotide.

site of pBR322 (see restriction map of Fig. 1)¹⁴. The subclone of the 3.7-kb *HindIII* fragment (*Hind A*), which contains the 5'-flanking region of the C_{μ} gene, was used for sequencing. The detailed restriction cleavage map of the *Hind A* fragment was constructed by digestion with combinations of various restriction endonucleases (see Fig. 1) and the nucleotide sequence was determined by the chemical modification method of Maxam and Gilbert¹⁵. The nucleotide sequence determined (Fig. 2) comprises a tandem array of two kinds of pentamer, GAGCT and GGGGT. The length of repeating units varies from 10 to 40 bp depending on the number of GAGCTs per GGGGT.

Because the 2.6-kb Sau 3A fragment of the Hind A fragment (see Fig. 1) does not contain adequate restriction cleavage sites (two MnII sites and abundant AluI, DdeI and HphI sites), we were unable to sequence a core portion of the fragment directly. However, the complete digestion of the Sau3A fragment with AluI, DdeI and HphI provided enough information for us to predict that the Sau3A fragment comprises tandem repetitive sequences similar to those shown in Fig. 2. When the 2.6-kb Sau3A fragment was digested with AluI (AGCT), a major band of 5 bp and a minor 10-bp band were produced in addition to faint bands of 15, 20 and 25 bp (Fig. 3, lanes a, d). The results indicate that the nucleotide sequence of the Sau3A fragment comprises abundant AGCT sequences, probably one every 5 or 10 bp. Obviously, AGCT appears in one of the basic pentamers (GAGCT) of the S_{μ} sequence shown in Fig. 2. Note that the 5-bp fragment was not detectable by ethidium bromide staining (Fig. 3, lane a) but it was clearly shown as the major band among ³²P-labelled fragments (lane d). When the 2.6-kb Sau3A fragment was digested with DdeI (CTNAG), a major band of 5 bp and a minor 15-bp band were produced in addition to faint bands of 20 and 25 bp (Fig. 3, lanes b, e). The results show the presence of a CTNAG sequence every 5 or 15 bp in the Sau3A fragment. CTNAG occurs at the junction of two basic pentamers, (GAGCT)-(GAGCT), in the S_u sequence. Digestion of the Sau3A fragment with HphI (GGTGA) yielded a major band of 20 bp and less intense bands of 15, 25, 30, 35, 40, 45, 50, 55 and 90 bp (Fig. 3, lanes c, f). As GGTGA appears at the junction of two kinds of basic pentamer, (GGGGT)-(GAGCT), in the S_{μ} sequence, the results indicate that GGGGT occurs once every three GAGCTs but that the frequency of GGGGT is rather irregular, ranging up to once every 17 GAGCTs. These results, taken together, indicate that the core portion of the 2.6-kb Sau3A fragment comprises two kinds of pentamer, GAGCT and GGGGT, and that its sequence is represented by $(GAGCT)_n(GGGGT)$. The number n ranges from 1 to 17, with the most frequent value being 3. Thus, the whole S_{μ} region, which lies 1.3-4.8 kb 5' to the C_{μ} gene, consists

Fig. 2 Nucleotide sequence of the S_u region. Nucleotide sequences are displayed from left to right with the direction of transcription of the structural sequence. The sequences are punctuated so that GGGGT is aligned at the same position in each repeating unit. DNA sequence was determined according to the method of Maxam and Gilbert¹⁵ with slight modifications²⁶. DNA fragments to be sequenced were digested with the enzymes indicated in Fig. 1, treated with bacterial alkaline phosphatase (Boehringer) and phosphorylated with T4 polynucleotide kinase (New England Biolabs) and $[\gamma^{-32}P]ATP$ (Amersham). ³²P-labelled DNA fragments were isolated by polyacrylamide gel electrophoresis and cleaved with second restriction enzymes. Fragments labelled only at one end were isolated by polyacrylamide gel electrophoresis and purified as described previously² The regions indicated I, II and III are shown in Fig. 1. Region II was determined using rearranged y3 gene of a γ 3-chain-producing myeloma J606 (unpublished data). Arrow a indicates the recombination site of IF-2¹⁰ while arrows b and c indicate the deletion site of MEP20312



of rather homogeneous repetitive sequences, with GAGCT and GGGGT as basic units.

Dunnick et al. ¹⁰ found repetition of $(GAGCT)_3(GGGGT)$ in the 5'-flanking region of the variant $\gamma 1$ gene of a $\gamma 1$ -chain-producing myeloma, IF2. As this $\gamma 1$ gene has lost a large segment (5.5 kb) of DNA, including the $C_H 1$ domain, a part of the 5'-flanking region of the $C_{\gamma 1}$ gene and portions of the S_μ and $S_{\gamma 1}$ regions, these workers were unable to locate the germ-line origin of $(GAGCT)_3(GGGGT)$ sequence. Nonetheless, they have discussed the possible role of these sequences in the class switch. Comparison of their sequences with ours indicates that the 5' deletion point of the IF2 $\gamma 1$ gene is located in the S_μ region at arrow α shown in Fig. 2 (also see Fig. 1).

In addition to three class switch recombination sites determined previously^{6,8,9,12,13}, we have recently determined two more such sites in the $\gamma 3$ -gene clone isolated from J606 myeloma and the ε-gene clone (IgE1) isolated from IgEproducing hybridoma²⁸ (see Fig. 1). The class switch sites of various classes are ordered as 5'-M141(γ 2b)-T15(α)-Ig ε -1(ε)-MC101(γ 1)-J606(γ 3)-3'. Although these recombination sites tend to cluster at the 5' side of the S_{μ} region, one cannot deduce any physiological significance from this fact because it is possible that a secondary deletion of the S region surrounding the recombination sites occurs during the propagation of myelomas and hybridomas. In fact, the α gene of MOPC603 has an internal deletion within the S_{μ} region⁸. The 5' recombination site in the S_{μ} region was located (see Fig. 1) but the 3'recombination site of the MOPC603 gene, which is the actual class switch recombination site, is not known. Comparison of the nucleotide sequences surrounding each class switch recombination site seems to indicate that H-chain switching is not highly specific to the nucleotides joined (T.N., in preparation). Again, this interpretation is limited if there has been secondary deletion around recombination sites.

We and others have observed that a major portion of the 3.7-kb *Hind* A fragment is deleted during its propagation in *Escherichia coli* ^{4,5,11,12}. Such deletion seems to depend on the

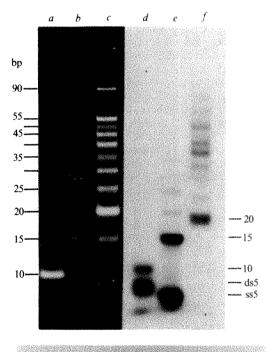


Fig. 3 Digestion of the S_{μ} region with restriction enzymes. $0.6~\mu g$ of 2.6-kb Sau3A fragment was cleaved with AluI~(a), DdeI~(b) or HphI~(c), electrophoresed in 10% polyacrylamide gel and stained with ethidium bromide. The sizes of fragments are shown in base pairs. $0.6~\mu g$ of the 2.6-kb Sau3A fragment was labelled with ^{32}P -dCTP by nick translation 29 and cleaved with AluI~(d), DdeI~(e) or HphI~(f). The sizes of the fragments were determined with pBR322 DNA digested with ThaI or AluI as standard. The 5-bp fragment of DdeI digests ran faster than that of AluI digests, probably because the former has only 2 bp and denatures easily. Nucleotide sequences of restriction sites of the enzymes used are shown at the bottom. ds, Double stranded; ss, single stranded.

Alui

GAGCTGGGGTGAGCTGAGC

Alui

Ddel Ddel Hphi Qdel

Alui

Alui

host strain; for example, deletion was very frequent in LE392 but rare in C600 regardless of whether they were recA or recA+. The deletion seems to take place at limited sites (indicated by A, B, C and D in Fig. 1). The cloning of the Hind A fragment in pBR322 with a host strain of LE392 has yielded 12 variant clones with inserts of different sizes. The deleted fragment was one of 2.8 kb between sites A and D. 2.3 kb between A and C, 2.4 kb between B and D or 1.9 kb between B and C. Embryonic C_μ-gene clone MEP203¹² deleted the 2.8-kb DNA fragment between arrows b and c in Fig. 2. The nucleotide sequences surrounding the deletion sites of A, B, C and D seem to correspond to the regions containing tandem repetition of regular 20-bp units (Fig. 2).

The S region was originally defined as the functional region responsible for the class switch recombination. We have shown that the structural basis of the S_v region is tandem repetition of homologous 49-bp units⁷. The S_{α} region contains tandem repetition of 80-bp units^{8,9}. It is now clear that tandem repetitive sequences of 20-30-bp units are present in the 5'-flanking region of the C, gene where the class switch recombination occurs. These nucleotide sequences contain abundant short sequences such as GAGCTG and TGGGG which are shared by repeating sequences in the S_{y3} , S_{y1} , S_{y2b} and S_{α} regions as shown in Table 1. It is known that the tandem repetitive sequence increases the chance of homologous recombination¹⁶. Accordingly, the recombination responsible for class switch from μ to α or from one γ to another γ , may be facilitated by such homology of repeating sequences occurring widely in the S region. Tens of short common sequences may be sufficient to make a transient join between two DNAs when they are brought close to each other. As the nucleotide sequences of the repeat units of the S regions are different, it is possible that specific enzymes catalyse the S-S recombination for different pairs of S regions. Both V-J (joining) and S-S recombinations are accompanied by the deletion of intervening DNA segments¹⁷⁻²⁴. We have recently proposed a model in which S-S recombination is mediated by an unequal crossing-over event between sister chromatids9

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- 1. Nossal, G. J. V., Warner, N. L. & Lewis, H. Cell. Immun. 2, 41-53 (1971).
- Pernis, B., Forni, L. & Amante, L. Ann. N.Y. Acad. Sci. 190, 420-431 (1971).
 Cooper, M. D., Kearney, J. F., Lydyard, P. M., Grossi, C. E. & Lanton, A. R. Cold Spring Harb. Symp. quant. Biol. 41, 139 (1976).
- 4. Kataoka, T., Kawakami, T., Takahashi, N. & Honjo, T. Proc. nam. Acad. Sci. U.S.A. 77,
- 5. Davis, M. M. et al. Nature 283, 733-739 (1980).
- Maki, R., Traunecker, A., Sakano, H., Roeder, W. & Tonegawa, S. Proc. natn. Acad. Sci. U.S.A. 77, 2138–2142 (1980).

- Kataoka, T., Miyata, T. & Honjo, T. Cell 23, 357-368 (1981).
 Davis, M. M., Kim, S. K. & Hood, L. Science 209, 1360-1365 (1980).
 Obata, M. et al. Proc. natn. Acad. Sci. U.S.A. 78, 2437-2441 (1981).
 Dunnick, W., Rabbitts, T. H. & Milstein, C. Nature 286, 669-675 (1980).
- 11. Marcu, K. B., Banerji, J., Penncavage, N. A., Lang, R. & Arnheim, M. Cell 22, 187-196 (1980)
- 12. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. Nature 286, 676-683
- Takahashi, N., Kataoka, T. & Honjo, T. Gene 11, 117-127 (1980)
- Kawakami, T., Takahashi, N. & Honjo, T. Nucleic Acids Res. 8, 3933–3945 (1980).
 Maxam, A. M. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 74, 560–564 (1977).
 Farabaugh, P. J. & Miller, J. H. J. molec. Biol. 126, 847–863 (1978).
- Honjo, T. & Kataoka, T. Proc. natn. Acad. Sci. U.S.A. 75, 2140-2144 (1978) Yaoita, Y. & Honjo, T. Nature 286, 850-853 (1980).

- Yaoita, Y. & Honjo, T. Biomed. Res. 1, 164-175 (1980). Coleclough, C., Cooper, C. & Perry, R. P. Proc. natn. Acad. Sci. U.S.A. 77, 1422-1426
- Cory, S., Jackson, J. & Adams, J. M. Nature 285, 450-456 (1980).
- Cory, S., Jackson, J. & Adains, J. M. Nature 263, 430-430 (1980).
 Rabbitts, T. H., Forster, A., Dunnick, W. & Bentley, D. L. Nature 283, 351-356 (1980).
 Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. Nature 280, 288-294 (1978).
 Seidman, J. G. et al. Proc. nam. Acad. Sci. U.S.A. 77, 6022-6026 (1980).
 Clewell, D. & Helinski, D. Proc. nam. Acad. Sci. U.S.A. 62, 1159-1166 (1969).

- Honjo, T. et al. Cell 18, 559-568 (1979).
- Kataoka, T., Yamawaki-Kataoka, Y., Yamagishi, H. & Honjo, T. Proc. natn. Acad. Sci U.S.A. 76, 4240-4244 (1979)
- Nishida, Y. et al. Proc. natn. Acad. Sci. U.S.A. 78, 1581-1585 (1981).
 Maniatis, T., Jeffrey, A. & Kleid. D. K. Proc natn. Acad. Sci. U.S.A. 72, 1184-1188 (1975).

Natural killer cells kill tumour cells at a given stage of differentiation

Magnus Gidlund, Anders Örn, Paul K. Pattengale*, Mats Jansson†, Hans Wigzell & Kenneth Nilsson‡

Department of Immunology, University of Uppsala Biomedical Centre, Box 582, S-751 23 Uppsala, Sweden *Department of Pathology, University of Southern California, School of Medicine, Los Angeles, California 90033, USA †Department of Microbiology, University of Uppsala Biomedical Centre, Box 581, S-751 23 Uppsala, Sweden ‡Wallenberg Laboratory, Box 562, S-751 22 Uppsala, Sweden

Natural killer (NK) cells have unique surface features and physiological characteristics and a selective ability to lyse some, but not other, target cells^{1,2}. However, the basis of this selectivity remains obscure at both effector and target cell levels. Proposed specific NK-cell target moieties3-5 include glycolipids, and glycoproteins unrelated to the major histocompatibility complex, while malignant and certain normal cells have been found to be susceptible to NK-cell-mediated cytolysis 6-8. There is good evidence that NK cells can inhibit the outgrowth of small numbers of transplanted tumour cells in vivo 9,10 and can restrict the establishment of secondary metastasis 11,12. It has thus been speculated that NK cells function as a primitive, thymusindependent immune system using phylogenetically preserved target structures to form a cell-mediated resistance barrier against the outgrowth of certain tumour cells1. The presence in the thymuses of neonatal mice and humans^{8,13} and in the marrow of human fetuses, of apparently normal cells which are quite sensitive to NK cells suggested that NK cells might have an increased ability to cause the lysis of cells at a particular stage of their differentiation. In agreement with this concept, embryonal carcinoma cells at various stages of differentiation display a strikingly different susceptibility to NK-cell-induced lysis14. Here only cell types representing early stages were sensitive to NK-cell-mediated cytolysis, whereas the more differentiated endodermal cell lines showed close to complete resistance. The above data would thus support the view that, in vivo, depending on the stage of differentiation, both normal and malignant cells are under surveillance by NK cells. Here we have considered the question of differentiation-related NK-cell susceptibility using defined cell lines known to undergo controlled differentiation in the presence of various agents. Three tumour cell-lines were investigated, and all demonstrated a striking positive correlation between a decrease in NK-cell susceptibility and the induction of differentiation by the various inducers. Our findings support the view that susceptibility to NK-cell mediated lysis may vary according to the stage of differentiation of the target cell.

Table 1 shows the three cell lines used in the present study and the impact of the inducing agents on NK-cell sensitivity in relation to various differentiation markers. The NK-cell sensitivity of K-562, the target cell line most commonly used to assess human NK-cell activity, decreased after exposure to sodium butyrate or haemin. This loss was well correlated in time and dose of the respective inducer with an increase in erythroid differentiation markers such as the expression of surface glycophorin A and haemoglobin synthesis 15. U-937 is a NK-cellsensitive human histiocytic lymphoma cell line16. The phenotype of this cell line suggests that it represents monocytic cells arrested at a differentiation stage close to the myelomonocytic stem cell^{17,18}. Treatment of U-937 cells with either 12-Otetradecanoyl phorbol acetate (TPA) or supernatant from 5-day mixed lymphocyte cultures (MLC) resulted within 2-3 days in the appearance of 30-60% of cells with a macrophage-like phenotype 18-20. Concomitantly, a drastically reduced NKsensitivity profile was noted. K-562 and U-937 show a similar loss of sensitivity regardless of the type of inducer used, demonstrating that 'unrelated' agents giving the same cellular

	•	•
Table 1	Effect of differentiation-inducing agents on NK-	-coll-susceptible target cells

Cell line	Inducer	Non-L	aduced	duced Induced		Phenotypic alterations during induced differentiation 18-21
_		: E	lector: tar	get cell ra	tlo ´	•
•		50:1	6.1	50:1	6:1	Benzidine staining reaction becomes positive
K-562 (human)	ВА	49 6	25 2	24 8	8.6	Plasmá membrane: specific changes in glycoprotein composition, increase in glycophorm A
K-562 (human)	Haemin	52 6	22.8	29 6	6.0	Function: globin production
				,		Morphology: acquisition of a macrophage-like morphology
U-937 (human)	MLC supernatant	38 0	-26.5	16.6	8.5	Plasma membrane, specific changes in surface glycoprotein composition increase in HLA and Ia-like antigen, β_1 -microglobulin and Fc recep-
U-937 (human)	TPA	21 4	7.3	29	0.0	tors Cytoplasmic enzymes: increase in nonspecific esterases Function increased especify for phagocytous and lyzoxyme secretion, induced activity as effector cell in ADCC
GM-86 (mouse)	НВМА	36.7	20.4	.18.1	2.4	Function: incresse in globin production

K-562, a human erythroleukaemia cell line¹², was cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and antibotics. U-937, a 'true' histocytic human cell line¹⁷, was cultured in F10 supplemented as above GM-86 (clone 745), a murine Friend virus-induced leukaemia originating from DBA/2I (ref. 21), was grown in Eagle's medium supplemented with 20% PCS and antibotics. Cells were tested for NK-cell sensitivity using Frcoll-Isopaque-isolated human peripheral blood cells when testing K-562 and U-937, and mouse (CBA/H) spleon cells when analysing GM-86. The sessy used was a 4-h ⁵¹Cr-release test performed in microtitre plates with a fixed number of target cells (10⁴ cells) and various effector cell concentrations as previously described¹⁰, using RPMI 1640 supplemented with 10% newborn calf serum and 10 mM HEPES. Calculations were done accordingly to the formula (c.p m in test—c.p m. in spontaneous control)/(c p m. m detergent—c.p.m. in spontaneous control). Before sessy, target cells were treated as follows. Each tumour cell population was split in half at day 0, one half being cultured in the presence of inducer, the other half serving as control in appropriate media. K-562 cells were treated with sodium buryrate at 1 mM for 3 days to give optimal differentiation²⁰. Haerium was added at 10⁻⁴ M and cells were cultured for 5 days. U-937 cells were cultured in medium supplemented with 20% day-6 supermatant prepared as described elsewhere ^{18,20}. Control cells were cultured in 20% supermatant derived from responder cells alone. Cells were seeded for optimal grown and differentiation at an initial concentration of 3×10⁵ cells ml⁻¹ and further cultured for 3 days²⁰. For TPA treatment cells were seeded at 3×10⁵ cells ml⁻¹ and grown in the presence of 1.6×10⁻⁷ M of TPA for 3 days as described elsewhere ¹⁸. Mouse GM-86 cells were grown in the presence of 4 mM HMBA for 5 days²¹. After induction the cells were washed free of inducer and labelled with ³¹Cr as described elsewhere

alterations also induce a similar increase in NK-cell resistance. Finally, following treatment with hexametabisacetamide (HMBA), GM-86, a mouse Friend virus-transformed erythroid stem cell line²¹, acquired the capacity for globin production. As with the differentiated K-562 and U-937 cell lines, a simultaneous decrease in NK-cell sensitivity was noted.

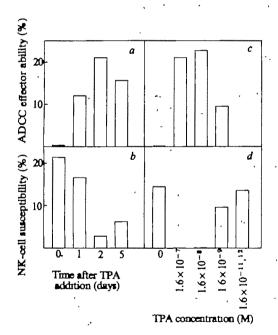


Fig. 1 Time and dose correlation between induced differentiation and loss of NK-cell susceptibility of U-937 cells. a, b, Time correlation. U-937 cells were cultured with TPA as in Table 1 for the indicated period of time. Induction was mitiated at different times to allow simultaneous collection at day 5. After cultivation the cell populations were washed and split. One part was tested for NK-cell susceptibility as in Table 1. The other part was used as effector cells in an ADCC test against antibody-coated chicken red blood cells (CRBC) in a 4-h ³¹Cr-release test in microtitre plates as described elsewhere². A rabbit anti-CRBC lgG fraction was used at 1.2×10⁻⁴ final dilution. Effector target ratio for assaying tumour susceptibility was 50:1 and for ADCC-ability determination 5. 1. Data represent one of two similar experiments. c, d, Dose correlation. U-937 cells were cultured in the indicated concentrations of TPA for 3 days and tested as above. Data represent one of two similar experiments.

Figure 1 shows the strong parallelism seen between the time and dose effect of TPA on U-937 cells with respect to induced differentiation (for other differentiation markers see Table 1), as exemplified by development of the ability to exert antibody-dependent cellular cytotoxicity (ADCC) and by the loss of NK-cell sensitivity. The highest degree of cytotoxic effector potential was found on days 2-3 (Fig. 1a), at which time the tumour cell populations also showed the lowest degree of NK-cell sensitivity (Fig. 1b). Doses of TPA inducing maximal ADCC ability, scored on day 3 (Fig. 1c), similarly produced a highly NK-cell-insensitive cell population (Fig. 1d).

Exposure of BM-86 cells for different periods of time to HMBA, in concentrations inducing optimal erythroid differentiation, resulted in a loss of NK-cell sensitivity (Fig. 2a) which was accompanied by the appearance of globin synthesis as detected by SDS polyacrylamide gel analyses of lysates of GM-86 cells labelled with 35S-methionine in identical conditions (Fig. 2c). In Fig. 2b the same GM-86 cell populations as in Fig. 2a were used as cold target competitors against the well known mouse NK target cell YAC-1 (ref. 6). The time-dependent decrease in NK-cell sensitivity (Fig. 2a) was paralleled by a gradual loss of competing ability. We interpret this to mean that the decreased NK-cell susceptibility of the GM-86 cells after induced differentiation is due to a loss of binding structure(s) for NK cells. However, a similar decrease in competing ability was not seen using differentiated U-937 cells in a parallel system (data not shown). This implies that the differentiation-related alterations seen on the target cell can be expressed either at the level of binding or as a change in susceptibility to lysis. The present system may thus allow the binding and lytic events to be distinguished at the NK target cell level.

The kinetics of the events argue against the possibility that the inducing agents per se cause a decrease in NK-cell sensitivity by some alteration of the target cell, not related to the induced differentiation. This possibility was further reduced by results obtained using spontaneous subclones of K-562 which, as judged by their higher expression of cell-surface glycophorin A, represented a more advanced stage of erythroid differentiation than the original K-562. As Fig. 3a shows, such subclones were also significantly more resistant to NK cytolysis. The clones and original populations could be further differentiated with sodium butyrate to reach the same, still lower, degree of NK-cell sensitivity, inducating the efficiency of the agent in promoting

differentiation to the same final stage in the three different K-562 populations.

To investigate further the correlation between the expression of a differentiation marker and the NK-cell sensitivity profile of cells within a non-cloned tumour cell line, TPA-treated U-937 cells were labelled with fluorescein isothiocvanate (FITC) rabbit anti-human HLA-DR IgG (Fab')2 fragments and sorted on a FACS II cell sorting machine. The population of cells showing markers indicative of greater differentiation (for example, large size and increased expression of HLA-DR¹⁸) were completely resistant to NK-cell lysis (Fig. 3b).

In conclusion, the human K-562 and U-937 cell lines and the mouse GM-86 tumour show a clear correlation between the expression of markers for induced differentiation and reduced NK-cell sensitivity. However, we do not know whether NK-cell sensitivity is always restricted to target cells at an early stage in their respective differentiation. In fact, examples of highly differentiated tumour cells such as myeloma cells, which are quite susceptible to NK lysis¹⁶, the wide variability of NK-cell sensitivity among tumours of the same histogenetic type 10 and the fact that treatment of tumour cells with sodium butyrate can in some cases lead to increased NK-cell sensitivity²⁴, strongly argue against too simplistic a view in this regard. The results do. however, demonstrate that the varying differentiation stages expressed by a particular cell type in a defined differentiation pathway are important in determining NK-cell susceptibility. The availability of the present in vitro systems in which controlled differentiation can be induced significantly increases

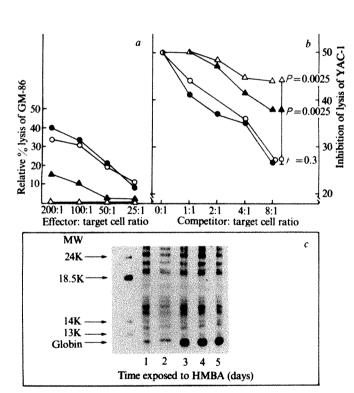


Fig. 2 Induced differentiation in mouse GM-86 cells is accompanied by a loss in NK-cell susceptibility and NK-target competing ability. a, GM-86 cells were cultured with HMBA as in Table 1 for $0 \oplus 0, 2 \oplus 0, 3 \triangleq 0$ and $5 \triangleq 0$ days. The cells were then collected and washed; one part was used as targets for mouse spleen cells as in Table 1, the other part was used (b) as cold competitors against the mouse NK target, the T lymphoma YAC-1, where indicated amounts of cold, unlabelled GM-86 target cells were added to a fixed 1:100 ratio of 51Cr-labelled YAC-1 target to mouse spleen cells to test for lysis as in Table 1. P values were calculated using paired t-statistics making versus day 0 values. c, GM-86 cells grown in identical conditions as in a and b were pulse labelled with 35S-methionine22. The lysates representing each time point were analysed by polyacrylamide gel electrophoresis as described elsewhere²². The globin band was confirmed by immunoprecipitation with a specific rabbit anti-mouse globin antibody (M.J., unpublished). MW, molecular weight.

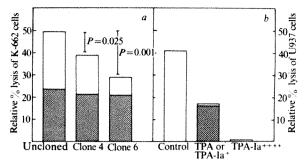


Fig. 3 The reduction in susceptibility of K-562 and U-937 cells is associated with more mature cells. a, Analysis of agarose-cloned K-562 cells. K-562 cells were cloned in agarose and cloned sublines analysed for the expression of surface glycophorin A by immunofluorescence using a rabbit anti-human glycophorin A antiserum (provided by Dr L. Andersson). Sublines expressing the highest amounts of glycophorin A as determined by quantitative cytofluorometric methods²³ were further subcultured. The original K-562 population and its cloned derivatives were tested as NK-cell targets before and after treatment with sodium butyrate (butyrate treatment hatched area) at a 50:1 ratio as in Table 1. Simultaneously the surface expression of glycophorin A was quantitated as described above. The P value after butyrate treatment was >0.5. Data represent one of two similar experiments within a 3-week interval. The P value was calculated as in Fig. 2. b, U-937 cells were induced with TPA as described in Table 1 legend. The cells were then labelled with 51Cr and incubated with an FITC-labelled rabbit F (ab')2 anti-HLA-DR antiserum (provided by Dr L. Klareskog) using phosphate-buffered saline with 0.02% NaN3 for 30 min on ice at a dilution of 1:5. Cells were then washed once in the same buffer and sorted at 4 °C on a FACS II sorter (Becton and Dickinson). Windows were set to obtain the upper 15% of the large, strongly fluorescent cells, denoted TPA-Ia+ giving 6.5×10^4 cells in the right channel. Small cells with insignificant fluorescence were obtained in the left window channel (left channel = $39 \times$ 104 cells), cutting out debris and dead cells (<10% of total). Cells were washed again after sorting, counted, diluted and used as targets in a short-term NK-cell lysis as described in Table 1 legend using a 50:1 effector: target ratio. Control = cells left in culture without TPA and not sorted; TPA = cells induced to differentiate with TPA but not sorted; TPA-Ia++++ = cells induced to differentiate with TPA followed by FACS-II sorting using the right channel. Hatched area = sensitivity of TPA-treated, weak Ia+ cells (left channel, 79% of total TPA cells are TPA-Ia+)

the possibility of analysing fine target structures relevant to NK-cell-mediated cytolysis at the level of binding and/or during the actual lytic event.

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- Kiessling, R. & Wigzell, H. Immun. Rev. 44, 165-208 (1979)
- Herberman, R. B. (ed.) Natural Cell-Mediated Immunity Against Tumours (Academic, New
- Roder, J. C., Ährlund-Richter, L. & Jondal, M. J. exp. Med. 150, 471-480 (1979). Young, W. W., Durdik, J. M., Urdal, D., Hakomori, S.-I. & Henney, C. S. J. Immun. 126, (1981).

- (1961). Kiessling, R. & Wigzell, H. Curr. Topics Microbiol. Immun. (in the press). Kiessling, R., Klein, E. & Wigzell, H. Eur. J. Immun. 5, 112-117 (1975). Cudkowicz, G. & Hochman, P. S. Immun. Rev. 44, 13-41 (1979). Hansson, M., Kiessling, R., Andersson, B., Kärre, & Roder, J. Nature 278, 174-176 (1979). Haller, O., Hansson, M., Kiessling, R. & Wigzell, H. Nature 270, 609-611 (1977). Riesenfeldt, I. et al. Im. J. Cancer 25, 399-403 (1980).

- Riesenfeldt, I. et al. Int. J. Cancer 25, 399-403 (1980).

 Talmadge, J., Meyers, K., Prieur, D. & Starkey, J. Nature 284, 622-624 (1980).

 Hanna, N. & Fiddler, I. J. natn. Cancer Inst. (in the press).

 Hansson, M. & Kiessling, R. in NK Cells: Fundamental Aspects and Role in Cancer (ed. Herberman, R. B.) (North-Holland, Amsterdam, in the press).

 Stern, P., Gidlund, M., Örn, A. & Wigzell, H. Nature 285, 341-342 (1980).

 Andersson, L. C., Jokinen, M. & Gahmberg, C. G. Nature 278, 364-365 (1979).

 Pattengale, P. K. et al. Int. J. Cancer (in the press).
- Pattengale, P. K. et al. Int. J. Cancer (in the press). Sundström, C. & Nilsson, K. Int. J. Cancer 17, 565-577 (1976).
- Nilsson, K. et al. Mod. Trends Hum. Leukaemia 26 (in the press). Koren, H. S., Andersson, S. J. & Larrick, J. W. Nature 279, 328-331 (1979).
- Nilsson, K., Andersson, L. C., Gahmberg, C. G. & Forsbeck, K. in New Trends in Human Immunology and Cancer Immunotherapy (eds Serrou, B. & Rosenfeld, C.) 271-282 (Doin, Paris, 1981)
- Gazitt, Y., Reuben, R., Deitch, A., Marks, P. & Ritkind, R. Cancer Res. 38, 3779-3785
- Persson, H., Jansson, M. & Philipson, L. J. molec. Biol. 136, 375-394 (1980).
 Nilsson, K., Evrin, P. E. & Welsh, K. I. Transplantn Rev. 21, 53-84 (1974).
 Blazar, B., Patarroyo, M., Klein, E. & Klein, G. J. exp. Med. 151, 614-627 (1980).

Cloned copy of the haemagglutinin gene codes for human influenza antigenic determinants in *E. coli*

Ingeborg Heiland & Mary-Jane Gething

Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK

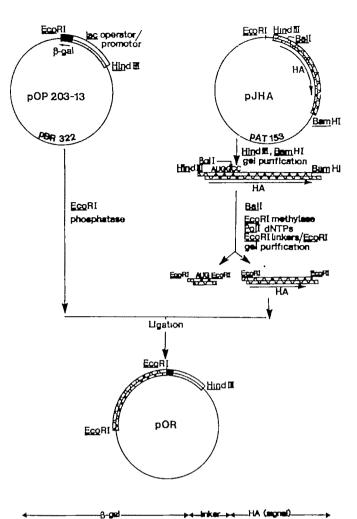
The haemaggintinin (HA) of infinenza virus is the major antigen towards which neutralizing antibodies are directed¹. Epidemics and pandemics of infinenza are associated with variation in the structure of HA^{2,3}. We have recently cloned and sequenced a DNA copy of the RNA gene coding for the HA from human strain A/Japan/305/57 (H2 subtype)^{4,5}. Recent advances in recombinant DNA technology have made it feasible to express in bacteria proteins coded by enkaryotic genes. Viral antigens synthesized in bacteria may ultimately be useful as vaccines. We now report the expression in Escherichia coli of HA antigenic determinants which might be the first approach towards production of a human vaccine. Expression was obtained from the cloned HA gene inserted into a plasmid under the control of the E. coli lac promotor.

Plasmid pJHA⁵ contains a copy of the entire Japan HA gene except for the terminal 11 nucleotides of the 5' non-translated region of the viral RNA. The gene contains an uninterrupted coding sequence specifying a protein of 562 amino acids including the amino-terminal hydrophobic signal peptide of 15 amino acids.

The plasmid pOP203-13 (ref. 6) contains a 203-base pair (bp) DNA sequence consisting of the $E.\ coll\ lac$ UV5 promotor, ribosome-binding site and codons for the first seven amino acids of β -galactosidase. This lac promotor will direct transcription of any DNA fragment inserted into the single EcoRI site of the plasmid (see Fig. 1).

The convenient location of a single Ball restriction site immediately after the AUG start codon in the HA gene⁵ allows the construction of a chimaeric plasmid which should code for a fusion protein consisting of the N-terminal seven amino acids of β -galactoridase followed by the complete HA amino acid sequence. The steps in this construction, together with the N-terminal sequence of the expected fusion protein, are shown in Fig. 1. E. coli HB 101 was transformed with the chimaeric plasmids, and ampicillin-resistant clones containing HA gene sequences were detected by hybridization with 32P-labelled HA DNA7. The orientation of the inserts was determined by restriction enzyme digestions of plasmid DNA isolated from 24 positive clones. In 13 clones the inserts were in the correct orientation for expression with respect to the lac operatorpromotor. Expression of HA antigenic determinants was analysed by solid-phase radioimmunoassay (RIA) using rabbit anti-Japan HA immunoglobulin. As shown in Fig. 2, plasmids pOR4, pOR9 and pOR19 expressed significant amounts of HA antigen.

The nucleotide sequences across the lac-HA juntion were determined for five clones, which are shown in Fig. 3. As was expected, in the three clones (pOR4, pOR9 and pOR19) which were positive in the RIA, the HA DNA was in the correct reading frame for expression. However, they lacked HA gene sequences corresponding to the N-terminal signal peptide and amino acids up to positions 10-15 of the mature protein. Those clones in which the HA DNA was not in the correct reading frame were negative in the RIA. Analysis by restriction enzyme digestion of a further 41 clones which contained the HA insert in



_ATQ_ACC_ATG_ATT_ACG GAT_TCA_CTG GAA_TTC_CCC_ATC_ATT_TAT_CTC__ Start Thr Met Me Thr Asp Ser Leu Glu Phe Pro Me Me Tyr Leu —13

Fig. 1 Construction of expression plasmids. The HradIII-BarnHI DNA fragment of pJHA5 (0.5 µg) was digorted with BelI (restriction enzymes from New England Biolabs, used in the conditions recommended). The EcoRI artes of the DNA were methylated with 0.2 U of S. coh RI methylase (New England Biolaba) for 1 h at 37°C. After phenol extraction and ethanol precipitation, the protrucing ends of the BamHI and HindIII aites were filled using 2U of E. coli DNA polymerase I (Klenow fragment; Boehringer) in a 40-μl reaction volume containing 0.2 mM dNTPx, 60 mM Tru-HClpH 7 5, 8 mM MgCl₂, 1.0 mM 2-mercaptoethanol, 1 mM ATP for 30 min at 10 °C 250 ng ³²P-EcoRI linkers (Collaborative Research; labelled with ³²P-ATP using polynucleotide kmase) in 15 µl of the above buffer were added and ligated to the DNA-using 0 3 U of T4 DNA ligase and 1 U of T4 RNA ligase for 16 h at 9 °C. The buffer was then adjusted to 100 mM Tris-HClpH 7.5, 50 mM NaCl and 5 mM MgCl, and the DNA completely digosted with EcoRL The DNA fragment coding for HA was purified on a 6% acrylamide gel⁴. The plasmid pOP203-13 (200 ng) was digested with *EcoRI* and the 5'-terminal phosphates removed by treatment⁴ with 0.2 U of bacterial alkaline phosphatase in 10 mM Tris-HCl pH 9 5 for 1 h at 37 °C. After phenol and chloroform extractions the DNA was ethanol precipitated and ligated to 50 ng of the HA DNA fragment for 16 h at 10 °C 03 ml CaCl2-treated E. col: HB101 were transfected with the ligated plasmids descrived in 0.2 ml TCM (10 mM CeCl₂, 10 mM MgCl₂, 10 mM Tris-HClpH $7.0)^{17}$ Transformants were selected on agar plates containing $30~\mu g$ ampicallin per ml. These colonies were transferred to introcallulose filters and analysed by in situ hybridization7 using 32P-labelled HA DNA. Positive clones were picked and grown overnight in 10 ml LB proth¹⁸ containing 20 μg ampicillin ml⁻¹ at 37 °C. Mmi-plasmid DNA preparations (1.5-ml cultures) were made up by a modification (lah-Horowicz, personal communication) of the procedure of Birnbolm and Dolyle. Aliquots ($\sim 0.1 \,\mu g$ plasmed) were taken for restriction enzyme discetions to determine the orientation of the inserts. In the HA DNA insert of pJHA there are only two PxtI sites near the 5' end³, so a PxtI digest of the pOR plasmeds with inserts in the correct ementation for expression of HA will give 5,800-, 1,000- and 100-bp fragments whereas those in the wrong orientation will give 4,400-, 2,400- and 100-bp fragments. The resultant fragments were stred on a 1.7% agarose goi (data not shown)

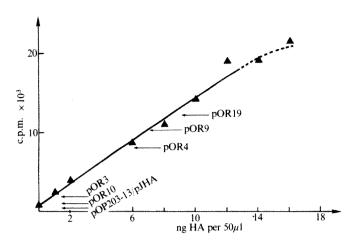


Fig. 2 Radioimmunoassay of HA in lysates of bacteria containing plasmids constructed as described in Fig. 1 legend. Bacterial lysates were prepared and assayed as described elsewhere 10,20. Rabbit anti-HA (IgG) was purified by chromatography on Protein A-Sepharose²¹ and labelled with ¹²⁵I using iodogen as described elsewhere 22. To quantify antigen, a standard curve was constructed using different amounts of purified HA. The results were normalized to the same optical density of cells $(A_{590} = 0.5)$.

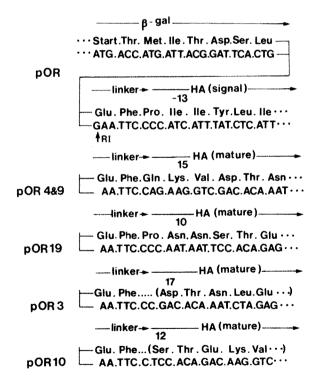


Fig. 3 Nucleotide sequences at the lac-HA junction of plasmids constructed as described in Fig. 1 legend. Only the sequence of the coding strand is given. pOR, expected sequence from the designed construction of plasmid. The nucleotide sequences were determined by the procedures of Maxam and Gilbert⁸. The plasmids were digested with EcoRI, treated with calf intestinal phosphatase (Boehringer) to remove the terminal 5'-phosphates²³ and then radiolabelled with ³²P-ATP using T4 polynucleotide kinase (PL Biochemicals). The fragments from a subsequent restriction digest with AvaI were separated on a 6% acrylamide gel and those containing the HA insertion point were eluted and sequenced.

the correct orientation did not yield any which contained sequences corresponding to the signal peptide or the entire mature HA protein, and showed that in no case had the clones lost any nucleotide sequences corresponding to the C-terminus of the protein. When the construction of the chimaeric plasmid was repeated with the modification that the EcoRI methylation step preceded the Ball digestion, the only clones with the HA insert in the correct orientation with respect to the lac promotor had small insertions at the *lac-HA* junction (results not shown). The reason for the difficulty in obtaining the desired construction is not yet clear. The possibility that the eukaryotic hydrophobic signal sequence of the HA protein is in some way inhibitory or toxic for growth of E. coli is being investigated.

The level of expression in pOR19 corresponds to $\sim 3,000$ molecules of HA per cell. Quantification by RIA assumes that the antigenicity of the hybrid protein synthesized in E. coli is identical with that of the natural HA. This may not be the case as the hybrid protein lacks some amino acids normally at the N-terminus of the mature HA even though they are remote from the major antigenic sites on the molecule9. In addition, the three-dimensional structure of the hybrid protein may differ from that of the natural protein if the correct folding requires transport through a membrane and glycosylation. These factors may affect both the antigenicity and the stability of the bacterially produced protein so that the above estimate of expression level will be a minimum value.

This is the first report of expression of a human influenza HA in bacterial cells. Antigenic determinants of the HA of a related avian orthomyxovirus, fowl plague virus, have been expressed in bacteria using a tryptophan operator/promotor system¹⁰. The level of expression of HA from our plasmid pOR19 is comparable with that of pWT111/FPV502 (ref. 10), although higher levels of expression were obtained after derepression of the trp promotor. The expression level is also comparable with those reported for other eukaryotic genes expressed in E. $coli^{6.11-16}$. As noted previously^{6.10}, the expression levels obtained are considerably lower (< 10%) than those theoretically attainable. It has been suggested that this is due to inefficient transcription, inefficient translation and proteolytic degradation of eukaryotic molecules6.

The HA gene expressed in E. coli does not originate from an influenza strain which is currently causing disease. However, the methods described here are widely applicable and might be a basis for producing a vaccine against a current strain if the levels of expression can be increased.

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- Laver, W. G. & Kilbourne, E. D. Virology 30, 493-501 (1966).

- Laver, W. G. & Kilbourne, E. D. Virology 30, 493–501 (1966).
 Laver, W. G. & Webster, R. G. Virology 34, 193–202 (1968).
 Laver, W. G. & Webster, R. G. Virology 34, 445–455 (1972).
 Gething, M.-J., Bye, J., Skehel, J. & Waterfield, M. in Structure and Variation in Influenza Virus (eds Laver, W. G. & Air, G.) 1–10 (Elsevier, Amsterdam, 1980).
 Gething, M.-J., Bye, J., Skehel, J. & Waterfield, M. Nature 287, 301–306 (1980).
 Fraser, R. H. & Bruce, B. J. Proc. natn. Acad. Sci. U.S.A. 75, 5936–5940 (1978).
 Grunstein, M. & Hogness, D. S. Proc. natn. Acad. Sci. U.S.A. 72, 3961–3965 (1975).
 Maxam, A. M. & Gilbert, W. Meth. Enzym. 65, 499–560 (1980).
 Wiley, D. C., Wilson, I. A. & Skehel, J. J. Nature 289, 373–378 (1981).
 Emplage, I. S. et al. Nature 283, 173–174 (1980).

- Emtage, J. S. et al. Nature 283, 171-174 (1980).
 Roberts, T. M., Bikel, I., Yogum, R. R., Livingston, D. M. & Ptashne, M. Proc. natn. Acad.

- Roberts, T. M., Bikel, I., Yogum, R. R., Livingston, D. M. & Ptashne, M. Proc. natn. Acad. Sci. U.S.A. 76, 5596-5600 (1979).

 Guarente, L., Lanes, G., Roberts, T. M. & Ptashne, M. Cell 20, 543-553 (1980).

 Goeddel, D. V. et al. Nature 287, 411-416 (1980).

 Goeddell, D. V. et al. Nucleic Acids Res. 8, 4057-4074 (1980).

 Taniguchi, T. et al. Proc. natn. Acad. Sci. U.S.A. 77, 5230-5233 (1980).

 Kupper, M. et al. Nature 289, 555-559 (1981).

 Mandel, M. & Higa, A. J. molec. Biol. 53, 159-162 (1970).

 Miller, J. H. Experiments in Molecular Genetics (Cold Spring Harbor Laboratory, New York, 1972)
- York 1972)
- York, 1972).
 Birnboim, H. C. & Doly, J. Nucleic Acids Res. 7, 1513–1523 (1979).
 Broome, S. & Gilbert, W. Proc. natn. Acad. Sci. U.S.A. 75, 2746–2749 (1978).
 Ey, P. L., Prowse, S. J. & Jenkin, C. R. Immunochemistry 15, 429–436 (1978).
 Fraker, P. J. & Speck, J. C. Jr Biochem. biophys. Res. Commun. 80, 849–857 (1978).
- Weaver, R. F. & Weissmann, C. Nucleic Acids Res. 7, 1175-1193 (1979).

Oestrogen receptor levels and vitellogenin synthesis during development of *Xenopus laevis*

Felicity E. B. May* & John Knowland

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

The synthesis of vitellogenin, the precursor of the major eggyolk proteins, becomes inducible by oestrogen in *Xenopus laevis* larval liver during metamorphosis¹⁻⁴, well before vitellogenin is required for oocyte growth. The synthesis in pipe of some liver proteins is responsive to oestradioi at earlier stages, but vitellogenin inducibility does not appear until a precise point during metamorphic climax, stage 62, when many 'adult' liver proteins first start to be synthesized. This suggests that vitellogenin inducibility is not determined simply by the presence of an oestrogen receptor. The receptor identified and characterized in fully inducible adult liver 4.7 (also detected by others) is unusual because it is present at very low concentrations and 50% is found in the nucleus in unstimulated animals^{6,7}, and is itself synthesized in response to oestrogen, raising the level in the nucleus'. Here we have measured nuclear receptor levels and the effect of oestrogen on these during Xenopus metamorphosis. Larvae which cannot be induced to synthesize vitellogenin contain an oestrogen receptor with the same affinity and specificity as the adult receptor. Treatment with oestrogen raises the level of nuclear oestrogen receptor in inducible but not in uninducible larvae. These and additional results from thyrostatic larvae suggest that an increase in the level of nuclear receptor is necessary for the induction of vitellogenin synthesis, and that the onset of vitellogenia inducibility might be determined by the ability of oestrogen to increase nuclear receptor

We first measured receptor levels in salt extracts of liver nuclei from untreated and oestrogen-treated larvae at six different stages during metamorphosis, using methods which maximize the yield of soluble receptor from adult liver nuclei, but which would not extract any insoluble receptor. Figure 1 shows examples of the Scatchard plots of total 3H-oestradiol binding for four of the six stages examined. In all cases, the curved Scatchard plots could be resolved into two components10 with the highaffinity component having a dissociation constant close to 0.50 × 10⁻⁹M, the value found previously for the oestrogen receptor in the adult. The relative amounts of low-affinity binding were estimated from the limiting bound/free values, which are proportional to the numbers of non-suppressible binding sites. The relative amounts of receptor and low-affinity binding were very different at different stages and the ratio was affected by oestrogen treatment. For example, the low-affinity component accounted for most of the binding in both untreated and oestrogen-treated stage 54 larvae, but for a much lower proportion in stage 66 animals and an even lower proportion in the same animals after oestrogen treatment. The larval livers were probably contaminated with plasma as it was not practicable to perfuse them; this could account for the often large amounts of low-affinity binding in the nuclear extracts. Neither progesterone, testosterone nor dexamethasone could bind to the larval receptor, and we conclude that the oestrogen receptors of larval and adult liver are indistinguishable by the criteria used here.

Figure 2 shows the number of receptor sites and the relative amounts of low-affinity binding present at various developmental stages. In untreated larvae the amount of receptor increases gradually from 49 sites per nucleus at stage 54 to 74 sites per nucleus at stage 66 (Fig. 2a). This compares with 100 sites per nucleus in the adult male and shows that there is, at the most, a twofold increase in the amount of nuclear receptor between stage 54 and the adult. In contrast, the amount of

low-affinity binding decreased about twofold between stage 54 and 3 months post metamorphosis (Fig. 2b). Vitellogenin synthesis is not inducible in larval liver before stage 62, but is clearly inducible at stage 62 during metamorphic climax, and inducibility increases during the completion of metamorphosis (stages 62–66) and the 3 months following it⁴. This study therefore establishes that the nuclear receptor is present in *Xenopus* liver before vitellogenin synthesis becomes inducible, and shows that the presence of the receptor is not by itself responsible for vitellogenin inducibility.

Figure 2a also shows the levels of nuclear receptor in oestrogen-treated larvae. In the three earliest stages, oestrogen had no effect on the nuclear receptor levels but from stage 62 onwards it progressively increased them, so that by 3 months after metamorphosis the increase in the nuclear receptor level was the same as that found in adult males after oestrogen treatment. Oestradiol treatment did not alter the affinity of the receptor at any stage. The amount of low-affinity binding was

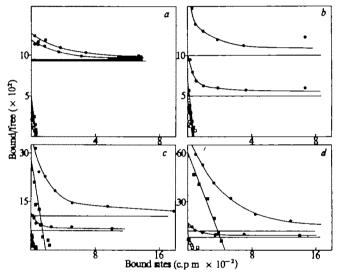


Fig. 1 Affinity of H-contradiol binding in nuclear extracts of liver from untreated or oestrogen-treated Xenopus larvae. All procedures were performed at 0-4 °C. Livers were removed from larvae (staged according to Nieuwkoop and Faber¹³), pooled and washed with saline¹⁴. They were then weighed and 250 mg (equivalent to ~120 hvers) were homogenized in 7 ml of homogenization buffer (0.25 M sucrose, 1 mM Trs-HCl, 1 mM MgCl, $150~\mu g~ml^{-1}$ phenylmethylsulphonylfinoride and 50~mM Bacitracin, pH7.3) using six strokes of a Tedion-glass homogenizer. The homogenate v filtered through two layers of cheesecloth, washed through with another 4 ml of homogenization buffer and centrifuged at 800g for 10 min. The pellet was resuspended in 20 volumes of homogenization buffer and the number of hepatocyte nuclei, which were distinguishable from contaminating crythrocytes, was estimated by microscopic examination of an aliquot. The suspension was recentrifuged at 800g for 10 min and the pellet resuspended in extraction buffer (0.5 M KCl, 0.01 M Tris-HCl, 1 mM 2-mercaptoethanol, 10% glycerol, 150 μg ml⁻¹ phenylmethylmlphonylfluoride and 50 mM Backtracin, pH 7.3) at 2 ml per g of original tissue, frozen and thawed, then homogenized in a small all-glass homogenizer. The nuclear extract was then centuringed in a cooled Beckmann microfuge for 4 min and the supernatant stored in Equal natrogen until use Glass columns (9.5×0 4 cm diameter) ere packed with Sephadex LH-20 which had been swollen in extraction buffer and then equilibrated at 4 °C. To remove endogenous oestrogen, 400 μl of the thawed extract were applied to the columns and cluted with 1 ml of extraction buffer. Abquots of the clustes were incubated with steroid at 20 °C for 1 h to allow exchange of bound steroid with "H-oestrachol". 100 µl were applied to each column and eluted with 750 µl extraction buffer. Eluates were counted with 50% efficiency after extraction of radioactivity into scintillant (10 ml containing. PPO (diphenyloxazole), 4 g; POPOP (p-phenylenephenyloxazole), 0.1 g per litre of tolinene) Equilibrium bindmg constants were estimated from the binding curves. We plotted the total binding to ³H-cestradiol according to Scatchard¹⁵ and determined the hmiting bound/free ratio reached after the high-affinity component becomes saturated. This ratio was used to calculate the low-affinity binding at each free ligand concentration, this was subtracted from the total binding to give the binding due to the high-affinity component, which was then analysed according to Scatchard 13 to give the K_a and number of binding sites. To study the effect of oestrogen, larvae were immersed for 3 days in 1 µM oestradiol in water, which was renewed daily, before staging them and removing their livers. The total H-oestradiol binding was determined at various concentrations of ³H-contradiol in untreated (O) or contragon-treated (O) larvae and the high-affinity binding assayed in untreated () or costrogen-treated

(III) livers (as described above) at stage 54 (a), 57 (b), 62 (c) or 66 (d).

^{*}Present address: Inserm (U 148), 60 rue de Navacelles, 34100 Montpelher, France

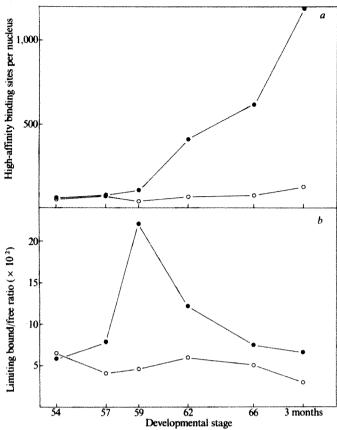


Fig. 2 Average number of high-affinity oestrogen-binding sites per nucleus (a) and the limiting bound/free ratio (b) as a measure of low-affinity binding in Xenopus liver during metamorphosis. At least two measurements were made at each stage. ○, Untreated and ●, oestrogen-treated larvae.

also increased by oestrogen treatment, but here the increase was greatest at the onset of metamorphic climax (stage 59) and much lower at later stages, being only twofold at stage 66. In contrast, the amount of nuclear oestrogen receptor was first increased by oestrogen treatment at stage 62, when vitellogenin synthesis becomes inducible. This suggests that the ability of oestrogen to induce vitellogenin synthesis at a certain stage during development is closely connected with its ability to increase receptor levels. We examined this relationship in further experiments.

An important advantage of developmental studies on Xenopus is that larval development can be arrested in late premetamorphosis using compounds such as propylthiouracil (PTU). As these animals age, events which would occur if they were developing normally will take place only if they do not depend on metamorphic development. This has allowed us to show⁵ that only half the alterations in liver protein synthesis normally associated with metamorphosis are dependent on developmental stage rather than on chronological age. If two events which occur during development retain the same temporal association in developmentally arrested animals, additional evidence is provided that one event requires the occurrence of the other. We therefore measured the level of oestrogen receptor and the effect of oestrogen on this level in liver from developmentally arrested larvae. The animals were first kept for 1 yr in water containing 10 µg ml⁻¹ (0.58 mM) PTU, which was changed every 2 weeks. This arrested development at stage 54, but did not inhibit growth, so that at the time of use the thyrostatic larvae were considerably larger than normal larvae of the same developmental stage. Vitellogenin synthesis was uninducible in the stage 54 thyrostatic animals, but Fig. 3 shows that there was as much receptor (100 sites per nucleus) in the livers of these larvae as in adult males, confirming that receptor alone cannot confer inducibility. Figure 3 also shows that the level could not be increased by oestrogen treatment, so that in this respect the thyrostatic larvae at stage 54 behaved like normal larvae of the same stage. The amount of low-affinity binding was somewhat higher in arrested larvae and was increased about twofold by oestrogen treatment whereas in normal larvae oestrogen had no effect on the amount of lowaffinity binding.

Animals were then removed from PTU and allowed to metamorphose, and the nuclear oestrogen binding was measured at various stages during this delayed metamorphosis (Fig. 3), before and after treatment with oestrogen. Whereas the level of induction of the low-affinity binding decreased (Fig. 3b), the nuclear receptor levels were increased by oestrogen treatment from about stage 60 (Fig. 3a), the stage at which vitellogenin synthesis becomes inducible in these animals⁴. The increased level of the receptor in stage 62 animals which had previously been kept in PTU was about the same as in normal animals of a similar stage (Fig. 2a). In all cases the dissociation constant of the receptor found in larvae that had been treated with PTU was $0.50 \pm 0.05 \times 10^{-9}$ M, the value found for normal larvae.

These results show that while the normal, small rise in receptor content to the level found in adult males does not require metamorphosis, the ability of oestrogen to increase receptor levels does. In addition, in both normal and arrested animals oestrogen can first increase the level of nuclear receptor at the developmental stage when it can first induce vitellogenin synthesis. This precise correlation suggests that an increase in the level of nuclear oestrogen receptor is required for the induction of vitellogenin synthesis, and that the ability of oestrogen to increase the level of its receptor is in some way responsible for the appearance of vitellogenin inducibility. These data are consistent with results from other steroidresponsive systems^{11,12} where the rate of accumulation of the steroid-induced protein and its mRNA is thought to be determined by the level of nuclear receptor. In these systems the increase in nuclear receptor levels after hormone treatment is largely the result of translocation from a pre-existing pool of cytoplasmic receptor whereas in Xenopus liver it is probably due to synthesis of the receptor9. We have previously described5 a

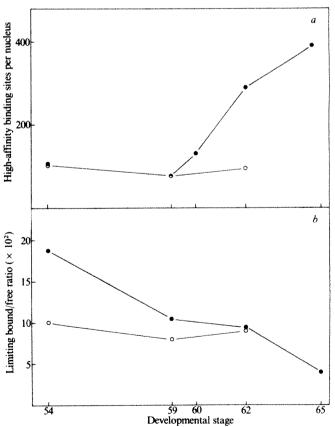


Fig. 3 Number of high-affinity oestrogen-binding sites per nucleus (a) and the limiting bound/free ratio (b) as a measure of the low-affinity binding in the livers of thyrostatic or previously thyrostatic Xenopus larvae.

O. Untreated and \bullet , oestrogen-treated larvae.

set of cellular proteins whose synthesis is influenced by oestrogen in larval liver before stage 62. Our data show that an elevated level of liver nuclear oestrogen receptor is not induced by oestrogen in animals of this stage. A model has been proposed11,12 in which different levels of nuclear oestrogen receptor are required to induce transcription of different genes, perhaps reflecting different affinities or numbers of nuclear acceptor sites, and our results are consistent with this type of model.

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- Knowland, J. Differentiation 12, 47-51 (1978).
- Huber, S., Ryffel, G. U. & Weber, R. Nature 278, 65-67 (1979).
- Skipper, J. K. & Hamilton, T. H. Science 206, 693-695 (1979).
- May, F. E. B. & Knowland, J. Devl Biol. 77, 419-430 (1980). May, F. E. B. & Knowland, J. Devl Biol. 82, 158-167 (1981).
- Westley, B. R. & Knowland, J. Cell 15, 367-374 (1978). Westley, B. R. Differentiation 15, 67-72 (1979).
- Westley, B. R. Differentiation 13, 6/- /2 (1979).
 Hayward, M. A., Mitchell, T. A. & Shapiro, D. J. J. biol. Chem. 255, 11308-11312 (1980).
 Westley, B. R. & Knowland, J. Biochem. biophys. Res. Commun. 88, 1167-1172 (1979).
 Channess, G. C. & McGuire, W. L. Steroids 26, 538-542 (1975).
 Mulvihill, E. R. & Palmiter, R. D. J. biol. Chem. 252, 2060-2068 (1977).

- Mulvihill, E. R. & Palmiter, R. D. J. viol. Chem. 255, 2000-2008 (1977).
 Mulvihill, E. R. & Palmiter, R. D. J. biol. Chem. 255, 2085-2091 (1980).
 Nieuwkoop, P. D. & Faber, J. Normal Table of Xenopus laevis (Daudin) 2nd edn (North Holland, Amsterdam, 1967
- Barth, L. G. & Barth, L. J. J. Embryol. exp. Morp. 7, 210-222 (1959).
 Scatchard, G. Ann. N.Y. Acad. Sci. 51, 660-672 (1949).

Mycoplasmas induce collagenase in BALB/c 3T3 cells

Barbara Kluve*, William C. Merrick*, Eric J. Stanbridge† & Howard Gershman*‡

*Department of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106, USA †Department of Microbiology, University of California, Irvine, College of Medicine, Irvine, California 92717, USA

The turnover of collagen, the major protein of the body, is controlled by the rate of collagen synthesis and the activity of collagenase, a highly specific protease which initiates collagen degradation. Because collagen is largely resistant to other proteases, collagenase is believed to have an important regulatory role in such normal processes as tissue remodelling, wound healing and ageing. In addition, the regulation of the activity of collagenase may be altered in diseases of connective tissue, particularly rheumatoid arthritis, and in malignant invasion and metastasis. Collagenase is an extracellular enzyme, and thus it has been necessary to study its regulation in cells maintained in tissue culture, by measuring accumulation of the secreted protein in the culture medium. In this way, collagenases have been isolated from many tissues and cells1-7. Little is known about the modulation of collagenase synthesis and secretion, but various treatments have been reported that induce its secretion in mammalian cells in monolayer culture⁸⁻¹⁷, possibly by a possibly by a mechanism involving membrane perturbation. We report here the production and secretion of collagenase by BALB/c 3T3 fibroblasts. Their ability to synthesize collagen 18,19 has been extensively studied, but there has been no previous report of their collagenolytic activity. During studies of collagenase activity in BALB/c 3T3 cells, we noted that certain cultures had greatly increased enzyme activity corresponding to cultures in which contamination with mycoplasmas had been detected during routine assay. Here we present evidence that infection of cultures of BALB/c 3T3 cells with mycoplasmas results in the accumulation of high levels of collagenase in the medium.

Figure 1 shows the time course of accumulation of collagenase activity in the culture medium of BALB/c3T3 cells known to be infected with mycoplasmas. Replicate cultures of BALB/c 3T3 cells were established and allowed to achieve confluence. Then the culture medium was changed and this point was taken as day 0 of culture. On each successive day from 1 to 9, the medium from duplicate dishes was collected and assayed for collagenase activity as described in Fig. 1 legend. Collagenase activity began to accumulate in the medium at about day 2, increased rapidly for the next 2 days, and then began to decline slowly. We do not know whether this decrease in accumulated collagenase activity is due entirely to an increase in rate of inactivation that eventually exceeds the rate of synthesis, or whether the rates of synthesis and secretion also decline. No collagenase activity was detected in medium not treated with trypsin; all the collagenase secreted was therefore in an inactive form. In addition, cell homogenates had no detectable collagenase activity, either active or trypsin-activable, indicating that most or all of the collagenase synthesized by 3T3 cells is secreted into the medium. Cell numbers did not, within experimental error, change in the period of collagenase accumulation (during the first 4 days after medium change), in uninfected, newly infected or chronically mycoplasma-infected cultures. Cell densities in infected and uninfected cultures were similar at confluence and throughout the period of collagenase production.

The contaminating microorganism in the 3T3 cultures with high collagenolytic activity was identified as Mycoplasma orale on the basis of a growth inhibition test using dried antiserumimpregnated disks20. The induction of collagenolytic activity in cultures of BALB/c 3T3 cells by infection with mycoplasmas was demonstrated by adding an aliquot of pure mycoplasma culture, obtained as described above, to uninfected cells, which had low but measurable activity. The time course of the appearance of collagenolytic activity in these newly infected cultures is shown in Fig. 2. In this experiment confluent cultures

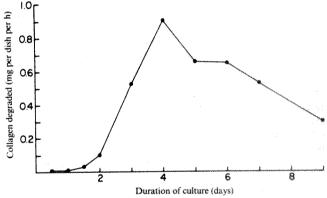


Fig. 1 Accumulation of collagenase activity in medium of BALB/c 3T3 cultures. BALB/c 3T3 cells were grown in Eagle's minimum essential medium (Gibco) with fourfold increased concentrations of vitamins and amino acids (Gibco) plus 10% fetal calf serum (Gibco), 100 U ml-1 penicillin and 100 µg ml-1 streptomycin (Sigma). The cells were plated into 10-cm plastic tissue culture dishes (Costar) containing 15 ml of medium. After the cells reached confluence, the medium in all dishes was replaced with fresh medium (day 0), and the cells maintained in these media for the time indicated. Media were collected from replicate cultures at the times indicated, centrifuged to remove debris, and brought to 0.1 M Tris-HCl, pH 7.5, and 0.05% sodium azide. Collagenase was precipitated from the conditioned medium by the addition of ammonium sulphate to 60% saturation. The precipitated protein was dissolved in, and dialysed against 0.05 M Tris-HCl, pH 7.5, 0.01 M CaCl₂, 0.05 M NaCl and 0.05% sodium azide. A 15-fold concentration of the starting volume was consistently achieved. All samples were then assayed for collagenase by measuring the release of soluble peptides from native collagen fibrils reconstituted at 37 °C (ref. 27). Each reaction mixture contained 36 µg of ¹⁴C-collagen in 10 µl of 0.1 M Tris-HCl, pH 7.5, 0.1 M NaCl, 0.01 M CaCl₂ and 0.05% sodium azide. The collagen had been radiolabelled by in vitro methylation to a specific activity of 667 c.p.m. per µg. A 30-µl aliquot of the collagenase preparation was added to the gelled collagen and treated with 30 µg of trypsin (Sigma no. T-1005) at room temperature for 45 min to activate the collagenase. A fourfold excess of soybean trypsin inhibitor (Sigma) was then added. Replicate reactions were incubated for increasing times at 37 °C and were terminated by centrifugation at 8,000g for 10 min. The entire supernatant fraction containing the digested collagen was counted in a liquid scintillation spectrometer. Blanks, which contained the same additions, including trypsin and trypsin inhibitor but no collagenase, were also run for each time of assay. Radioactivities released in these control reactions, which was generally less than 5% of the experimental (collagenase-containing) samples, were subtracted from the data shown. Units of collagenase activity were determined from the linear portion of each rate curve. Identical results were obtained in samples activated by dialysis against 1 mM APMA at 4 °C, in place of trypsin treatment.

‡To whom correspondence should be addressed.

were established as described in Fig. 1 legend and the medium replaced with 15 ml of fresh medium to which was added 0.45 ml of a broth culture containing mycoplasmas. Control cultures received only fresh medium. Subsequently, the media were collected every 4 days and replaced by 15 ml of fresh medium. The media were assayed for collagenase activity as described in Fig. 1 legend.

As shown in Fig. 2, uninfected cells secreted relatively low levels of collagenase activity during each of the 4-day collection periods, whereas newly infected cells secreted increasing amounts of collagenase with time after infection. During the first 4 days, medium collected from both newly infected and control cultures had equivalent collagenase activity. However, during the second 4-day period, cultures infected with samples of mycoplasmas contained substantially increased collagenase activity. Medium from these infected cultures collected during the third 4-day period showed even higher collagenase activity, equal to that produced by cultures that had been infected before the start of the experiment (also shown in Fig. 2) and in which the level of collagenase activity had stabilized. In addition, parallel uninfected cultures received 0.45 ml of medium from infected 3T3 cultures instead of pure cultures of mycoplasmas. Collagenase activity in these cultures, also shown in Fig. 2, increased with a time course similar to that of the cultures treated with pure mycoplasmas. The increased level of collagenase activity in the third collection of medium from control cultures may be due to stimulation by accumulated collagen in the cultures; these cells are known to secrete and deposit collagen, which has been recently reported to stimulate the synthesis and secretion of collagenase8. On subculture, the level of collagenase in the control (uninfected) cell cultures dropped to the original level, while those in infected cultures remained high. The levels of collagenase that accumulate in culture medium exposed for 4 days to mycoplasma-infected cells are ~ 4.3 U per 10^6 cells (1 U = 1 µg collagen degraded per min at 37 °C). Uninfected (control) 3T3 cells produce < 0.25 U per 106 cells. This is similar to the unstimulated/stimulated levels calculated from data reported for human skin fibroblasts exposed to collagen (0.36/7.14 U per 106 cells; see ref. 8), for rabbit corneal cells stimulated by lymphocytes from alkaliburned animals (0.7/6.1 U per 10⁶ cells; ref. 16) and for human synovial fibroblasts stimulated by cytochalasin B (0.64-1.6/7.7-7.1 U per 10⁶ cells; ref. 12).

The various cultures used in the experiment shown in Fig. 2, were re-examined for the presence of mycoplasmas. The control

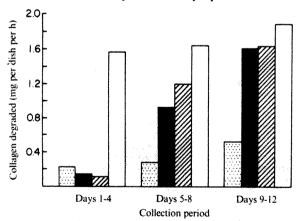
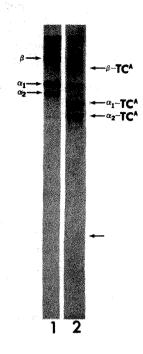


Fig. 2 Induction of collagenase activity in BALB/c 3T3 cultures after infection with mycoplasmas. BALB/c 3T3 cells were grown as described in Fig. 1 legend. After the cells reached confluence, the medium in all dishes was replaced with fresh medium (day 0). At this time, duplicate cultures received 0.45 ml of fresh medium (stippled bars); 0.45 ml of medium from infected BALB/c 3T3 cultures (solid bars); or 0.45 ml of a broth culture of mycoplasmas (cross-hatched bars). Parallel cultures of BALB/c 3T3 cells were grown in which mycoplasma infection had been previously established (open bars). Media were collected from each of these cultures every 4 days over a 12-day period and replaced with fresh media. After collection, the media were centrifuged to remove debris and brought to 0.05 M Tris-HCl, p H 7.5, and 0.05% sodium azide. Collagenase was measured as described in Fig. 1 legend.

Fig. 3 SDS-polyacrylamide gel electrophoresis of collagen after reaction with BALB/c 3T3 collagenase. Lane 1. 24 μg of ¹⁴C-collagen before incubation with collagenase. Lane 2, 24 µg 14Ccollagen incubated with partially purified collagenase at 23 °C for 24 h. The reaction mixture contained 12 µg of partially purified collagenase and 24 µg of ¹⁴C-collagen in a solution consisting of 0.05 M Tris-HCl, pH 7.5, 0.01 M CaCl₂, 0.05 M NaCl, 1 mM phenylmethylsulphonyl fluoride, 0.04% sodium azide and 6.25% glycerol in a final volume of 32 µl. The collagenase used was purified by precipitation with (NH₄)₂SO₄ (40-60% saturation), batch elution from phosphocellulose, gradient clution from QAE-Sephadex A-50, gradient elution from phosphocellulose. and gel filtration on Sephadex G-100. The collagen had been radiolabelled by in vitro methylation28 to a specific activity of 667 c.p.m. per µg. The reaction was stopped by the addition of icecold 10% trichloroacetic acid. The protein was allowed to precipitate and then collected by centrifugation at 8,000g for 15 min. The protein pellet was rinsed twice with acetone, dried and dissolved in 20 µl of electrophoresis sample buffer containing 10% glycerol, 0.1 M dithiothreitol, 2% SDS, 0.08 M Tris-HCl, pH 6.8, and 0.2% bromophenol



blue. The sample was heated to 95 °C for 5 min then subjected to SDS-polyacrylamide gel electrophoresis (0.1% SDS in 12% polyacrylamide) according to the method of Laemmli²⁹. The gels were stained with Coomassie blue, dried and autoradiographed using Kodak X-Omat R film. The figure shows the autoradiograms of these gels. In lane 1, the positions of the $\alpha_1\beta$ -dimer, α_1 and α_2 are indicated. In lane 2, almost all the β -dimer, as well as most of the α_1 and α_2 polypeptides, have been digested. The positions of β -TC^{Δ}, the large cleavage product of β , and α_1 -TC^{Δ} and α_2 -TC^{Δ}, the large cleavage products of α_1 and α_2 are indicated. The unlabelled arrow indicates the position of TC^{Δ}, which can be visualized by overexposing the autoradiogram or by incubating reaction mixtures with collagenase for shorter times.

cultures contained no detectable mycoplasmas, whereas the remaining three cultures, all of which showed increased collagenolytic activity, were infected. We have therefore concluded that infection of BALB/c 3T3 cells with mycoplasmas results in the increased accumulation of extracellular collagenase.

The collagenase produced by cultures of BALB/c 3T3 cells infected with M. orale seems to be a product of the murine 3T3 line, rather than that of the mycoplasma. We base this conclusion on the following evidence. (1) BALB/c 3T3 cells produce collagenase, although at a much lower level, before infection. (2) Mycoplasma organisms in broth, or transferred to culture medium in dishes containing no 3T3 cells, demonstrated no collagenase activity when assayed in the same conditions as BALB/c 3T3 culture medium. (3) We have partially purified the enzyme and found it to be similar to previously reported mammalian collagenases. Briefly, it degrades collagen at neutral pH; in crude preparations it requires treatment with trypsin or p-aminophenylmercuric acetate (APMA) for maximal activity; and it is completely inhibited by EGTA, orthophenanthroline and serum, partially inhibited by cysteine and dithiothreitol, and not inhibited by phenylmethylsulphonyl fluoride or soybean trypsin inhibitor. Most significantly, and in direct contrast to bacterial collagenases, collagenase purified from culture medium of mycoplasma-infected BALB/c 3T3 cleaves collagen into characteristic three-quarter length TCA and one-quarter TCB fragments, as shown in Fig. 3.

Thus, it seems that BALB/c 3T3 cells can synthesize and secrete relatively low levels of collagenase in normal circumstances. Infection of BALB/c 3T3 cells with mycoplasmas results in a significant increase in the accumulation of collagenase in the culture medium. Infected cultures of confluent cells show uniformly high levels of collagenase activity even after subculturing. In addition, the ability of mycoplasmas

to induce the synthesis and/or secretion of collagenase is not limited to BALB/c 3T3 cells. We have shown that cultures of chick fibroblasts also give dramatic increases in the level of extracellular collagenase activity on infection with mycoplasmas (data not shown). The induction of collagenolytic activity in cells by infection with mycoplasmas may have important clinical implications in terms of the development and persistence of connective tissue diseases. Mycoplasmas are recognized as agents possibly contributing to the establishment of rheumatoid arthritis in humans21,22 and are known to cause chronic arthritis

Received 13 March; accepted 23 June 1981.

- Harris, E. D. & Krane, S. M. New. Engl. J. Med. 291, 557-563, 603-609, 652-651 (1974).
 Stricklin, G. P., Bauer, E. A., Jeffrey, J. J. & Eisen, A. Z. Biochemistry 16, 1607-1615
- Woolley, D. E., Glanville, R. W., Roberts, D. R. & Evanson, J. M. Biochem. J. 169, 265-276 (1978).
- Cawston, T. E. & Tyler, J. A. Biochem. J. 183, 647-656 (1979).
- 5. Woolley, D. E., Glanville, R. W., Crossley, M. J. & Evanson, J. M. Eur. J. Biochem. 54, 611-622 (1975).
- 6. Sakamoto, S., Sakamoto, M., Goldhaber, P. & Glimcher, M. J. Archs Biochem. Biophys. 188, 438-449 (1978)

- Halme, J., Tyree, B. & Jeffery, J. J. Archs Biochem. Biophys. 199, 51-60 (1980).
 Halme, J., Tyree, B. & Jeffery, J. J. Archs Biochem. Biophys. 199, 51-60 (1980).
 Biswas, C. & Dayer, J.-M. Cell 18, 1035-1041 (1979).
 Brinckerhoff, C. E. & Harris, E. D. Excerpta med., Arthritis Rheum. 21, 745-753 (1978).
 Brinckerhoff, C. E., McMillan, R. M., Fahey, J. V. & Harris, E. D. Excerpta med., Arthritis Rheum. 22, 1109-1116 (1979).
- Moscatelli, D., Jaffe, E. & Rifkin, D. B. Cell 20, 343-351 (1980).
 Harris, E. D., Reynolds, J. J. & Werb, Z. Nature 257, 243-244 (1975).
 Wahl, L. M., Wahl, S. M., Mergenhagen, S. E. & Martin, G. R. Proc. natn. Acad. Sci. U.S.A.
- 71, 3598-3601 (1974).

in certain animal species23; increased levels of collagenase have been associated with joint destruction in rheumatoid24,25 and osteoarthritic26 conditions. It seems reasonable to hypothesize that the involvement of mycoplasmas in disease states stems, at least in part, from their induction of collagenase.

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- 14. Wahl, L. M., Wahl, S. M., Sandberg, A. L. & Mergenhagen, S. E. Proc. natn. Acad. Sci. wani, L. M., wani, S. M., Sandberg, A. L. & Mergenhagen, S. E. Proc. n. U.S.A. 74, 4995-4958 (1977).
 Dayer, J.-M., Russell, R. G. & Krane, S. M. Science 195, 181-183 (1977).

- Newsome, D. A. & Gross, J. Cell 16, 895–900 (1979). Werb, Z. & Reynolds, J. J. J. exp. Med. 140, 1482–1497 (1974). Green, H. & Goldberg, B. Proc. natn. Acad. Sci. U.S.A. 53, 1360–1365 (1965).
- Peterkofsky, B. Archs Biochem. Biophys. 152, 318-328 (1972). Stanbridge, E. & Hayflick, L. J. Bact. 93, 1392-1396 (1967).
- Stanbridge, E. A. Rev. Microbiol. 30, 169-187 (1976). Cassell, G. H. & Cole, B. C. New Engl. J. Med. 304, 80-89 (1981).
- Cassail, G. H. & Cole, B. C., Gelman, M. I. & Ward, J. R. Excerpta med., Arthritis Rheum. 23, 825–836 (1980).
- Dayer, J.-M., Krane, S. M., Russell, G. G. & Robinson, D. R. Proc. natn. Acad. Sci. U.S.A. 73, 945-949 (1976).
- Steer, A. C. et al. Excerpta med., Arthritis Rheum. 23, 591-599 (1980).
 Erlich, M. G., Houle, P. A., Vigliani, G. & Mankin, H. J. Excerpta med., Arthritis Rheum 21, 761-765 (1978).
- 27. Nagai, Y., Lapiere, C. M. & Gross, J. Biochemistry 5, 3123-3130 (1966).
- 28. Jentoft, N. & Dearborn, D. G. J. biol. Chem. 254, 4359-4365 (1979) 29. Laemmli, U. K. Nature 227, 670-685 (1970).

Two distinct candidate transforming genes of lymphoid leukosis virus-induced neoplasms

Geoffrey M. Cooper* & Paul E. Neiman†

* Sidney Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA † The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104, USA

Avian lymphoid leukosis viruses (LLVs) are oncogenic retroviruses which induce tumours with relatively long latent periods and which seem to lack viral transforming genes1. We previously reported that high molecular weight DNAs of seven LLV induced tumours efficiently transformed NIH 3T3 mouse cells on transfection and that the transformed NIH cells did not contain viral DNA sequences detectable by hybridization with probes homologous either to the entire LLV genome or to the long terminal redundancy (LTR) of LLV DNA². These results suggested that oncogenesis by LLVs involved indirect activation of cellular transforming gene(s) which were not linked to LLV DNA. Hayward et al. recently found that 80-90% of LLVinduced metastatic bursal lymphomas contained exogenous LTR sequences near the cellular gene (c-myc) homologous to the presumptive transforming gene of myelocytomatosis virus strain MC29, a highly oncogenic avian acute leukaemia virus. Integration of LTR sequences containing the viral transcriptional promoter apparently increases transcription of c-myc in these lymphomas3. We now report that the LLV-induced tumours used as donors of DNA in our previous transfection experiments also contain LTR sequences near c-myc. However, further analysis of the NIH cells transformed by DNAs of LLV-induced bursal neoplasms indicates that transformation was not mediated by transfer of the tumour c-myc gene. These observations suggest that at least two different cellular genes with potential oncogenic activity are activated by different mechanisms in LLV-induced neoplasms.

DNAs of LLV-induced tumours were digested with EcoRI, electrophoresed in agarose gels, transferred to nitrocellulose filters and hybridized to 32P-DNA probes for either the 5' LTR sequences of LLV DNA (p53-5' probe) or for the chicken c-myc gene (pmyc probe, a gift of C. Pachl and M. Groudine) (Fig. 1).

32P-DNA of pmyc hybridized to two EcoRI fragments of normal chicken DNA with molecular sizes of ~14 and ~20 kilobases (kb) (Fig. 1). The 14-kb EcoRI fragment of normal chicken DNA is similar to the c-myc-containing EcoRI fragment4 and appears to contain all the chicken c-myc sequences. In addition to the 14- and 20-kb EcoRI fragments of normal chicken DNA, ³²P-DNA of pmyc hybridized to a tumour-specific EcoRI fragment in DNAs of two neoplastic bursal nodules (T^c and T^d 2993), a metastatic bursal lymphoma (2902) and a nephroblastoma (3000) (Fig. 1). In each case, the tumour-specific c-myc-containing EcoRI fragment also hybridized to p53-5' probe (Fig. 1). Similar results were obtained with DNAs of a second metastatic lymphoma (B7362) and of a cell line derived from such a lymphoma (RP9) (not shown).

These observations are consistent with the findings of Hayward et al.3. As an EcoRI site is present within the LTR of exogenous LLVs5,6, integration of an LTR near chicken c-myc in the tumour DNAs results in formation of a tumour-specific EcoRI fragment containing both 5' LTR and c-myc sequences.

EcoRI-digested DNAs of NIH cells2 transformed by DNAs of the lymphoma cell line RP-9, metastatic lymphoma B7362, bursal nodule Td 2993 and bursal nodule 2999 were hybridized to pmyc probe to determine whether the transformed NIH cells contained c-myc sequences derived from the tumour DNAs (Fig. 2). The c-myc-containing EcoRI fragment of 14 kb was readily detectable in normal chicken DNA, which was included as a positive control. In contrast, c-myc sequences were not detected in NIH cells transformed by LLV-induced tumour DNAs. Although we have detected normal mouse sequences homologous to chicken c-myc as faintly hybridizing EcoRI fragments of ~10 kb in NIH 3T3 DNA, these bands are too faint for photographic reproduction. The absence of chicken c-myc sequences in NIH cells transformed by LLV-induced tumour DNAs indicates that the transforming gene of these tumours detected by transfection is different from the c-myc gene activated in the tumours by integration of LTR sequences.

LLV-induced tumours thus appear to contain at least two different genes with potential oncogenic activity: a c-myc gene activated by integration of a viral LTR and a distinct cellular gene capable of efficiently transforming NIH 3T3 cells. As both genes are present in most or all LLV-induced tumours so far studied^{2,3}, including a nephroblastoma, premetastatic bursal nodules and metastatic bursal lymphomas, it would seem that both genes are important in LLV-induced oncogenesis.

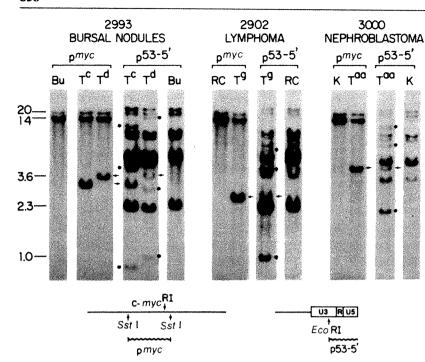


Fig. 1 Analysis of LLV-induced tumour DNAs. EcoRI-digested DNAs (10 μg) from two discrete bursal nodules $(\breve{T}^c \text{ and } T^d)$ and surrounding normal bursa (Bu)from bird no. 2993, from a metastatic bursal lymphoma (T8) and erythrocytes (RC) from bird no. 2902, and from a small nephroblastoma (Taa) and normal kidney from bird 3000 were subjected to agarose gel electrophoresis and Southern blot hybridization using ³²P-DNA probes prepared by nick-translation of pmyc and p53-5', pmyc contains a 3.2-kb Sstl fragment which was subcloned in a derivative of pBR322 containing a single Sst1 site from a bacteriophage λ clone of c-myc isolated from the λ library of random chicken DNA fragments prepared by Dodgson et al. 10, p53-5' was a fragment of p5311 and contains the sequences (165 bp) from the EcoRI site in viral LTR to the terminus of linear LLV DNA. Tumour-specific fragments which contain both c-myc and p53-5' sequences are indicated by arrows. Additional tumour-specific fragments containing p53-5 sequences are indicated by circles. Molecular sizes (kb) are indicated for the normal chicken DNA fragments detected with pmyc probe (14 and 20 kb) and for the internal EcoRI fragments of LLV DNA detected previously¹² with probes representative of the entire LLV genome (1.0, 2.3 and 3.6 kb).

Activated cellular transforming genes which are not linked to viral DNA have also been detected by transfection of DNAs of mammary carcinomas induced by mouse mammary tumour virus which, like LLV, induces tumours with long latent periods and does not appear to contain a viral transforming gene7.

The pathogenesis of LLV-induced lymphomas suggests a multi-step process probably involving more than a single transformation event8. Activation of c-myc may therefore be involved in a stage of tumour development which precedes or complements activation of the transforming genes detected by transfection. Further studies will be required to define the roles of these two potential transforming genes in the disease.

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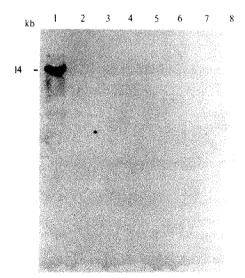


Fig. 2 Absence of chicken c-myc sequences in transformed NIH cells. EcoRI-digested DNAs (20 µg) of normal chicken embryo fibroblasts (lane 1), NIH 3T3 cells (lane 2), two independent lines of NIH cells transformed by DNA of bursal nodule Td 2993 (lanes 3 and 4), two independent lines of NIH cells transformed by DNA of bursal nodule 2999 (lanes 5 and 6), NIH cells transformed by DNA of metastatic bursal lymphoma B7362 (lane 7) and NIH cells transformed by DNA of lymphoma cell line RP9 (lane 8) were analysed by blot hybridization with pmyc probe.

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- Hanafusa, H. Compreh. Virol. 10, 401-483 (1977).
- Cooper, G. M. & Neiman, P. E. Nature 287, 656-659 (1980). Hayward, W. S., Neel, B. & Astrin, S. M. Nature 290, 475-480 (1981)
- Sheiness, D. K., Hughes, S. H., Varmus, H. E., Stubblefield, E. & Bishop, J. M. Virology 105, 415-424 (1980).
- 5. Hsu, T. W., Sabran, J. L., Marks, G. E., Guntaka, R. V. & Taylor, J. M. J. Virol. 28, 810-818
- Shank, P. R. et al. Cell 15, 1383-1395 (1978).
- Lane, M. A., Sainten, A. & Cooper, G. M. Proc. nam. Acad. Sci. U.S.A. 78 (in the press). Neiman, P. E., Payne, L. N., Jordan, L. & Weiss, R. A. Cold Spring Harb. Conf. Cell Proliferation 7, 519-528 (1980).

- Proliperation 1, 519-528 (1980).

 9. Southern, E. M. J. molec. Biol. 98, 503-517 (1975).

 10. Dodgson, J. B., Strommer, J. & Engel, J. D. Cell 17, 879-887 (1979).

 11. Neiman, P., Beemon, K. & Luce, J. A. Proc. natn. Acad. Sci. U.S.A. 78, 1896-1900 (1981).

 12. Neiman, P. E., Payne, L. N. & Weiss, R. A. J. Virol. 34, 178-186 (1980).

A tryptophan-containing peptide recognizes and cleaves DNA at apurinic sites

Tula Behmoaras, Jean-Jacques Toulmé & Claude Hélène

Laboratoire de Biophysique, INSERM U201, Muséum National d'Histoire Naturelle, 61, Rue Buffon, 75005 Paris, France

Oligopeptides containing aromatic amino acids can preferentially form stacked complexes with single-stranded nucleic acids^{1,2}. Moreover, the peptide lysyl-tryptophyl- α -lysine (Lys-Trp-Lys) binds efficiently to locally destabilized regions in DNA after UV irradiation^{3,4} or modification by N-acetoxy-N(2)-acetylaminofluorene⁵. Lys-Trp-Lys also photosensitizes the cleavage of pyrimidine dimers in DNA³. We have recently shown that removal of purines in DNA introduces strong binding sites for this tripeptide through very efficient stacking of the tryptophyl residue with nucleic acid bases at apurinic sites⁶. We report here that incubation of partly depurinated DNA with Lys-Trp-Lys results in a specific cleavage of the DNA backbone probably due to the presence of the amino groups brought by the peptide into close proximity with the apurinic sites. This could provide a model for the enzymatic activity of AP endonucleases which are involved in the first step of the in vivo repair of apurinic site-containing DNA7.8.

The binding of the peptide Lys-Trp-Lys (P) to nucleic acids (N) involves the formation of two complexes C1 and C2 according to a two-step model proposed earlier1:

$$P+N \stackrel{\kappa_1}{\Longrightarrow} C_1 \stackrel{\kappa_2}{\Longrightarrow} C_2$$

The tryptophyl ring of the peptide is stacked with nucleic acid bases in complex C₂ whereas only electrostatic interactions occur in complex C1. With a DNA containing apurinic sites the value of K_2 , which measures the ratio of the concentrations of stacked and unstacked complexes, is much higher than for any form of DNA damage previously studied^{3,6}. The values of K_2 for a native and an apurinic site are 0.3 and =200, respectively⁶. Because of the high value of K_2 and the unmodified value of K_1 (ref. 6), the overall association constant $[K_1 (1+K_2)]$ is more than two orders of magnitude higher for an apurinic site than for a native site. The peptide Lys-Trp-Lys can therefore recognize apurinic sites in a double-stranded structure through stacking interactions. The tryptophyl ring occupies the vacant site left by the removal of a purine6

To test the ability of Lys-Trp-Lys to cleave the DNA backbone at apurinic sites, an average of two purines were removed from supercoiled covalently closed PM2 DNA molecules (see Fig. 1 legend and ref. 9). Cleavage of one strand per DNA molecule converts the supercoiled DNA (form I) into the relaxed form (form II). As the electrophoretic mobilities of these two forms are different, agarose gel electrophoresis can be used to visualize single-strand breaks. Incubation of PM₂ DNA containing two apurinic sites in the presence of Lys-Trp-Lys resulted in a decrease in the intensity of the band corresponding to form I and a simultaneous increase in the intensity of the band corresponding to form II (results not shown). This effect was not

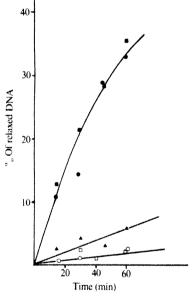


Fig. 1 Kinetics of single-strand break production in closed supercoiled PM2 DNA containing two apurinic sites on incubation at 37 °C with Lys-Trp-Lys (●), Lys-Tyr-Lys (■), Lys-Gly-Lys (▲) or alone in the same conditions (control, 0). PM2 DNA without apurinic sites was also incubated with Lys-Trp-Lys (□). PM2 DNA containing 70% supercoiled molecules was incubated for 8 min at 70 °C in 0.1 MNaCl, 10 mM phosphate, 10 mM citrate (pH 5) to introduce about two apurinic sites per molecule9. These DNA samples were precipitated with ethanol and redissolved in 1 mM NaCl, 0.2 mM EDTA and 1 mM sodium cacodylate (pH 6). 10 µl of 10^{-4} M peptide solutions were added to $10 \,\mu l$ of 2×10^{-4} M DNA substrate at 37 °C. After the indicated time the solutions were withdrawn and diluted with 6 µl of a solution containing 50% glycerol and 0.1% bromophenol blue. The mixtures were loaded onto 0.8% agarose gels and the gels stained with ethidium bromide. Agarose gels were photographed and negatives were scanned with a spectrodensitometer. The ratio of relaxed and supercoiled DNA was estimated from the areas under the corresponding peaks.

observed when native DNA without apurinic sites incubated with the peptide, indicating that the cleavage is related to the presence of apurinic sites in DNA. The amount of relaxed DNA molecules increased with the time of incubation: 35% of supercoiled DNA was cleaved after 60 min at 37 °C (Fig. 1). In the absence of Lys-Trp-Lys, incubation at 37 °C had a negligible effect on DNA containing apurinic sites (Fig. 1), whereas 1 h incubation of apurinic DNA with the peptide Lys-Gly-Lys resulted in the conversion of only 6% supercoiled DNA containing two apurinic sites to open DNA. The endonucleolytic activity is therefore clearly related to the specificity conferred to Lys-Trp-Lys by stacking interactions between the indole ring and nucleic acid bases, with the indole ring replacing the missing purine⁶. A tyrosine-containing peptide, Lys-Tyr-Lys, exhibited the same catalytic activity as Lys-Trp-Lys (Fig. 1), providing indirect evidence that the tyrosyl residue does stack with nucleic acid bases at apurinic sites.

Curves similar to those shown in Fig. 1 were obtained using fluorescence emission of intercalated ethidium bromide as a probe10. Intercalation of ethidium bromide molecules is favoured in relaxed relative to supercoiled DNA¹⁰. The ethidium bromide fluorescence emission was higher for apurinic DNA samples incubated with Lys-Trp-Lys than for DNA alone or for DNA incubated in the presence of Lys-Gly-Lys (results not shown). The time dependence of ethidium bromide fluorescence enhancement was similar to that of cleavage measured by gel electrophoresis.

The presence of an aromatic amino acid residue brings amino groups of lysyl residues into the vicinity of apurinic sites. At an apurinic site in DNA, the deoxyribose residue occurs in equilibrium between the free aldehyde and the furanose forms11, and the previously reported effects of amines on the rate of chain cleavage probably depend on an interaction with these aldehydic groups¹². The present data suggest that the specific binding of Lys-Trp-Lys to apurinic sites enhances the local amine concentration. The different amines investigated by Lindahl and Andersson which promoted chain breakage at apurinic sites in DNA were added at 10⁴-fold higher concentrations than those used with Lys-Trp-Lys¹²

The mechanism of Lys-Trp-Lys-induced strand breakage is unknown. Cleavage could involve the formation of a Schiff base between the aldehydic group of the ribosyl moiety at apurinic sites and an amino group of the peptide and/or a β -elimination reaction, as has been proposed for the cross-linking of histones to DNA after methylation followed by depurination¹³. To locate the NH₂ group involved in cleavage we are now investigating the activity of peptides in which the indole ring is separated from the potential 'active site' by glycyl residues (results to be reported elsewhere).

Our results show that the peptide Lys-Trp-Lys mimics both the damage specificity and the catalytic activity of endonucleases specific for apurinic sites (AP endonucleases). Stacking interactions provide a simple tripeptide with the essential recognition specificity required for this activity.

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- Brun, F., Toulmé, J. J. & Hélène, C. Biochemistry 14, 558-563 (1975)
- 2. Mayer, R., Toulmé, F., Montenay-Garestier, T. & Hélène, C. J. biol. Chem. 234, 75-82
- 3. Toulmé, J. J., Charlier, M. & Hélène, C. Proc. nam. Acad. Sci. U.S.A. 71, 3185-3188
- 4. Toulmé, J. J. & Hélène, C. J. biol. Chem. 252, 244-249 (1977)
- Toulmé, F., Hélène, C., Fuchs, R. & Daune, M. Biochemistry 19, 870-875 (1980).
 Behmoaras, T., Toulmé, J. J. & Hélène, C. Proc. nam. Acad. Sci. U.S.A. 78, 926-930
- 7. Laval, J. Nature 269, 829-832 (1977).
- Verly, W. G. & Rassart, E. J. biol. Chem. 250, 8214-8219 (1975).

- Verty, W. U. & Russart, E. J. mat. Crem. 250, 5214-523 (1972).
 Lindahl, T. & Nyberg, B. Biochemistry 11, 3610-3618 (1972).
 Paoletti, C., Le Pecq, J. B. & Lehman I. R. J. molec. Biol. 55, 75-100 (1971).
 Overend, W. G. J. chem. Soc., 2769 (1950).
 Lindahl, T. & Andersson, A. Biochemistry 11, 3618-3623 (1972).
 Mirsabekov, A. D., Shick, V. V., Belyavsky, A. V. & Bavykin, S. G. Proc. nam. Acad. Sci. 17 C A 78, 4184-4188 (1078). U.S.A. 75, 4184-4188 (1978).

Sequence specificity of methylation in higher plant DNA

Yosef Gruenbaum, Tally Naveh-Many, Howard Cedar & Aharon Razin

Department of Cellular Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel 91010

Although plant DNA has a high content of 5-methylcytosine (5mC¹) very little is known about the distribution of this modification. Many of these methylations are found in the dinucleotide sequence C-G (refs 2-5) which is also the major modified site in animal cell DNA. It is clear, however, that C-G methylation cannot account for all the 5mC in DNA, in which this modified base may represent over 30% of the cytosine residues (as in certain varieties of plant¹). It was this observation that prompted the search for other methylated sites in the DNA. The results presented here show that methylated cytosine is indeed present at a variety of cytosine-containing dinucleotides, all of which, however, are part of the basic trinucleotide C-X-G. This sequence is probably essential for modification as it provides the symmetrical cytosines necessary for ensuring the inheritance of methylation at these sites.

The first step in characterizing plant DNA methylation was to determine the dinucleotide distribution of all the 5mC present in this organism. This was achieved by a modification of the standard nearest neighbour analysis which allows the detection of 5mC⁶. Plant DNA contains 5mC at C-A, C-T and C-C as well as C-G sites and the extent of methylation at these sequences is summarized in Table 1 (all sequences are in the $5' \rightarrow 3'$ direction). In lieu of the nearest neighbour frequencies for this DNA⁷, the methylation observed in Table 1 accounts for the total methylcytosine of wheat-germ DNA, $\sim 23-29\%$ of all cytosines¹. Similar results were obtained for 2-week seedling wheat DNA and for other higher plant varieties, including tobacco.

Studies on the inheritance of 5mC in animal cells stongly suggest that the symmetrical presence of 5mC on both strands of the DNA is necessary to ensure the inheritance of this modification, at least at the sequence C-G. Because none of the dinucleotides C-A, C-T or C-C can accommodate this type of symmetry, we postulated that methylation at these sites might be based on a trinucleotide symmetry at the sequence C-X-G. This hypothesis is supported by the nearest-neighbour analyses for C-A and C-T (see Table 1), as it predicts that these dinucleotides

will be found in the sequence $\frac{C-A-G}{G-T-C}$ and would therefore be

methylated to the same extent. To examine this possibility, we developed an extension of the nearest-neighbour analysis technique which allows the detection of methylation at specific cytosine containing trinucleotide sequences. As shown in Table 1, over 80% of the trinucleotide sequences C-T-G and C-A-G were found to be modified, whereas the non-symmetrical sequence C-A-T was less than 4% methylated.

These partial sequence data strongly suggest that most, if not all, of the methylated C-A and C-T sequences in plant DNA are found adjacent to G in the prototype symmetrical trinucleotide

 $\begin{cal}C-A-G\\G-T-C\end{cal}$ To validate this observation, we searched for restriction

enzymes which might recognize this site and be sensitive to cytosine methylation. Several restriction enzymes have recognition sequences which contain the sequence C-G but do not cleave when this site is methylated, for example, *HpaII*, *HhaI* and *AvaI* which have been used to analyse the methylation pattern of total mammalian DNA as well as specific genes^{8,9}. As expected, both *HpaII* and *HhaI* cleave plant DNA very poorly (data not shown). Individual restriction enzymes may be useful for detecting methylation at other sites. *Eco* RII, and *PstI* have

recognition sequences containing the trinucleotide sequence C-A-G, but do not cleave when cytosine is methylated¹⁰. As

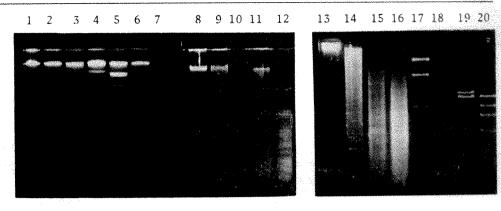
shown in Fig. 1, wheat-germ and tobacco DNA are very poorly digested by these three enzymes, all of which digest animal DNA as expected. To determine the extent of methylation at these and other restriction sites, it was necessary to quantitate the degree of DNA digestion. To this end, the average molecular size of the

Table 1 Nearest-neighbour analysis of 5mC in plant DNA

DNA sequence	% Methylation	
C-G	82	
C-A	19	
C-T	19	
C-C	7	
C-A-G	>80	
C-T-G	>80	
C-A-T	<4	

DNA from wheat-germ was prepared as follows. Frozen wheat germ was ground under liquid nitrogen by mortar and pestle. The powder was dissolved in a mixture containing 10 mM Tris-HClpH 7.9, 0.4 M NaCl, 5 mM EDTA, 0.5% SDS and 100 μg ml⁻¹ proteinase K (Merck), incubated for 1 h at 37 °C, extracted three times with phenol followed by three extractions with chloroform/isoamyl alcohol (24:1 v/v) and ethanol precipitated. The DNA preparation was treated for 30 min with RNase A and further extracted with phenol and chloroform/isoamyl alcohol. The plant DNA (5 µg) was randomly nicked by sonication and nick translated with E. coli polymerase I (Biolabs) in the presence of a single $[\alpha^{-32}P]$ -deoxynucleoside triphosphate (200–500 Ci mmol⁻¹, NEN)⁶. The $[\alpha^{-32}P]$ -labelled DNA was digested to deoxynucleoside 3' monophosphates using micrococcal nuclease and spleen phosphodiesterase and applied to thin layer cellulose sheets (Eastman Kodak). The nucleotides were separated by two-dimensional chromatography and analysed by autoradiography as previously described⁶. Following TLC, the cytosine and 5-methylcytosine spots were scraped from the plastic sheet and the amount of radioactivity in each was determined by liquid scintillation counting. The percentage of cytosine methylation for each dinucleotide was then calculated $[(C/5mC+C)\times$ 100]. In each case the nearest-neighbour frequencies of other dinucleotides appearing on the chromatogram were also measured to ensure that these correspond to published data on nearest-neighbour analyses. Partial trinucleotide sequencing was performed using the same basic technique modified to select specific trinucleotide sequences. DNA was nick translated in the presence of a single nucleotide as described above using either $[\alpha^{32}P]dATP$ or $[\alpha^{32}P]dTTP$. Following a 5-min incubation at 15 °C, ddATP, ddCTP and ddTTP were added (6 mM each) and the reaction was continued for an additional 30 min. After this treatment only sequences containing Xp*TpG or Xp*ApG will have a free 3'-OH group. The DNA was isolated by phenol extraction, Sephadex column chromatography and ethanol precipitation. This DNA was denatured and those molecules containing a free 3'-OH end were elongated with poly(dA) tails using 18 units terminal transferase (PL Biochemicals). The specific molecules which contained the desired labelled trinucleotide sequence were then isolated by oligo(dT) cellulose chromatography²⁰. This DNA was then subjected to nearest-neighbour analysis⁹. Whereas ~20% of the C-A or C-T residues in plant DNA were found to be methylated, >80% of the trinucleotide sequence C-T-G and C-A-G were modified. In keeping with this, the DNA which did not stick to the oligo(dT) column and therefore did not contain poly(A) tails was only 5% methylated. This, of course, would include DNA containing all Cp*A and Cp*T residues which are not adjacent to G. For the C-A-T triplet, the procedure was modified slightly by using the dideoxynucleotides ddATP, ddGTP and ddCTP and the standard deoxynucleotide dTTP to select the correct sequence. The results are expressed as the percentage of methylation of the cytosine present in each sequence and should be considered as reliable estimates of the methylation content. The values shown for these triplets were obtained from the chromatogram and adjusted to take into account the instances where the second nucleotide is repeated. Thus, in addition to C-A-G the sequences C-A-A-G and C-A-A-G will also be selected by the oligo(dT) column and therefore contaminate the desired triplet. As indicated in Table 1, the values represent minimal estimates because these data have not been corrected for the fact that the dideoxynucleotide triphosphates are not 100% efficient even at the high concentration used in this experiment (unpublished results).

Fig. 1 Detection of plant DNA methylation by restriction enzyme analysis. DNA was prepared as described in Table 1 legend and digested by various restriction enzymes at a ratio of 2 units per ug DNA for 2 h using the buffers and conditions recommended by the suppliers. Restricted DNA was separated by electrophoresis on 1.5% (lanes 1-12) or 1.8% (lanes 13-20) agarose (Seakem) gels stained with ethidium bromide and photographed using Polaroid positive-negative type 665 film. Undigested tobacco DNA (lane 1) and undigested wheat-germ DNA (lanes 8 and 13) are shown for comparison. Tobacco DNA was cleaved with Pst I (lane 2), PvuII (lane 3), Eco RII (lane 6) and Bst NI (lane 7). Tobacco DNA was also digested with



Pst I (lane 4) and Pvu II (lane 5) in the presence of the marker λ phage DNA. Wheat-germ DNA was cleaved with EcoRII (lane 9), BstNI (lane 10), MspI (lane 14), HaeIII (lane 15) and Taq I (lane 16). This DNA was also digested with EcoRII (lane 11) and BstNI (lane 12) in the presence of the marker herpes simplex virus DNA. Lanes 17-20 contain molecular weight markers (250-4,500 bp) obtained by restriction digestion of various plasmid and phage DNAs.

DNA resulting from each restriction enzyme digestion was calculated from optical density scans of the ethidium bromidestained agarose gels. The average experimental length of DNA obtained for each restriction enzyme can then be compared with the expected size computed on the basis of nearest-neighbour frequencies. For the enzymes HpaII and HhaI the experimental length is 10 times greater than expected, indicating that this site is $\sim 90\%$ methylated in wheat-germ DNA. The restriction sites for PstI, PvuII and EcoRII were also found to be extensively methylated (Table 2). Note that the degree of methylation at PstI and PvuII sites represents an underestimate, because the average size of the DNA resulting from these digestions is probably higher, but not within the range of sensitivity for this particular gel. In the case of EcoRII the decreased digestion cannot be due to a lack of sites for this enzyme, because Bst NI, an isoschizomer of EcoRII that cleaves even if cytosine is methylated, cleaves this DNA normally (Fig. 1, Table 2).

In addition to methylation at C-A, C-T and C-G dinucleotides, we observed a small amount of methylation at C-C. It is tempting to suggest that these methylations are also localized in the trinucleotide site $\frac{C-C-G}{G-G-C}$. One might expect that both

cytosines could be methylated in this sequence, as the internal cytosine is part of a C-G dinucleotide. The enzyme *MspI* (C-C-G-G), which is inhibited by methylation at the external cytosine¹⁰, may be useful for probing methylation at C-C-G sites. The average expected molecular size of plant DNA after digestion with *Msp I* is 440 base pairs (bp). As shown in Fig. 1 and Table 2, the average size of DNA resulting from *MspI* digestion is over twice that expected, suggesting that the external cytosine of this site is about 50% methylated.

BstNI may be used to visualize directly the extent of methylation at $\frac{C-A-G}{G-T-C}$ sequences. As this enzyme cleaves the pentanucleotide sequence $CC \stackrel{\downarrow}{T} GG$ on the 3' side of the inter-

nal C, restriction digestion leaves DNA tails consisting of one nucleotide, A or T. These tails may be labelled with $[\alpha^{-32}P]$ dATP or $[\alpha^{-32}P]$ dTTP using the large subunit of *Escherichia coli* DNA polymerase I. When this DNA is subjected to nearestneighbour analysis, the labelled phosphate is transferred to the internal C of the EcoRII sequence. If this cytosine is methylated, it should be revealed by chromatographic analysis of the 3'-monophosphate nucleotides. Autoradiography clearly shows that cytosine is the only labelled nucleoside phosphate, and that it is extensively methylated (90%) (Fig. 2). This experiment

provides direct evidence that this particular site is highly methylated and suggests that other ${C \cdot A \cdot G \over G \cdot T \cdot C}$ sites are similarly

modified. Because the cytosine residues adjacent to both A and T are 90% methylated, we also conclude that this site is symmetrically methylated on both DNA strands.

In animal cells 5mC accounts for about 2-7% of the total cytosine¹¹, whereas over 25% of the cytosine residues are methylated in plant cells¹. This difference is due to two major factors. Although in both plants and animals the C-G sequences are methylated to about 70-80%, the C-G dinucleotide is much more frequent in Plant DNA (3-4%) than animal DNA (0.5-1%)⁷. The second element which contributes to the high 5mC content of plants is methylation at other sites. Partial sequence analysis and restriction enzyme studies clearly show that almost all methylated C-A, C-C and C-T residues are found in the symmetrical trinucleotide sequence C-X-G.

It has previously been noted that C-G is a dinucleotide which contains symmetrical cytosine residues in both strands of the DNA and other evidence suggests that when this dinucleotide is modified both cytosines are indeed methylated^{9,12}. This symmetry is probably what enables the cell to transmit its methyl-

Table 2 DNA methylation at specific restriction enzyme sites

	-				and the second second second second
Source of DNA		Restriction enzyme	Expected size (bp)	Experimental size (bp)	% Methyl- ation
Wheat germ	Hpall	(CCGG)	440	5,100	90
	EcoRII	$(CC_{\mathbf{T}}^{\mathbf{A}}GG)$	530	6,000	90
	Bst NI	$(CC_{\overline{T}}^{A}GG)$	530	550	nah-
	Pst I	(CTGCAG)	3,870	15,000	70
	$Pvu\Pi$	(CAGCTG)	3,870	14,000	70
	Mspl	(CCGG)	440	920	50
	TaqI	(TCGA)	. 380	330	
	Hae III	(GGCC)	330	390	enter.
Mouse liver	Pst I	(CTGCAG)	3,250	3,900	lien
	PvuII	(CAGCTG)	3,250	3,900	

Wheat-germ or mouse liver DNA was digested with various restriction enzymes at a ratio of 2 enzyme units per µg DNA for 2 h in the specific buffers recommended by the enzyme manufacturers (New England Biolabs, Bethesda Research Laboratories and Boehringer-Mannheim). The restricted DNA (5-10 μg) was subjected to gel electrophoresis on 0.6% agarose (HpaII, EcoRII, BstNI, PstI, PvuII), 1.5% agarose (MspI, BstNI) or 1.8% agarose (MspI, TaqI, BstNI, HaeIII). Each gel was run together with at least 20 molecular length marker bands obtained by restriction enzyme digestion of various plasmid and phage molecules. Following electrophoresis, the ethidium bromidestained gels were photographed using positive-negative Polaroid film and the film negative assayed using a scanning spectrophotometer. The resulting graphs were computer digitalized and the average molecular size calculated by reference to the molecular weight markers20 results were then corrected to take into consideration the size of the undigested DNA which was of the order of 50 kilobases. These results are accurate to about ±10%. Expected average molecular size of each restriction enzyme was calculated from nearest-neighbour data. The per cent methylation at each of these sites was determined by comparing the experimental and the expected size.

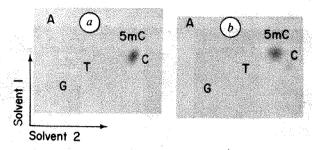


Fig. 2 The extent of methylation of the sequence CC_TAGG in wheat-germ

DNA. DNA (5 μ g), prepared as described in Table 1 legend, was cleaved with 10 units of BstNI for 60 min at 60 °C. The DNA fragments were labelled at their 3' end by filling the sticky ends using E. coli DNA polymerase I (large fragment) with either $[\alpha^{32}P]dATP(a)$ or $[\alpha^{32}P]dTTP(b)$ for 10 min at 37 °C (ref. 21). The labelled fragments were digested to deoxynucleoside 3' monophosphates, chromatographed by two-dimensional TLC and autoradiographed6.

ation pattern to succeeding generations. In general, replicating DNA is in a state of hemimethylation since the parental strand is methylated at specific sites, which the newly replicated strand is as yet unmodified. The cellular 'maintenance methylase' would then act on the new strand using the parental strand methylation pattern as template 11,13-15. This model has received recent support from experiments in which unmethylated and in vitro methylated DNA were introduced into mouse L-cells by DNAmediated gene transfer. Whereas unmethylated DNA remains unmodified, methylated foreign DNA faithfully inherits its in vitro acquired methylated pattern16,17. We propose that the same symmetry that characterizes the dinucleotide C-G must be an integral part of any inheritable methylated residue. All the methylated sequences in plants seem to fit this criterion, as all methylations are either at the dinucleotide sequence C-G or the symmetrical trinucleotide sequence C-X-G.

The site ${C\text{-}C\text{-}G}\atop{G\text{-}G\text{-}C}$ may be methylated at either of two different

cytosines or at both. In either event, the single cytosine on the complementary strand would have to serve as a template for both cytosine methylations. The fact that this single methylated cytosine is part of a C-G residue suggests that in such cases the internal cytosine on the C-C-G strand will always be methylated, but the external cytosine may or may not be modified. This was indeed found to be the case for Scilla satellite DNAs analysed for methylation by DNA sequencing⁵. Methylation at the external cytosine of the trinucleotide C-C-G raises problems with regard to the inheritance of this methylation. As the complementary strand used as a template during replication contains only one methylated cytosine in the sequence C-C-G, it is difficult to understand how the methylase decides whether or not to methylate the external cytosine in addition to the internal cytosine. This decision may be random or determined by adjacent DNA sequences. Note that two specific C-C-G-G sites in the human globin gene battery were found to be resistant to MspI and therefore methylated at the external cytosine in a tissue-specific manner^{18,19}. Because, in general, vertebrate DNA does not contain a detectable amount of C-C methylations, this type of animal DNA methylation is probably very rare.

As in vertebrate cells, the methylatable sites of plant DNA are not fully methylated. Approximately 80% of all C-G or ${\buildrel C-A-G}$ sites are methylated and only 50% of the C-C-G sites are modified at the external cytosine. It is this incomplete methylation which makes these sites potential factors in cellular regulation. A large body of evidence supports the view that DNA methylation may indeed play a part in the control of vertebrate gene expression. It is tempting to speculate that both the methylated C-G and C-X-G sites may also be involved in the regulation of gene expression in higher plants, but there is no evidence to support this. The enzymes PstI, PvuII and EcoRII

should prove useful for studying the relationship between gene expression and methylation in plant cells.

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- Shapiro, H. S. CRC Handbk Biochem. molec. Biol. 2, 259 (1976).
- Bonen, L., Huh, T. Y. & Gray, T. W. FEBS Lett. 111, 340-346 (1980). Burton, W. G., Grabowy, C. T. & Sager, R. Proc. natn. Acad. Sci. U.S.A. 76, 1390-1394
- Royer, H. D. & Sager, R. Proc. natn. Acad. Sci. U.S.A. 76, 5794-5798 (1979).
- Deumling, B. Proc. natn. Acad. Sci. U.S.A. 78, 338-342 (1981).
 Gruenbaum, Y., Stein, R., Cedar, H. & Razin, A. FEBS Lett. 124, 67-71 (1981).
 Setlow, P. CRC Handbk Biochem. molec. Biol. 2, 312-318 (1976).
 Bird, A. P. & Southern, E. M. J. molec. Biol. 118, 27-48 (1978).

- Cedar, H., Solage, A., Glaser, G. & Razin, A. Nucleic Acids Res. 6, 2125-2132 (1979). Gruenbaum, Y., Cedar, H. & Razin, A. Nucleic Acids Res. 9, 2509-2515 (1981).
- Razin, A. & Riggs, A. D. Science 210, 604-610 (1980).
- Bird, A. P. J. molec. Biol. 118, 46-60 (1978).
- Riggs, A. D. Cytogenet. cell. Genet. 14, 9-14 (1975). Holliday, R. & Pugh, J. E. Science 187, 226-232 (1975).
- Razin, A. & Friedman, J. Prog. Nucleic Acids Res. molec. Biol 25, 33-52 (1981).
 Pollack, Y., Stein, R., Razin, A. & Cedar, H. Proc. nam. Acad. Sci. U.S.A. 77, 6463-6467 (1980)

- Wigler, M., Levy, D. & Perucho, M. Cell 24, 33-40 (1981).
 van der Ploeg, L. H. T. & Flavel, R. A. Cell 19, 947-958 (1980).
 van der Ploeg, L. H. T., Groffen, J. & Flavel, R. A. Nucleic Acids Res. 20, 4563-4574 (1980)
- Naveh, T. & Cedar, H. Proc. natn. Acad. Sci. U.S.A. (in the press)
 Sneider, W. T. Nucleic Acids Res. 8, 3829-3840 (1980).

Active multi-subunit ACh receptor assembled by translation of heterologous mRNA in Xenopus oocytes

K. Sumikawa, M. Houghton, J. S. Emtage*, B. M. Richards & E. A. Barnard†

Molecular Genetics Department, Searle Research and Development, PO Box 53, Lane End Road, High Wycombe, Buckinghamshire HP12 4HL, UK

† Department of Biochemistry, Imperial College, London SW7 2AZ, UK

The acetylcholine receptor (AChR) mediates synaptic transmission on binding to the acetylcholine neurotransmitter. To gain further information on the composition and biosynthesis of this receptor (for review of properties see refs 1-3), we have extracted AChR mRNA and translated it in a cell-free system and also in Xenopus oocytes. The latter have been shown to be not only highly efficient translation systems for microinjected, heterologous mRNAs but they can also faithfully execute posttranslational processes such as glycosylation, sequestration, prepeptide cleavage and secretion of appropriate products⁴⁻⁷. As native AChR is found glycosylated and sequestered in the plasma membrane, this system is of potential interest in studying its biosynthesis. We report here that Xenopus oocytes efficiently assemble intact, multi-subunit AChR molecules which show properties characteristic of the native AChR, including the binding of α -bungarotoxin (α -BTX).

Initially, cell-free translation⁸ products of mRNA extracted from the electric organ of the ray, Torpedo marmorata, were immunoprecipitated with rabbit antiserum raised against the native AChR. AChR-specific polypeptides with apparent molecular weights (MWs) of 40,000 (40 K), 51 K and 59 K were clearly identified; two smaller and less abundant species were also found (Fig. 1). These sizes differ from those of the subunits thought to comprise native AChR1-3; unlike the latter, the cell-free translation products were unable to bind α -BTX, which agrees with previous work9. Thus, the cell-free system seems to be incapable of faithfully synthesizing intact receptor molecules.

Experiments in which Torpedo mRNA was injected into Xenopus oocytes gave better results. Figure 2 shows that

^{*} Present address; Celitech Ltd. 250 Bath Road, Slough, Berkshire, SL1 4DY, UK

 α -BTX binding activity could be detected in the translation products of the oocytes, the actual level of activity increasing with the amount of heterologous mRNA injected. To characterize the α -BTX binding component, ³⁵S-methionine-labelled translation products were purified by affinity chromatography on α -BTX-Sepharose (for details see Fig. 3 legend), and analysed by SDS-polyacrylamide gel electrophoresis ¹⁰ (Fig. 3b, lane 1). As a control for nonspecific binding to the Sepharose, an aliquot of the extract was preincubated with a large excess of α -BTX before chromatography and electrophoresis (Fig. 3b,

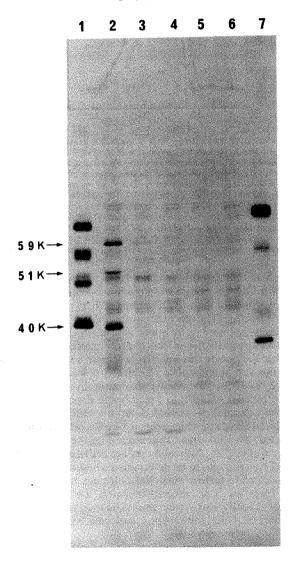


Fig. 1 Electrophoretic analysis of AChR polypeptides synthesized in a cell-free system. Poly(A) mRNA (25 µg ml⁻¹) extracted from the electric organ of Torpedo marmorata 16 was translated in a nuclease-treated rabbit reticulocyte lysate8 in the presence of 35S-methionine (~1 mCi ml-1). The translation products were immunoprecipitated using formalin-fixed Staphylococcus aureus cells (SAC)¹³. The SAC antibody-antigen complexes were dissolved in SDS sample buffer ¹⁰ and SAC removed by centrifugation at 10,000g for 1 min. The supernatants were electrophoresed through a 10% (w/v) SDS-polyacrylamide gel10 before fluorography17. Lane 1, AChR purified from T. marmorata electric organ by affinity chromatography then radioactively-labelled with succinimidyl [2,3-3H]propionate11. radiolabelling process gives the appearance of doublets after gel electrophoresis, which are absent in non-radioactive purified preparations of the native AChR (see Fig. 3a). Lane 2, immunoprecipitate obtained with antibody raised against native Torpedo AChR. Lane 3, immunoprecipitate obtained with antibody raised against native Torpedo AChR but in the presence of a large excess of native Torpedo AChR; control for lane 2. Lane 4, immunoprecipitate obtained with normal rabbit serum; lane 5, immunoprecipitate produced with antibody against α-BTX (control for lane 6). Lane 6, immunoprecipitate obtained by pre-incubating with α -BTX, before addition of antibody raised against α -BTX. Lane 7, ¹²⁵I-labelled standard proteins-bovine serum albumin (68 K), catalase (58 K), ovalbumin (43 K) and lactate dehydrogenase (35 K). Titres of antibody raised against native Torpedo AChR and α -BTX were $\sim 2 \times 10^{-6}$ M and $\sim 4 \times 10^{-6}$ M, respectively.

lane 2). Lanes 1 and 2 of Fig. 3b show four toxin-specific polypeptides of MWs 40 K, 49 K, 58 K and 66 K; these sizes are very similar to those of the native Torpedo AChR polypeptides that had been purified by α -toxin-gel affinity chromatography and then radioactively labelled by H-propionylation (Fig. 3b, lane 3). The native subunit sizes (Fig. 3a) were estimated to be 40 K, 49 K, 57 K and 65 K, as generally found for the α , β , γ and δ subunits heart similar electrophoresis pattern was also obtained when oocyte translation products were purified by binding to α -BTX, followed by immunoprecipitation with α -BTX antibody and protein A¹³.

The size of the AChR molecule synthesized in oocytes was analysed by centrifugation through sucrose density gradients. As Fig. 4 shows, the AChR purified from the Torpedo electric organ exists in two molecular forms having sedimentation coefficients of 9S and 13S (monomer and dimer), an observation generally made 1-3 for Torpedo AChR preparations, the 9S form being predominant in reducing conditions. The monomeric 9S form has been shown^{2,3,12} to contain five subunits $(\alpha_2, \beta, \gamma, \delta)$, corresponding to a MW of ~250,000. It was therefore interesting that on a parallel sucrose density gradient (Fig. 4), the translation products from the microinjected oocytes produced a peak of toxin-binding activity at the same position (9S) as the native monomer. This result clearly indicates the ability of the oocyte to assemble the newly synthesized subunits into intact receptor molecules. After sedimentation of 35S-methioninelabelled translation products and immunoprecipitation of these gradient fractions with the antiserum raised against native AChR, SDS-gel electrophoresis showed that the receptorspecific peptides were present only in the 9S peak (data not shown). Assuming that this antibody can recognize newly synthesized, unassembled AChR polypeptides, which is the

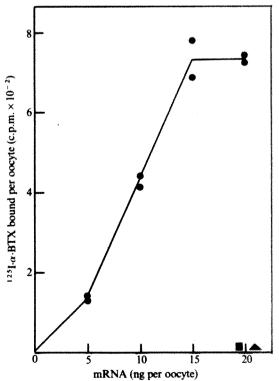


Fig. 2 α-BTX binding activity in Xenopus oocytes after microinjection with Torpedo mRNA. Duplicate batches of 15 oocytes were microinjected⁵ with various amounts of poly(A) mRNA that had been extracted¹⁶ from the electric organ of T. marmorata and cultured at 21 °C in 150 μl modified Barths' medium⁵ for 48 h. The oocytes were then homogenized in 200 μl of 50 mM phosphate buffer, pH 7.2/1% Triton X-100/1 mM EDTA/1 mM EGTA/0.1 mM phenylmethylsulphonyl fluoride (PMSF). The 1251-α-BTX¹⁸ binding activity (♠) in the resulting supernatants was measured¹⁹ after centrifugation at 10,000g for 30 min. ♠, Control in which a large excess of unlabelled α-BTX was added just before the assay. ♠, As for experimental sample except that before the assay, the extract was incubated with Con A-Sepharose²⁰.

likely interpretation of the data from the cell-free system (see Fig. 1 and ref. 9), it seems that the assembly process in oocytes is efficient.

As in the case of the native Torpedo receptor 12,14, the AChR synthesized in microinjected oocytes is also glycosylated, as it binds completely to concanavalin A(ConA)-Sepharose (Fig. 2). The y-subunit synthesized in oocytes may be slightly larger than that in the purified native AChR, thus it is possible that glycosylation and/or other processing events in the oocyte are not

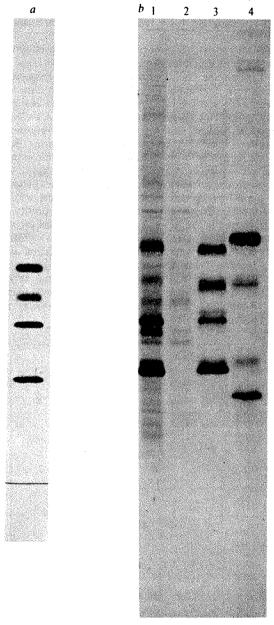


Fig. 3 Electrophoretic analysis of the AChR synthesized in oocytes as purified by affinity chromatography on α -BTX-Sepharose. a, A stained gel containing nonradioactive AChR purified from T. marmorata 11 . (Note that this gel is shorter than that in b.) b, Batches of 40 oocytes were microinjected with *Torpedo* poly(A) mRNA (20 ng per oocyte) and cultured in 300 μl Barths' medium containing 0.3 mCi ³⁵S-methionine at 21 °C for 48 h. They were then homogenized in 500 µl of 50 mM phosphate buffer, pH 7.2/1% Triton X-100/1 mM EDTA/1 mM EGTA/5 µg ml-1 soybean trypsin inhibitor/1 mM benzamidine/100 µg ml-1 bacitracin/0.1 mM PMSF, centrifuged at 10,000g for 30 min. The supernatant was shaken with 0.1 ml α -BTX-Sepharose, which was then washed 11 . All purifications were done as rapidly as possible in the cold and in the presence of protease inhibitors to limit proteolysis¹¹. Lane 1, AChR was eluted from the α -BTX-Sepharose using $50 \,\mu$ l of 2% SDS in sample buffer¹⁰, and 25 μ l electrophoresed. Lane 2, a control preparation containing the same ³⁵S-labelled translation products, preincubated with a large excess of α-BTX before application to α -BTX-Sepharose. Lane 3, 3 H-propionylated native *Torpedo* AChR (see Fig. 1). Lane 4, 125 I-labelled standard proteins (see Fig. 1). A 10% (w/v) SDS-polyacrylamide gel 10 was used; b was subjected to fluorography 17 and ato silver staining2

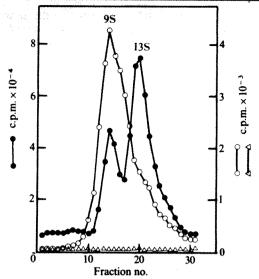


Fig. 4 Analysis of the molecular form of the AChR newly synthesized in oocytes. 40-50 oocytes were microinjected with 20 ng of mRNA per oocyte and cultured for 48 h. The oocytes were then extracted in 200 µl buffer as described in Fig. 2 legend. Samples of oocyte extracts or of AChR purified from *T. marmorata* were loaded on to 5-20% (w/w) sucrose gradients containing 50 mM phosphate buffer, pH 7.2/100 mM NaCl/0.2% Triton X-100/1 mM EDTA/0.02% NaN₃ and centrifuged in a Beckman SW60 Ti rotor at 59,000 r.p.m. for 6 h (ref. 11). Fractions (0.13 ml) were collected by upward displacement and analysed for α-BTX binding activity using 125 I-α-BTX as described in Fig. 2 legend. , Purified AChR from Torpedo; A, extract from uninjected oocytes; O, extract from oocytes injected with Torpedo mRNA

completely faithful for this product. However, when detergent (Triton X-100) was omitted from the homogenization medium (Fig. 2), over 90% of the α -BTX binding activity remained in the membrane fraction. The receptor molecule is transported in vivo to the plasma membrane by a mechanism which shares many common features with the process of protein secretion 15. As it is known that the oocyte can selectively secrete a wide variety of homologous and heterologous proteins^{6,7}, it seems likely from the above result that the oocyte also faithfully sequesters the newly synthesized AChR into a membrane structure.

Thus the heterologous mRNA determines the production of multi-subunit receptors which seem to be efficiently synthesized, glycosylated, assembled and sequestered by the oocyte with a high degree of fidelity. Although they are active in that they can bind α -BTX, it remains to be determined whether the receptors synthesized in oocytes are functional molecules in terms of being able to form an acetylcholine-mediated ion channel in a membrane. Clearly, this system should be used to elucidate the mechanisms by which the receptor molecule is assembled and inserted into the membrane.

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- Heidmann, T. & Changeux, J. P. A. Rev. Biochem. 47, 317-357 (1978).
 Karlin, A. in Cell Surface Reviews Vol. 6 (eds Poste, G., Nicolson, G. L. & Cotman, C. W.) 192-242 (North Holland, New York, 1980).
 Raftery, M. A., Hunkapiller, M. W., Strader, C. D. & Hood, L. E. Science 208, 1454-1456
- 4. Gurdon, J. B., Lane, C. D., Woodland, H. R. & Marbaix, G. Nature 233, 177-182 (1971)
- Gurdon, J. B. The Control of Gene Expression in Animal Development (Clarendon, Oxford
- Lane, C. D., Shannon, S. & Craig, R. Eur. J. Biochem. 101, 485-495 (1979). Lane, C. D. et al. Eur. J. Biochem. 111, 225-235 (1980).
- Lane, C. D. et al. Eur. J. Biochem. 111, 225-235 (1980).
 Pelham, H. R. B. & Jackson, R. J. Eur. J. Biochem 67, 247-256 (1976).
 Mendez, B., Valenzuela, P., Martial, J. A. & Baxter, J. D. Science 209, 695-697 (1980).
 Laemmli, U. K. Nature 277, 680-685 (1970).
 Sumikawa, K., Barnard, E. A. & Dolly, J. O. Eur. J. Biochem. (submitted).
 Lindstrom, J., Merlie, J. & Yogeeswaran, G. Biochemistry 18, 4465-4469 (1979).
 Ivarie, R. D. & Jones, P. P. Analyt. Biochem. 97, 24-35 (1979).
 Vandlup, P. L. W. W. C. S. Eigensch, L. G. Bratter, M. A. Biochemistry 18, 1845-1856.

- Vandlen, R. L., Wu, W. C. S., Eisenach, J. C. & Raftery, M. A. Biochemistry 18, 1845-1854

- Rotundo, R. L. & Fambrough, D. M. Cell 22, 595-602 (1980).
 Houghton, M. et al. Nucleic Acids Res. 8, 1913-1931 (1980).
 Laskey, R. A. & Mills, A. D. Eur. J. Biochem. 56, 335-341 (1975).
 Vogel, Z., Sytkowski, A. J. & Nirenberg, M. W. Proc. natn. Acad. Sci. U.S.A. 69,
- 3180-3184 (1972).
- Weinberg, C. B. & Hall, Z. W. Proc. nam. Acad. Sci. U.S.A. 76, 504-508 (1979) Lyddiatt, A. et al. FEBS Lett. 108, 20-24 (1979).
- 21. Switzer, R. C., Merril, C. R. & Shifrin, S. Analyt. Biochem. 98, 231-237 (1979).

TERS ARISING

The arrival of Equus

EVEN though papers which attempt to draw together a lot of diverse evidence are most important for the scientific community the authors of such papers should not neglect to acknowledge the basic analytical work on which they base their discussion. I feel that J. Brunet and I deserve to be quoted in discussion of the arrival of Equus in the Old World at least for Roccaneyra², probably the earliest European site to have yielded Equus, and probably the only one where Equus and Hipparion coexist. So far as I know, it was not V. J. Maglio³ but D. A. Hooijer⁴ and myself⁵ who, independently, stated that the first occurrence of Equus in the Omo beds was in member G of the Shungura Formation. Since 1973, we have often repeated that the arrival of Equus in Africa was about two million years ago⁴⁻⁹.

Lindsay et al.'s bibliography is quite instructive. Most of the papers cited on the first occurrence of Equus in Europe and Africa are themselves reviews, rather than original papers describing new material or stating new facts. People like J. Brunet, who has worked for years with equids, or D. A. Hooijer and myself, who have published about 30 papers dealing with equids, are ignored, although we were responsible for the basic descriptions and determinations.

I am sure that any specialist whose colourless original work has been neglected, involuntarily or not, in more appealing papers will understand why I decided, even so late, to write about such a trifle.

VERA EISENMANN

Institut de Paléontologie (LA 12 du CNRS). 8 rue de Buffon, 75005 Paris, France

- 1. Lindsay, E. H., Opdyke, N. D. & Johnson, N. M. Nature
- 287, 135-138 (1980).
 Eisenmann, V. & Brunet, J. in Int. Colloquium on the Problem 'The Boundary Between Neogene and Quaternary' 4, 104-122 (Moscow, 1973). 3. Maglio, V. J. Nature 239, 379-385 (1972).
- Hooijer, D. A. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y., Howell, C. F., Isaac, G. Ll. & Leakey, R. E. F.) 209-213 (University of Chicago Press, 1976)
- Eisenmann, V. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y., Howell, C. F., Isaac, G. Ll. & Leakey, R. E. F.) 225-233 (University of Chicago Press, 1976).
- Hooijer, D. A. Zool. Verh., Leiden 142, 1-75 (1975).
 Hooijer, D. A. Zool. Verh., Leiden 148, 1-39 (1976).
 Eisenmann, V. Bull. Mus. natn Hist., nat., Paris 438,
- 9. Eisenmann, V. Bull. Soc. geol. Fr. 21, 277-281 (1979).

LINDSAY ET AL. REPLY—We regret that the important palaeontological contributions of Dr Eisenmann and others were slighted in our references. This was unintentional, but resulted from a bias towards selection of references with a chronological rather than a palaeontological message.

Certainly, the paper by Eisenmann and Brunet on the co-occurrence of Equus and Hipparion at Roccaneyra is an important palaeontological contribution for recognition of the appearance of Eauus in Europe. Our study was initiated with the expectation that the record of Equus at Montopoli would be demonstrably earlier than that at Roccaneyra, and we were more impressed with the proximity of their age assignment than with the palaeontological identity of the equids at Roccaneyra and Montopoli.

We cited Maglio² as an early review of East African biochronology in which faunal levels were characterized, including the Mesochoerus limnetus zone, with the appearance of Equus. Correlation of this faunal sequence had been questioned because of similar faunas with conflicting radiometric limits in the Shungura and Koobi Fora Formations—that conflict was resolved after further work on the radiometric dating, as discussed by Drake3. Our emphasis was on resolution of the conflict, and we concluded that the appearance of Equus in deposits of the Omo Basin, east of Lake Turkana, was contemporaneous with that at Olduvai Gorge. Unfortunately, we did not palaeontological the acknowledge contributions of Hooijer4, Eisenmann5, Churcher⁶, and others.

We think there might be a strong tendency for reviewers to cite other reviews, and similarly for analytical contributions to cite other analytical contributions. In spite of this, we recognize and appreciate the numerous palaeontological, radiometric, and stratigraphic studies of many researchers whose work we drew on for our review.

EVERETT H. LINDSAY Department of Geosciences, The University of Arizona, Tucson, Arizona 85721, USA

NEIL D. OPDYKE Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York 10964, USA

NOYE M. JOHNSON Department of Earth Sciences, Dartmouth College, Hanover, New Hampshire 03755, USA

- 1. Eisenmann, V. & Brunet, J. in Int. Colloquium on the Problem 'The Boundary between Neogene and Quater-
- nary' 4, 104-122 (Moscow, 1973). Maglio, V. J. Nature 239, 379-385 (1972).
- Drake, R. E., Curtis, G. H., Cerling, T. E., Cerling, B. W. & Hampel, J. Nature 283, 368-372 (1980).
- Hooijer, D. A. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y., Howell, C. F., Isaac, G.Ll. & Leakey, R. E. F.) 209-213 (University of Chicago Press, 1976).
- Eisenmann, V. in Earliest Man and Environments in the Lake Rudolf Basin (eds Coppens, Y., Howell, C. F., Isaac G. Ll. & Leakey, R. E. F.) 225-233 (University of Chicago Press, 1976).
- Churcher, C. S. Zöol. Meded., Leiden 55, 265-280 (1980); Can. J. Earth Sci. 18, 330-341 (1981)

Pulsar birthrates

NARAYAN AND VIVEKANAND1 have obtained a minimum estimate for the birth rate of pulsars in the Galaxy of 1 pulsar per (100^{+100}_{-30}) f yr, where $f(=K^{-1}) \le 1$ is the beaming factor. They have obtained this estimate from the flow rate in period space, without recourse to the spin-down age $\tau := P/2\dot{P}$. At the same time, their estimated number of pulsars in the Galaxy is $N = 1.4 \times 10^{5\pm0.3}/f$, and they find that τ is a good measure of age for $\tau \leq$ 0.5×10^6 yr. Their method is elegant, but I find it hard to trust their result quantitatively, for the following reason.

Their birth rate N implies a mean pulsar age $N/\dot{N} \approx 4 \times 10^7$ yr which is some 10 times larger than the average age determined2 both from the fraction of young pulsars ($\tau < 10^6$ yr, for which τ is held to be a good measure of age) and from the kinematic ages z/\dot{z} , and also³ from the histogram of spin-down ages. It would imply that \(\tau \) underestimated the true age. However, according to our understanding of pulsars, τ measures their age for a dipole-coupling to their surroundings, and can only lose its property of an age indicator in the presence of some overtaking ageing mechanism (such as spin alignment4), in which case it would overestimate the true age.

If the birth rate derived by Narayan and Vivekanand can be trusted vaguely, it means that τ is not always as large an overestimate of age as suggested by kinematic ages. Such a trend does not surprise me in view of the two populations of pulsars which are expected if pulsars are born in binary systems⁵. A large fraction of all τ -old pulsars may be 'elder twins' born with a large τ_0 (>106yr, instead of $\leq 10^3$ yr), and for which τ is not a significant overestimate of age. At the same time, if pulsars are in general the younger twins, the birth rate of neutron stars should be approximately twice that

Another word of caution concerns the beaming factor whose value is often assumed to be 0.2. This estimate follows from the assumption of an almost circular beam cross-section, and independently from the fact that most supernova remnants lack a central pulsar. However, supernova remnants housing a pulsar would almost certainly have a filled-centre appearance, that is, be plerions, whereas shell-type remnants are expected⁵ to contain binary system neutron stars (like W50 around SS433). Moreover, pulsar beams may well have banana-shaped cross-sections, with $f \approx 1$. A beaming factor f near unity is likewise indicated by the high occurrence rate of interpulses $(\approx 5\%)$ if the latter come from the opposite magnetic pole.

With these modifications and ref. 2 in

mind. I arrive at a galactic neutron star birth rate of 1 in 20 ± 10 yr.

W. KUNDT

Institut für Astrophysik. Universität Bonn, 5300 Bonn, FRG

- Narayan, R. & Vivekanand, M. Nature 290, 571 (1981).
- Lvne, A. G. IAU Symp. 95, (1980).
- Kundt, W. Naturwissenschaften 68, 63 (1981). Kundt, W. Astr. Astrophys. 98, 207 (1981).
- Kundt, W. Naturwissenschaften 64, 493 (1977).

NARAYAN AND VIVEKANAND REPLY-It is known1 that the radio luminosities of pulsars decrease with age. Therefore, the observed sample of pulsars is biased towards the more luminous younger pulsars. Consequently, the average age of pulsars determined2 from the observed fraction of young pulsars $(\tau < 10^6 \text{ yr})$ underestimates the true mean pulsar age. The bias is probably even more stronger in the kinematic ages determined from z/\dot{z} because proper motions have been reliably obtained only for relatively luninous pulsars. On the other hand, in our analysis, we have accounted for radio luminosity selection effects and thereby obtained an approximation to the complete galactic population of pulsars. Consequently, our result $N/N \approx 11 \times 10^6$ yr is unbiased and a more reliable estimate of the mean pulsar age than the earlier approaches which typically gave 4×10^6 yr. Note, however, that age is not a very relevant parameter in our analysis, which is based entirely on pulsar flow in the P-P diagram (pulsar period to its time derivative). Moreover, the concept of mean pulsar age itself may not be very meaningful or useful if pulsars belong to more than one class with widely different active lifetimes.

Regarding the beaming factor K, we consider that the 5% occurrence of interpulses is consistent with a circular beam cross section and a value of K = 5 rather than with banana-shaped cross sections and K = 1.0

> RAMESH NARAYAN M. VIVEKANAND

Raman Research Institute. Bangalore 560 080 India

1. A. G. Lyne, R. T. Ritchings & F. G. Smith Mon. Not. R.

astr. Soc. 171, 579-597 (1975). 2. A. G. Lyne, IAU Symp. 95, (1980).

Solar-flare produced ³He in lunar samples

RAO AND VENKATESAN³ have reported a method for deducing the absolute solar cosmic ray (SCR) proton fluxes in the past few million years using SCR protoninduced ³He in the top surface layers of Moon rocks. To quote the authors: "even if there are minor diffusion losses, this method holds good as long as the losses are not depth dependent". I would like to point out that Moon rocks studied in this laboratory which are similar to those used by Rao and Venkatesan show severe 3He diffusion losses, up to 99% (refs 2-6), and the losses show strong depth dependence³⁻⁶. Another problem arises from the directly implanted solar flare ³He found in the top few millimetres of these Moon rocks³⁻⁶. This ³He component shows depth dependence and is about one order of magnitude higher in concentration than the SCR proton-induced ³He, therefore masking it in the top few millimetres of rock³. The rocks which we have studied, 68815 and 65315, excavated from south ray crater, have GCR and SCR exposure ages of 2 Myr, contain plagioclase as the major mineral and therefore are similar in many characteristics to the rocks studied by Rao and Venkatesan, 61016 and 64435. The near constancy of ³He/²¹Ne ratios which they suggest as an indication that depth dependence diffusion effects are not significant can also be misleading as the ²¹Ne diffusion pattern shows in our rocks a similar depth dependence to the 3He and

therefore the ³He/²¹Ne ratio might stay quite constant. We believe that Rao and Venkatesan were unable to observe the two effects, depth dependence diffusion and the directly solar flare-implanted ³He because first, only three layers of different depths were taken from each rock, and second, the layers were too thick (≥1.5 mm). We can judge from our fine sampling, nine for each rock in the range 0-15 mm and ~ 0.2 mm thick layers in the first 0-1 mm of range, that neither the diffusion pattern nor the SCR-implanted ³He would have been observed using the sampling technique of Rao and Venkatesan. I therefore consider ³He to be the wrong isotope for the use proposed unless the effects mentioned are taken into account.

AKIVA YANIV

Department of Physics and Astronomy Tel Aviv University. Ramat Aviv. Israel

- 1. Rao, M. N. & Venkatesan, T.R. Nature 286, 788-790
- Yaniv, A. & Marti, K. Meteoritics 15, 390 (1980)
- 4. Yaniv, A. & Marti, K. Astr. Phys. J. Lett. 247, L1-L4
- 5. Yaniv, A., Kirsten, T. & Richter, H. Astr. Phys. J. Lett. (submitted).
- 6. Yaniv, A. & Kirsten, T. Proc. 12th lunar planet. Sci. Conf., 1224-1226 (1981).

RAO AND VENKATESAN REPLY-We point out that we discussed these issues in detail elsewhere1. Relevant points are briefly outlined below.

Our inferences regarding ³He losses are based on experimental results in rocks

61016 and 64435. The general agreement (within a factor of two) of exposure ages based on SCR-produced 21Ne and 38Ar and ³He, with the surface exposure ages based on particle tracks, could not have been observed if almost total loss of ³He had taken place by diffusion in these samples. Further, the diffusion effects of ³He and ²¹Ne are mass-dependent. Yaniv may not be observing such mass-dependent effects because 99% of the gas has been lost from his samples. The depthdependent diffusion will lead to variations in the ³He/²¹Ne and ³He/³⁸Ar ratios with depth, which is not borne out by the nearuniformity of these observed ratios in our samples. (Details are discussed in refs 1.

The peak temperatures to which some of these rocks were exposed on the lunar surface is ~370 K and the average temperature is 210 K; He loss can be partial but not almost total, at such temperatures, in these samples.

In some lunar plagioclases, 3He was lost and in some it was well retained. In a sensitive Gas ion-probe analysis of ⁴He in plagioclases, Müller et al. observed large amounts of ⁴He in two plagioclases (P4 and P2), with a capability of retention similar to that of ilmenite, whereas in some other plagioclases from soil 76501, no 4He was found. Shock effects also seem to be involved.

The occurrence and composition of directly implanted solar flare He and Ne in lunar samples is important. We are investigating the composition of solar flare Ne in these samples by selective chemical etching and subsequent stepwise thermal release methods. The long-term solar flare Ne composition seems to be similar to fractionated solar wind4. The long-term solar flare He composition is not well known, but the contemporary solar flare ³He/⁴He ratios (measured from spacecraft) vary from 1 to 0.01 and differs between flares5. Most of the 3He-rich flares are very weak and their contribution to the total He seems to be limited. It is not clear whether the long-term solar flare He composition is similar to the fractionated solar wind as in the case of Ne or varies drastically as observed in contemporary flare studies. Further controlled experiments are under way to understand these complexities.

> M. N. RAO T. R. VENKATESAN

Physical Research Laboratory, Navrangpura, Ahmedabad 380 009. India

- 1. Venkatesan, T. R. et al. Proc. 11th lunar planet. Sci. Conf. 1271-1284 (1980).
- 2. Rao, M. N. & Venkatesan, T. R. Nature 286, 788-790 (1980).
- 3. Müller, H. W. et al. Proc. 7th lunar Sci. Conf. 937-951 4. Nautiyal, C. M. et al. Proc. 12th lunar planet. Sci. Conf.
- (1981) (in the press). 5. Ramaty R. et al. NASA, Tech. Rep. 79660 (1978).

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Volume Holography and Volume Gratings

L. Solymar and D.J. Cooke

September/October 1981, x+468pp., £39.60 (UK only)/\$95.50, 0.12.654580.4

The book is devoted mainly to the study of the properties of volume holograms, such as efficiency, fidelity of reproduction and the generation of higher order modes, including also a chapter on the materials used for recording. The treatment is extended to volume gratings in general, in which diffraction effects at or near the Bragg angle are significant. Applications in the form of displays, optical elements and couplers, acousto-optic and electro-optic devices, computer memories and integrated optics are discussed.

Goat Production

edited by C. Gall

September/October 1981, xx+620pp., £41.40 (UK only)/\$99.50, 0.12.273980.9

The renewed interest in goats for both intensive production in temperate and extensive production in tropical countries has inspired this book. The 19 chapters are written by 18 leading authorities. On the basis of the widely scattered literature they discuss what is known in their fields drawing heavily on own research results, some of which are published here for the first time.

Stress and Fish

edited by A.D. Pickering

September/October 1981, xiv + 368pp., £24.60 (UK only)/\$59.50, 0.12.554550.9

This book extends knowledge by presenting original research in the context of a detailed review of the existing information. The endocrinological/physiological responses of fish to stress, fundamental to our understanding of the subsequent performance of fish, are given particular attention as are the problems of stress associated with intensive fish cultivation and the harmgul influences of man's activities on the aquatic environment.

Aquarium Systems

edited by A.D. Hawkins

September/October 1981, x+452pp., £27.80 (UK only)/\$67.00, 0.12.333380.6

This book was written as a practical guide to fish-keeping in the laboratory and is the result of collaboration between working scientists with first-hand experience in the subjects they have presented. It considers the basic aspects of aquarium design and construction, the supply of aquarium water and its treatment to make it suitable for the fish, the choice of materials for building the aquarium in order to minimize corrosion and contamination of the water, and other practical aspects of fish husbandry.

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Series editors: Robin Sibson and Joel E. Cohen

This new series seeks to promote collaborative work among mathematicians, statisticians and biologists by providing a forum for the publication of important new work in this area on the boundary of two major disciplines. Both mathematical modelling and the analysis of biological data will be covered, especially in relation to areas of currently active interest in biology.

Mathematics in Biology Series

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Edward Batschelet[†]

August/September 1981, xvi+372pp., £28.80 (UK only)/\$69.50, 0.12.081050.6

There have been considerable advances in the study of circular statistics in the last twenty years. Circular Statistics in Biology seeks to provide a comprehensible guide to this field for the biologist and scientist who have a large amount of circular data to deal with but a limited knowledge of mathematical and statistical theory. The emphasis is on interpretation and analysis rather than unnecessary theory and many illustrative examples are provided.

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BOOK REVIEWS

Chinese puzzles

John Maddox

THE time has gone when people could bring conversation in the West to a halt by saying that they were just back from China. Indeed, the conversational gambit is dangerous, for there is as likely as not to be somebody in the gathering who can boast of having found a better hotel, or of having been allowed to visit Chinese Mongolia or Tibet. Yet China remains a puzzle, not only because mutual travelling is still only a trickle but because of language and the sheer strangeness of China as seen from the West and, no doubt, vice versa.

Science in Contemporary China is, in the circumstances, a welcome book even though its defects, sometimes serious and often irritating, also command attention. The book is a compilation of travellers' tales from the late 1970s brought back from China by 26 scholars of various disciplines, many of whom seem to have made two or three journeys under the auspices of the Committee on Scholarly Communication with the People's Republic of China, itself an offshoot of the National Academy of Sciences in Washington but funded, for this purpose, by the National Science Foundation. But since most of the contributions are well laced with handlists of laboratories, and the names of their directors, the book is likely to be most used as a kind of Baedeker — a guide by which intending travellers from the West can plan their journeys. That is not something to be scorned.

The most glaring flaw is that the book. according to its editors and Professor Walter Rosenblith, the chairman of the supervisory committee, is uneven. In many ways, that is an understatement. Some contributions are merely handlists. Others sensitively relate the condition of some discipline now (or in 1978 or 1979) with the great events of the Cultural Revolution which then seems more than ever a calculated madness. A more forgivable defect of the book is that most of the journeys on which it is based were made in that dreadful period when the Cultural Revolution had come to an end but when Mao Zedong (according to the pin-yin transliteration) was still nominally in the saddle. Some travellers were plainly mystified by what was happening around them, at least until the changing scene was crystallized and made familiar by the great National Science Conference in Peking (sorry, Beijing) in March 1968.

Travellers to foreign lands must be wary of the three most obvious traps — those of generalizing from impressions, of writing patronizingly and of writing with stars in their eyes. This bunch of travellers has been

Science in Contemporary China. Edited by Leo A. Orleans with the assistance of Caroline Davidson. Pp.599. ISBN 0-8047-1078-3. (Stanford University Press: 1981.) \$35.

reasonably circumspect. Now and again, first impressions enliven the separate catalogues of information — Leo Goldberg was obviously shocked to learn of the ups and downs of the Purple Mountain Observatory (Nanjing) from which the astronomers were driven after the Japanese occupation in 1937. The general first impression that Chinese researchers use less sophisticated equipment than their colleagues in the West has, however, been carefully confirmed; and there is a thorough account of what is being done by the Chinese Academy of Science to put things right.

For the most part, devotional prose has also been avoided. Fang Yi, the vice-premier with responsibility for science and technology in 1968, made a great impression on several of the travellers as did Deng Xiaoping (deputy premier at the same time) on the smaller number who had the luck to meet him. Some seem to have regarded the national plan announced in March 1978 as having the force of Mosaic tablets, but collectively the travellers have given a good account of how the plan is having to be matched against economic and social reality.

Now and again, the travellers have fallen into the trap of patronizing those whom they observed. The impression is heightened by the way in which China is so often called the PRC. One writer asks how it can be that the Chinese choose to lavish surgical skill on intricate operations when they have so few Western-style doctors per 10,000 population. Even, in an extended footnote, Joseph Needham (the author of the monumental Science and Civilization in China) is patronized because his book is not "philologically sound". But these are minor points.

For the rest, this Baedeker, based though it is on 26 snapshots of China, is full of absorbing information. Not merely can one learn that C.N. Yang (the field theoretician) is the son of K.C. Yang, who took a PhD in mathematics in Chicago in the 1920s, but that Chen Jingrun was the first to prove (in 1966) that, above a certain size, every even number can be written as the sum of a prime number and another which is either itself prime or the product of two prime numbers; that China is heavily committed to superconducting technology for reasons not readily understood; that

the commitment to high-energy physics derives from Mao's interest in the philosophical implications (but that the 50 GeV accelerator will not now be finished until 1985); that there is a project to synthesize a transfer-RNA molecule and it appears to be customary, when an active compound has been isolated from some traditional medicinal herb, to synthesize the pure chemical so as to avoid the associated side-effects. China's need of military electronic equipment is urgent. Computers still languish.

Collectively, the snapshot is clear. But what is happening to the people? The Cultural Revolution lasted for 15 years, almost a generation, and university posts were either not filled or filled with the wrong people. Science in Contemporary China records in several places that Chinese hosts were aware of the problems foolishly created, and that they were doing their best to put things right. How quickly, one must wonder, will they succeed?

Anecdotally, this book provides some evidence of change in the right direction. I was pleased to find in it the name (almost unrecognizable behind the pin-yin) of a graduate student at Manchester many years ago. He was so homesick that he travelled every other week to Liverpool for a decent meal in St George's Square. In 1953, he caught Mao's student boat back to China. We were surprised to learn that as an F-centre expert he planned to help with the "building of a dam"; only afterwards did we understand that it was a project to do with weapons. But when seen at the height of the Cultural Revolution, he was working as a kind of filing clerk at the Academy. Now, it seems, he has been rehabilitated, the head of a division of an institute in Beijing.

Only occasionally do the contributors have the time to reflect on what they describe, but Saunders Mac Lane (who writes about mathematics) does best. He gives an account of how his delegation chose to argue with their Chinese hosts about the virtues of mathematics - is it elegance ("beauty") or utility that matters most? The encounter was apparently rowdy, and the question unresolved. The reason seems to have been a difference of idiom - which is not merely a name for a pretty turn of words. What remains to be learned, from the West, about China is whether present puzzles will melt away with the passage of time or whether, on the other hand, the idiomatic gulf will prove to be unbridgeable.

John Maddox is Editor of Nature.

Physics for philosophers and philosophy for physicists

D. ter Haar

Superposition and Interaction: Coherence in Physics. By Richard Schlegel. Pp.302. ISBN 0-226-73841-8. (Chicago University Press: 1981.) \$22.50, £13.50.

This is an interesting book but - to my mind — very much a curate's egg. The first question which comes to mind is: for whom is the book meant? The author himself states that although at times he had thought of philosophers as his audience, his primary prospective audience is among physicists or other physics-minded scientists "who have some technical knowledge of special relativity theory and of quantum theory - as given, say, in introductory courses in those topics in American universities". The next question is: what is the message which the author wants to convey? The answer is that he wishes to suggest that there is a fundamental coherence in physics which can be shown especially to exist when we consider the two main pillars of what is still called "modern" physics, namely, the special theory of relativity (now 76 years old) and non-relativistic quantum theory (in its late sixties). The suggestion is that in both of these theories superposition (that is, the fact that if one has two separate solutions of the equations, a line or combination of them is also a solution) plays an essential part and interactions with an observer are a deciding factor. This is orthodoxy as far as quantum theory is concerned, but takes some swallowing when we come to special relativity. However, while this book does not give any compelling reasons - in my view, not even aesthetic reasons - to accept this thesis, there do not seem to be any experimental data which contradict it. This is, perhaps, not surprising, as no experimenta crucis are suggested to decide for or against the author's views.

Let me first of all list those aspects of the book which I found most admirable and from which I benefited most. For a careful and well-informed reader it contains much that is illuminating. In particular, I found the first and last chapters of great interest. The discussion in the first chapter of the contents of classical physics - going back to Aristotle - and of the importance of models in theoretical physics in general and in classical physics in particular is excellent. The last chapter containing a "summertime conversation" between two physicists, the wife of one of them and a philosopher gives a cogent and on the whole clear account of most of the important points made in the main body of the book.

The second volume in the series Genetic Engineering (for a review of Vol.1 see Nature 292, 480) has recently appeared. Genetic Engineering 2 contains four contributions on gene evolution, genomic libraries, restriction enzymes and gene cloning in yeast. Prices are: £9.80, \$24.

One might object to quantum mechanics as a deus ex machina and the relegation of God to a superior watchmaker — miracles seemed to have no place in the Universe inhabited by these four people — but these are minor objections. Another excellent discussion is the one of the clock paradox, where the author follows a suggestion made to him by Einstein himself and uses three uniformly moving inertial systems to resolve this paradox.

I now come to a number of points where I feel the author has seriously diminished the usefulness of this mainly philosophical treatise, especially for younger physicists. I would hesitate to give the book to my own pupils without a number of caveats, since in a number of places the theory given is wrong (or at least so poorly formulated as to be seriously misleading). In the account of measurement in quantum theory there is, in my opinion, insufficient emphasis on the difference between the preparation for a measurement and the measurement itself. However, the most fundamental error and one which crops up several times — is in the discussion of pure states and mixtures. A mixed state cannot be represented by a wavefunction, but needs a density matrix. The concept of a density matrix - so central in the theory of measurement - is, in fact, nowhere mentioned in this book.

Other points where the text is, to say the least, misleading are where the author states that the Maxwell equations are linear (homogeneous) differential equations for which the superposition principle holds, without stating that this is only true for the Maxwell equations in vacuo. It is also confusing to stress de Broglie's introduction of the relations between energy/momentum

and frequency/wavevector as against Schrödinger's introduction of the wave equation in order to find a close relation between special relativity and non-relativistic quantum theory. After all, we still do not have a complete relativistic wave equation, but only approximate equations such as the Dirac and Klein-Gordon equations which are valid in the not-very-relativistic domain.

In a book which is mainly philosophical in tone, one would expect careful use of language and it is disappointing to find a number of sloppy expressions. It is probably too much to expect that a careful distinction is made between utilize ("to make useful, turn to account" according to the OED) and use, but a vital point is missed when it is stated that "the Greek word atomos means 'individual'." As an important issue in the book is the distinction between macroscopic and microscopic systems, somewhere a careful definition of those terms should be given and the dividing line drawn between them - or it should be stated that it is often impossible to draw such a line. The general problem of what are physically meaningful questions and which are mainly philosophical ones, although hinted at several times, could also have been considered more carefully. For instance, I feel strongly that in a book of this kind there should be a clear discussion of the fact that a wavefunction only has a meaning in as far as it can predict the outcome of an experiment - this, after all, makes quantum theory a theory which can be falsified.

D. ter Haar is a Fellow of Magdalen College and Reader in Theoretical Physics at the University of Oxford.

What price the diversity of species?

A.D. Bradshaw

Conservation and Evolution. By O.H. Frankel and Michael E. Soulé. Pp.327. ISBN hbk 0-521-23275-9; ISBN pbk 0-521-29889-X. (Cambridge University Press: 1981.) Hbk £25, \$49.50; pbk £7.95, \$17.95.

THE natural ecosystems of the world are disappearing at a frightening speed. Tropical rain forests are being destroyed at a rate of 4.7 ha min⁻¹ and will be gone within 50 years. Every year sees a further 6 million hectares of arable land. Although some areas will be almost impossible to cultivate, the ingenuity of people pressed for space must not be underestimated; and even if cultivation is not possible, hunting, logging and grazing will inevitably increase drastically beyond their present high levels.

Against this scenario, what will be left of

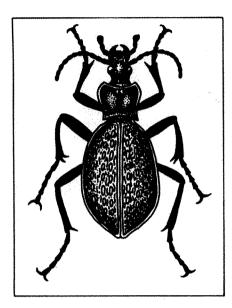
the wealth of plants and animals on this planet? Some species, r selected, of small size and with tolerance of man-made environments, will certainly survive. There are plenty of species left in England and China. But the great diversity will inevitably be reduced. This book is a critical analysis of what is likely to happen, written by two well-known population geneticists, highly experienced between them in both wild and cultivated species.

If species are to be conserved at all, the authors argue that they must be conserved without loss of genetic variability. For outbreeding organisms, from considerations of the effects of inbreeding/mutation/selection equilibria the authors conclude that a minimum effective population size of 50 is necessary.

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Editor: T.A. Miller With a Foreword by G.S. Fraenkel With contributions by numerous experts 1980. 90 figures, 23 tables. XXIV, 282 pages Cloth DM 79,—; approx. US \$33.90 ISBN 3-540-90451-4

This book discusses the broad range of current neurochemical research on neurohormones. Although insects are used as models, the information offered is applicable to a variety of animal systems.

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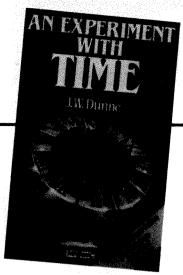
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Sampling Methods in Soybean Entomology

Editors: M.Kogan, D.C.Herzog 1980. 252 figures, 65 tables. XXIII, 587 pages Cloth DM 88,-; approx. US \$40.00 ISBN 3-540-90446-8

This book summarizes the fundamental, experimental, and analytical procedures which are indispensable for studying demographic data on insect populations. Practical procedures for obtaining relative and absolute data on arthropod parasites, predators, and diseases are examined in-depth. Construction of various sampling devices is explained, as well as selection of the most appropriate collecting techniques based on the particular characteristics of the species, cost of collection, and accuracy of collected data. Development of operational standards for running these tests is stressed.

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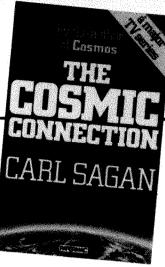
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But if conservation is to include the possibility of continuing evolution then the minimum population size is 500. Once this conclusion is reached the problems of conservation become apparent. The minimum territory of a pair of mountain lions is 52 km²; to conserve this species requires an area about twice the size of Yellowstone National Park. There are therefore few reserves in the world that are large enough to ensure the survival of the dominant carnivores, and there is no doubt the majority are doomed to disappear.

Yet nature reserves present the only possible solution. In which case there are criteria of population genetics and biology which must be observed if reserves are to be designed to function properly. Size is crucial according to island biogeographical theory, but so is migration. As a result there are arguments that small reserves are possible if there are connecting corridors. But the authors point out that this will only work in a limited number of cases.

Even the r selected species, notably our crop plants and their wild relatives, have problems. It is crucial that we maintain genetic diversity to satisfy our present and future needs for food production. This means genes to cope with specific individual problems such as day length or disease, and genes by which we can build up co-adapted complexes for characters such as yield. The authors show that these genes are scattered in wild and cultivated material, often in places that cannot be predicted.

For conservation and continued evolution, a diversity of populations must be maintained, if possible in natural conditions. So far, because of geographical expansion from migration and trade, the diversity in these species has actually increased. But now the success of modern agriculture and plant breeding has sent the process into reverse, and whole groups of cultivars, such as the Welsh upland wheats and barleys, have completely disappeared. The solutions endorsed by the authors are various: in situ and ex situ dynamic conservation, and static conservation by storage, of which seed storage is perhaps the most efficient. But they do not offer any easy solutions to the problem of what to store, except to emphasize the need for international co-operation.

This book is a remarkable survey of a problem which is crucial to us all. Not only does it list over 600 references but it achieves a remarkable synthesis and perspective, based on a masterly understanding of theory as well as practice. It is enormously readable because of its simple and enthusiastic style. It is critical reading for plant and animal breeders, and all ecologists, so that they can better argue with politicians about what has to be done, before it is too late.

Liquid crystal measurement

J. W. Emsley

Physical Properties of Liquid Crystalline Materials. By W.H. De Jeu. Pp.133. ISBN 0-677-04040-7. (Gordon and Breach: 1980.) \$35.25.

EVERY schoolboy knows that there are liquid crystal display devices, but a relatively few graduates are familiar with this "fourth state of matter". There is certainly a need for introductory texts on the physical properties of these materials. to supplement George Gray's excellent Molecular Structure and the book, Properties of Liquid Crystals (Academic, 1962), which covers their general properties. This short book by De Jeu is the first of what promises to be a series, edited by Professor Gray, on liquid crystals. It has, however, a misleading title since it does not cover all physical properties and is confined to discussing only the simplest mesophase, the nematic. This does have the advantage of making it a book that will be read rather than one too complex and long to appeal to those interested in other aspects of liquid crystals.

Five physical properties are discussed: magnetic susceptibility, refractive index, dielectric permittivity, elastic constants and viscosity coefficients. Each topic is introduced by a discussion of the basic physics, which makes heavy demands on the background knowledge of the reader.

There follows a survey of methods of measurement and a discussion of the work which has been done. The author draws heavily on his own research and does not attempt a general review, but this again adds to the readability of the book and conveys a sense that much remains to be done.

Physicists, chemists and engineers interested in liquid crystallinity will welcome this book as providing a short, readable account of the five physical properties. It is written primarily with experimentalists in mind and it has a particularly useful chapter on sample preparation. It is less successful at explaining the significance of the results. mainly because of a deliberate decision not to get involved with the details of the various theoretical models developed in recent years. Restricting the scope of the book to those topics in which the author has been directly involved was a wise decision, for although one might wish for more discussion of cholesteric or smeetic phases, or a more critical review of theory, what is included is discussed with an authority derived from a long experience in the laboratory.

J. W. Emsley is a Senior Lecturer in the Department of Chemistry at the University of Southampton.

Consolidating fungal biochemistry

P.G. Mantle

Lipid Biochemistry of Fungi and Other Organisms. By J. D. Weete with contributions by D.J. Weber. Pp.388. ISBN 0-306-40570-9. (Plenum: 1981.) \$45, £28.35.

THIS volume is a reincarnation of the authors' previous book, Fungal Lipid Biochemistry, published by Plenum in 1974. The text has been completely rewritten and revised but still centres on the distribution and biochemistry of fungal lipids — fatty acids, acylglycerols, sterols, phospholipids, aliphatic hydrocarbons and sphingolipids. New aspects covered in the present volume include a brief history of fungal lipid research, more comprehensive treatments of lipid taxonomy and substrate conversion into stored lipid, a description of the multifunctional yeast enzyme for fatty acid synthesis, the biosynthesis of polyprenols and carotenoids, and a review of the role of fungal lipids in sporulation and spore germination. The bibliography has been correspondingly increased and, where appropriate, reference is made to contrasting bacterial, plant and animal systems.

A few minor typographical errors can be

recognized and I was somewhat surprised to find myself credited (Table 3.7) with research in the field of phycomycete fatty acids. Nevertheless, the authors should be congratulated on condensing the essence of about 1,000 research papers into a lucid text supplemented liberally by comprehensive tables and figures.

It is often only when someone gives the time and care to review a subject that the gaps in our knowledge of an otherwise diffuse topic become well defined. Fungal lipid metabolism is a case in point. Thirty years ago this book would have been simply a pamphlet listing some of the oils contained in fungi. Biochemistry has since moved so fast that the lipid composition of representatives of the major groups of fungi is now fairly well documented and the biosynthetic routes are generally understood. It is, however, notable that although regulatory mechanisms for fatty acid synthesis have been well researched, the regulatory interactions involving lipids in whole-organism metabolism are largely unknown. This is perhaps not surprising since it is bound to be very complex. Nevertheless, it is such areas of knowledge which

A.D. Bradshaw is Professor of Botany at the University of Liverpool.

will be invaluable in fully understanding fungi as organisms. This is particularly relevant to species which interact parasitically with other organisms or which have the ability to produce complex secondary metabolites, whether suitable for exploitation as pharmaceuticals or agrochemicals or whether hazardous on account of their propensity to act as mycotoxins. Often these functions are closely associated with lipid metabolism, frequently yielding metabolites from a common pool of key intermediates.

In an age when biochemistry is concentrating so much on the molecular level, however exciting, it would appear that metabolic biochemistry is being neglected. This book will be valuable in focusing attention on fungal biochemistry — students, teachers and researchers in the field of microbial biochemistry will do well to consult it.

P.G. Mantle is a Senior Lecturer in the Department of Biochemistry, Imperial College, University of London.

Academic synergism

J.B. Butterworth

Universities in Partnership. By I.C.M. Maxwell. Pp.480. ISBN 0-7073-0270-6. (Scottish Academic Press: 1980.) £15.

Universities in Partnership describes the first 25 years of the work of the Inter-University Council for Higher Education Overseas, from the time when it was set up by the British universities in 1946 to assist in the creation of new universities in colonial territories which were rapidly becoming independent. Gradually the IUC was called upon to fulfil a wider role in helping these new institutions to develop their academic potential and train their staff. More recently, it became an important vehicle in facilitating better two-way communication between the universities in the UK and some 35 others in developing countries, an important partnership, based in equality, giving benefits to both sides by a number of different forms of relationship of which links between departments have become particularly important.

This record of the universities with which the IUC has had association is impressively comprehensive and portrays their development as seen from the standpoint of the IUC. One is grateful to the author, Mr Maxwell, for his immense work of distilling the records of the IUC and, from a voluminous amount of material, producing such a readable and full account. At a time when the IUC is becoming the IUPC and merging with the British Council, it is important that all of this material should be available within the covers of one volume.

The book is divided into three parts, the first of which is particularly interesting in

that it gives a short and attractive account of the basic theories upon which the work of the IUC has been based, and of the way in which those theories or principles have changed with time. In Part 2, "The Foundation and Growth of the Universities", Mr Maxwell presents the universities in considerable detail, much of which comes from the archives of the IUC. The book seems to change gear between Part 1 and Part 2, the earlier section dealing in a lively way with the principles and policies which evolved over the 25 years. The second part, no doubt because it is dealing with each individual university and is condensed from the IUC records, does not always say what one might wish to hear. In the development of any institution people are crucial, and the end of the period covered by Mr Maxwell was particularly important because indigenous Vice-Chancellors were taking over from expatriates; one would have liked to know more about those individuals whose influence had been crucial in the development of particular universities.

The book, of course, is intended to be a history of the IUC and not of the individual universities with which it was associated. But too close an adherence to the reports of commissions and the decisions of Senates and Councils may not always reveal the reality which is the university. Moreover, it would have been interesting if Part 2 or Part 3 on the role of the IUC had explored more fully some of the important themes referred to earlier in the book, especially the historical growth of the concept of a developmental university. The ideas and plans already current in the 1960s, as the Kericho Conference in 1966 bears evidence, were having and were to have considerable effect upon the new universities in developing countries. A prime example is the concept of an umbrella university such as Malawi, taking within iteself virtually all post-school education. Another is the single university catering for a number of different countries, evolving from the University of the West Indies, to the University of Botswana, Lesotho and Swaziland, and the experience there acquired being applied in the University of the South Pacific. The concept of a developmental university advanced rapidly after 1970 and it might have been advantageous if Mr Maxwell had not stopped abruptly at that date, for a deeper exploration of these themes could have given guidance and illumination to university development both now and in the future.

Nonetheless, we should be grateful to the author for the valuable work of reference he has made available to us. Those who consult it about particular events in the history of any of the universities are unlikely to be disappointed; it is an extensive and objective record.

J.B. Butterworth is Vice-Chancellor of the University of Warwick, was Chairman of the IUC from 1968 to 1977, and becomes Chairman of the IUPC from 1981.

Of bugs and brains

Howard C. Berg

Bacterial Chemotaxis As a Model Behavioral System. By Daniel E. Koshland. Pp. 193. ISBN 0-89004-468-6. (Raven: 1981.) \$19.50.

BACTERIA, the most primitive free-living organisms, continuously monitor the concentrations of chemicals in their environment, decide whether these concentrations are rising or falling, shift gears in their rotary engines and swim toward regions that they find more favourable. The modern armament of genetics, biochemistry and biophysics has been brought to bear on this remarkable behavioural system, and some of the processes involved are now understood in molecular detail. Professor Koshland surveys this work in terms intended for both the educated layman and the specialist, striving, as he puts it, to bridge the gap between general interest and scientific accuracy. He then moves with some temerity to a discussion of the function and malfunction of the human brain, arguing that the biochemistry of bacterial chemotaxis is relevant to the nervous system of human beings themselves.

This is a provocative book. It speaks with authority about the interactions of ligands with receptors, receptors with signallers, and signallers with enzymes that methylate and demethylate. It describes models for signalling and adaptation that, although incomplete, have led to a number of fruitful biochemical studies. It contains lively speculations on necessity and chance, memory and learning, and even love and hate.

But I have serious reservations. This is a personal account. The book does not adequately treat pioneering work on bacterial motility and chemotaxis done outside Berkeley. Nor does it make any attempt to place in proper historical perspective the idea that the nervous system of higher organisms evolved from reactions that can be found in the most primitive living things, or the hope that a knowledge of the mechanisms of bacterial chemotaxis might contribute to our understanding of neurobiology and psychology. These concepts are not new. Neurobiologists already know that they need to learn more about the biochemistry of the brain. A biochemist of Professor Koshland's experience might have been expected to offer more insights into problems that they might profitably pursue.

I prefer to think of bacteria as objects of fascination in their own right. Indeed they may prove to be more sophisticated than cells of the human brain. Bacteria have been around for billions of years, and individuals in each generation have had so survive on their own merits.

Howard C. Berg is Professor of Biology at the California Institute of Technology, Pasadena.

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BARAGOZZI, C. Vito Volterra Symposium on Mathematical Models. Lecture Notes in Biomathematics, Vol. 39. Proceedings of a Conference held at the Centro Linceo Inter-disciplinare, Accademia Nazionale dei Lincei, Rome, December 1979. Pp.417. Flexi ISBN 3-540-10279-5. (Springer-Verlag: 1980.) DM 48.50, \$28.70.

DEVLIN, K. J. Sets, Functions and Logic. Basic Concepts of University Mathematics. Pp.90. Hbk ISBN 0-412-22660-X; pbk ISBN 0-412-22670-7. (Chapman & Hall: 1981.) Hbk np; pbk £2.95.

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ARMSTRONG, J.E.. Geological Survey Bulletin 322. Post-Vashon Wisconsin Glaciation, Fraser Lowland, British Columbia. Pp.34. Pbk ISBN 0-66-10709-0. (Geological Survey of Canada, Ottawa: 1981.) Pbk \$4.80.

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BRITISH MUSEUM (NATURAL HISTORY)/CAMBRIDGE UNIVERSITY PRESS. Origin of Species. Pp.120. Hbk ISBN 0-521-23878-1; pbk ISBN 0-521-28276-4. Np.

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HERSCOWITZ, H. B. et al. (eds). Manual of Macrophage Methodology. Collection, Characterization, and Function. Immunology Series, Vol. 13. Pp. 560. ISBN 0-8247-1222-6. (Marcel Dekker: 1981.) SwFr, 175.

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HORZINEK, M. C. Non-arthropod-borne Tagaviruses. Pp. 200. ISBN 0-12-356550-2. (Academic: 1981.) £16.40, \$39.50.

HOWARD, A. N. and BAIRD, I. M. (eds). Recent Advances in Clinical Nutrition. 1, Proceedings of the First International Symposium on Clinical Nutrition, July 1980, Royal College of Physicians, London. Pp. 298. ISBN 0-86196-009-2. (John Libbey, London: 1981.) £18.

ISMAIL, A. A. Biochemical Investigations in Endocrinology. Methods and Interpretations. Pp. 275. ISBN 0-12-374850-X. (Academic: 1981.) £14.20, \$34.50. JÄGER, W., ROST, H. and TAUTU, P. (eds). Biological Growth and Spread.

Mathematical Theories and Applications. Lecture Notes in Biomathematics. Vol. 38. Proceedings of a Conference held at Heidelberg, July 1979. Pp. 511. Flexi ISBN 3-540-10257-4. (Springer-Verlag: 1980.) Np.

Psychology

DICKSON, W.P. (ed.) Children's Oral Communication Skills. Pp.394. ISBN 0-12-215450-9. (Academic: 1981.) \$29.50.

FREEDMAN, J.L., SEARS, J.L. and CARLSMITH, J.M. Social Psychology. 4th Edn. Pp.686. ISBN 0-13-817783-X. (Prentice-Hall: 1981.) £12.95.

HOFER, M.A. The Roots of Human Behavior. An Introduction to the Psychobiology of Early Development. Pp.331. Hbk ISBN 0-7167-1277-6; pbk ISBN 0-7167-1278-4. (W.H. Freeman: 1981.) Hbk \$22.50, pbk \$11.50. HOFFMEISTER, F. and STILLE, G. (eds). Psychotropic Agents. Part 1:

Antipsychotics and Antidepressants. Handbook of Experimental Pharmacology, Vol.55/1. Pp.734. ISBN 3-540-09858-5. (Springer-Verlag: 1980.) DM232, \$136.90. HOUSTON, J.P. et al. Essentials of Psychology. Pp.529. Flexi ISBN 0-12-356858-7. (Academic: 1981.) \$14.95.

LA BARBA, R.C. Foundations of Developmental Psychology. Pp.545. ISBN 0-12-432350-2. (Academic: 1981.) \$18.95.

LOCKER, D. Symptoms and Illness. The Cognitive Organization of Disorder. Pp.193. ISBN 0-422-77460-X. (Tavistock, London: 1981.) £12.

MENDELS, J. and AMSTERDAM, J.D. (eds). The Psychobiology of Affective Disorders. Pfizer Symposium on Depression, Boca Raton, Florida, February 1980. Pp.220. Flexi ISBN 3-8055-1400-X. (Karger, Basel: 1980.) SwFr.39, DM47, \$23.50.

WALKER, C.E. et al. Clinical Procedures for Behaviour Therapy. Pp.400. ISBN 0-13-137794-9. (Prentice-Hall: 1981.) £12.95.

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History of Science

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MOLELLA, A.P. et al. (eds). A Scientist in American Life: Essays and Lectures of Joseph Henry. Pp.136. Pbk ISBN 0-87474-641-8. (Smithsonian Institution, Washington, D.C.: 1981.) Pbk \$6.95.

NEWELL, H.E. Beyond the Atmosphere. Early Years of Space Science. The NASA History Series. Pp.197. (Superintendent of Documents, U.S. Government Printing Office, Washington: 1980.) Pbk np.

STEARN, W.T. The Natural History Museum at South Kensington, A History of the British Museum (Natural History) 1753-1980. Pp.414. ISBN 434-73600-7. (William Heinemann, London: 1981.) £15.

Anthropology

ANDERSON, D.C. and SEMKEN, H.A. Jr. (eds). The Cherokee Excavations Holocene Ecology and Human Adaptations in Northwestern Iowa. Pp.277. ISBN 0-12-058260-0. (Academic: 1980.) \$23.

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MUCKELROY, K. Discovering a Historic Wreck. Handbooks in Maritime Archaeology, No.1. Pp.47. Pbk ISBN 0-905555-51-1. (Trustees of the National Maritime Museum, London: 1981.) Pbk £1.20.

ROBINSON, W.S. First Aid for Marine Finds. Handbooks in Maritime Archaeology, No.2. Pp.40. Pbk ISBN 0-905555-52-X. (Trustees of the National Maritime Museum, London: 1981.) Pbk £1.20.

STYLES, B.W. Faunal Exploitation and Resource Selection, Early Late Woodland Subsistence in the Lower Illinois Valley. Northwestern University Archaeological Program: Scientific Papers, No.1. Pp.312. (Northwestern University Archaeological Program, Evanston, Illinois: 1981.) Pbk \$12.

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HARRIS, E. and HARRIS, J. The Guinness Book of Trees, Britian's Natural Heritage. Pp. 160. ISBN 0-85112-303-1. (Guinness Superlatives, Enfield, Middlesex:

MIDDLETON, W. E. K. Radar Development in Canada: The Radio Branch of the National Research Council of Canada 1939-1946. Pp.148. ISBN 0-88920-106-4. (Wilfrid Laurier University Press: 1981.) \$9.75.

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Flexi ISBN 0-85139-596-1. (Architectural Press, London: 1981.) £12.95. PERSINGER, M. A. The Weather Matrix and Human Behavior, Pp.327. ISBN 0-03-057731-4. (Praeger/Holt-Saunders, New York: 1980.) £18.

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ROSENTHAL-SCHNEIDER, I. Reality and Scientific Truth. Discussions with Einstein, von Laue, and Planck. Pp.148. ISBN 8143-1650-6. (Wdayne State University Press: 1980.) Np.

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WEIRZBICKA, A. Lingua Mentalis. The Semantics of Natural Language. Pp.367. ISBN 0-12-750050-2. (Academic: 1981.) \$54.

WOLF, J. A., CAHEN, M. and DE WILDE, M. (eds). Harmonic Analysis and Representations of Semi-Simple Lie Groups, Mathematical Physics and Applied Mathematics, Vol.5. Lectures given at the NATO Advanced Study Institute on Representations of Lie Groups and Harmonic Analysis, held at Liège, Belgium, September 1977, Pp.495, ISBN 90-277-1042-2. (Riedel: 1980.) Dfl.125, \$66.

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Applications are invited for this post, which is available immediately, to work on mechanisms that inhibit the transposition of transposable elements, particularly those encoding drug-resistance. Experience in recombinant DNA technology is desirable. The appointment will be for three years.

Starting salary will be in range 1A of the scales for Research and Analagous Staff (£6,070 — £6,880 pa).

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent, as soon as possible, to Dr P M Bennett, University of Bristol, Department of Bacteriology, The Medical School, University Walk, Bristol BS8 ITD. (9408)A

UNIVERSITY OF OTAGO Dunedin, New Zealand LECTURER (Part-time) IN FOOD CHEMISTRY

Applications are invited for a lecturership (part-time) in Food Chemistry in the Department of Human Nutrition of the Faculty of Home Science.

The appointee will contribute to the Food Science sections of Departmental teaching. The post is suitable for a Food Scientist to combine with Consulting or R and D work, or it could be combined with postgraduate study. Up to a half-time position is available.

Salary: Lecturer — Pro Rata NZ\$19,140 — \$23,520 per annum.

Further detailed information is available from Professor J A Birkbeck, University of Otago, Department of Human Nutrition, Dunedin, New Zealand. A Statement of General Information and conditions of appointment including method of application is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WCIH OPF, or from the Registrar of the University, PO Box 56, Dunedin, New Zealand.

Applications close on 30 September 1981. (9422)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF BOTANY POSTDOCTORAL RESEARCH FELLOW

Applications are invited for this SERC funded post tenable for three years from November 1981 to work on the taxonomy of perennial bromegrasses under the supervision of Dr P M Smith. The work will include anatomical and morphological investigations and the applicants should have a good Honours degree in Botany and have a PhD or similar experience in plant taxonomy.

Applications, together with the names of three referees, should be sent by 30th September 1981 to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL. Please quote Reference 5050. (9427)A

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The University of Queensland invites applications for a Chair of Physiology in the Department of Physiology and Pharmacology which became vacant in January, 1981, with the resignation of Professor G D Thorburn. Applicants should be able to present evidence of substantia research achievement and acknow ledged leadership in the field o Physiology. The successful applican will be expected to demonstrate an foster excellence in research, and wi be responsible to the Head of th Department of undergraduate an postgraduate teaching in keepir with Faculty Board and Depar mental requirements.

Salary: \$A41,509 per annum. To position is subject to the availabil of funds.

Additional information a application forms are obtains from the Association of Comm wealth Universities (Appts), Gordon Square, London WC OPF, (9404).

FRESHWATER BIOLOGICAL ASSOCIATION BIOMETRICIAN/ STATISTICIAN

required for Windermere Laboratory to initiate research in biometry as applied to pupulation dynamics of benthos, plankton and fish, sampling freshwater organisms and chemical/physical variables and experimental studies in collaboration with biologists and chemists. Minimum of 2.1 honours degree in statistics/biometry/mathematics or science degree plus statistical training or experience. Conditions analogous to HSO/SSO in Civil Service, starting salary between £6,075 and £7,644 pa.

Full details: Secretary, FBA, The Ferry House, Ambleside, Cumbria LA22 0LP (096 62 2468). Closes 7 Sept. (9314)A

KING'S COLLEGE LONDON RESEARCH TECHNICIAN GRADE 3

Applications are invited from persons interested in working on the control of globin gene expression in the frog. Xenopus laevis, in our Biophysics Department at Drury Lane WC2. Experience of work with nucleic acids, enzymes and bacteria would be an advantage. The successful applicant will also help with the administrative running of the laboratory and with the preparation of undergraduate practical classes. Salary on a scale rising from £5,688 pa (incl) to £6,489 pa (incl). 37½ hour week, Monday to Friday. Five weeks' annual holiday. Annual season ticket loan. Superannuation Scheme.

Apply in writing as soon as possible with a curriculum vitae and the names and addresses of two referees, to The Head Clerk (Ref: N226996), King's College London, Strand, London WC2R 2LS. (9415)A

... UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY TEMPORARY RESEARCH ASSOCIATE

Applications are invited for an MRC-funded position of temporary Research Associate in the above department. The post is tenable for three years from 1st October, 1981 or as soon as possible thereafter.

The project will investigate the regulation of the activity of branched-chain 2-oxo acid dehydrogenase by phosphorylation — dephosphorylation. Experience of enzyme purification and/or protein phosphorylation would be beneficial.

Initial salary will be at the rate of £6,070 per annum within the range £6,070 — £6,475 — £6,880 on the Range 1A scale.

Applications, including full curriculum vitae and the names and addresses of three persons to whom reference may be made, should be sent, as soon as possible, to Dr S J Yeaman, Department of Biochemistry, Ridley Building, University of Newcastle upon Tyne NE1 7RU, from whom further details may be obtained. (9402)A

UNIVERSITY OF NATAL DEPARTMENT OF BOTANY PIETERMARITZBURG

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin, for appointment to the post of:

SENIOR LECTURER

Minimum qualifications would be a PhD in Botany or closely allied field and/or suitable academic or research experience.

The successful incumbent will be responsible for the organisation and teaching of courses in the fields of Plant Taxonomy, Plant Biosystematics and Population Biology of Plants.

Major areas of research within the Department include Angiosperm Taxonomy, Experimental Morphology, Phycology, Plant Physiology and Terrestrial and Aquatic Ecology.

The salary will be in the range of: R14,370 — R20,850.

The commencing salary will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual service bonus equivalent to 93% of one month's salary is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the London Representative, South African Universities Office, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of Natal, PO Box 375, Pietermaritzburg, 3200, (telephone 63320), with whom applications must be lodged not later than 16 October 1981, quoting the reference PMB 51/81. (W407)A

UNIVERSITY OF OXFORD NUFFIELD DEPARTMENT OF ORTHOPAEDIC SURGERY MRC BONE RESEARCH LABORATORY

Applications are invited for two posts to work on the isolation and characterization of factors that induce the differentiation of bone cells.

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Starting salary (Range 1A) — £6,070 — £7,290 pa for biochemists with experience in protein isolation + cell culture techniques

(2) MEDICAL LABORATORY SCIENTIFIC OFFICER

Salary range — £4,851 — £5,334 for technical assistance with above project

The posts are supported by MRC for a three year period. Applications with full curriculum vitae and names and addresses of three referees to Dr J T Triffitt MRC Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford OX37LD, as soon as possible. (9367)A



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Scientific Research Position

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A vacancy exists in the Pathology Division of ILRAD for a Research Scientist to continue studies on the inductive mechanisms for cell-mediated immunity against the protozoan parasite *Theileria parva* in cattle and to analyse the antigenic composition of the parasite. Opportunities exist for collaborative research on surface markers of bovine lymphocytes and means of eliciting protective immunity against *Theileriosis*.

The applicant should possess a degree in Veterinary Science (or be familiar with experimentation in livestock) together with a PhD. Relevant postdoctoral experience would also be an advantage. Salary will be paid in US dollars according to experience. Perquisites include movement, housing and commutation allowances, medical and retirement benefits.

Applications with curriculum vitae and the names of three referees should be addressed to: The Director of Administration, ILRAD, PO Box 30709, Nairobi.

Closing Date: 15 September, 1981.

(W408)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOW

Crystallography

Applications are invited for the above SRC-funded post to investigate the crystal structures of some simple volatile inorganic compounds.

Applicants should have experience in X-Ray Crystallography. Familiarity with techniques of low temperature crystal preparation would be an advantage.

The appointment will be for two years starting on or after 1st January 1982. The initial placing will be on the 1A scale, starting at a point that will depend on age and qualifications.

Applications, including the names of two referees, should be sent to Professor E A V Ebsworth or Dr A J Welch, Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, from whom further particulars may be obtained. Please quote reference 5049. (9428)A



Royal Postgraduate Medical School

(University of London)

Department of Clinical Pharmacology

SENIOR RESEARCH OFFICER (Post-Doctoral)

required to join a multi-disciplinary team involved in the characterisation of purified forms of human drug metabolising enzymes, in particular forms of cytochrome P-450. The successful applicant will have a PhD in Biochemistry, preferably with experience of protein chemistry. The post is sup-ported by the Medical Research Council until 31 August 1982. Salary on range IA between £6,070 - £7,700 plus £967 London Allowance a year. Applications forms and further details may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 20/458. (9419)A

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(9370)A

TISSUE IMMUNOLOGY SPECIALIST

for a senior post in Cape Town, perhaps the world's most attractive coastal city.

The Hospitals Department within the Cape of Good Hope's Privincial Administration seeks a Senior Specialist for the Tissue Immunology Laboratory, situated at the University of Cape Town Medical School.

This well established laboratory renders services to two major teaching hospitals, namely the Groote Schuur Hospital/University of Cape Town and Tygerberg Hospital/University of Stellenbosch (just outside Cape Town) as well as to the State Health Department.

Candidates should be experienced in all laboratory aspects of HLA typing and transplantation immunology. Knowledge of red cell serology and genetics would be a recommendation.

A fixed salary of R24,450 p.a. is offered (1 = R1.74) and, if necessary, the successful candidate will be assisted in finding accommodation. Cape Town itself offers comprehensive schooling and shopping facilities together with a high standard of living at low cost, some of the world's most attractive scenery, beaches and a Mediterranean-style climate.

Further details can be obtained from Dr. du Toit, Provincial Laboratory for Tissue Immunology, Private Bag 4, Observatory 7935, Cape Town, Republic of South Africa.

(9412)A

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London) Courtauld Institute of Biochemistry

POST-DOCTORAL RESEARCH ASSISTANT & RESEARCH ASSISTANT

Applications are invited for the above positions to work on an MRC supported project concerning the biosynthetic pathway of membrane proteins of the Golgi apparatus. The project will utilize a wide range of techniques including tissue culture, cell fractionation, cell-free protein synthesis and recombinant DNA technology. Previous experience in some of these techniques would be advantageous. Applicants should have a PhD or a good BSc Honours degree dependent on the position sought. The appointment three years from 1 October 1981 with a starting salary of £6,880 per annum plus £967 London Allowance (Postdoctoral Research Assistant) and £5,285 per annum plus £967 per annum London Allowance (Research Assistant).

Applications should be sent together with a curriculum vitae and the names of two referees to Dr R K Craig, Courtauld Institute of Biochemistry The Middlesex Hospital Medical School, London W1P 7PN.

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL SCIENCES

POSTGRADUATE RESEARCH ASSISTANT

Applications are prited for the above ARC funded post to investigate the role of cell wall degrading enzymes during abscission. The post is for a period of 3 years and suitable candidates may register for a higher degree.

Applicants should have a good honours degree in a biological science and should send a Curriculum Vitae, together with the names of two referees to: Dr J A Roberts, Department of Physiology and Environmental Sciences, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE12 5RD. (9426)A



PROJECT MANAGER (PM) TEN METER TELESCOPE (TMT) UNIVERSITY OF CALIFORNIA (UC)

Applications are invited for the current opening of project manager for the UC TMT. The PM will have overall project responsibility for planning, engineering design, cost estimation, scheduling, and budget management for procurement, fabrication, and construction during the design and construction phases of the Ten Meter Telescope and related facilities. The PM will work in close cooperation with the project scientist in the determination of design and performance specifications and the development of the detailed engineering design. Applicants should have demonstrated ability to complete major projects on time within budget. Experience in siting and site support requirements of standalone research facilities is valuable. Knowledge of electromechanicaloptical systems, computer environments, structural engineering, and laboratory research facility designs is desirable. Experience as manager of major research construction projects is a requirement. Applicants with degrees in engineering or physical sciences are preferred.

The PM will be an associate director of Lick Observatory reporting to a UC Executive Management Committee. Salary will be commensurate with experience and qualifications.

Applicants should send résumé and the names of three persons who have been asked by the applicant to submit letters of professional reference directly to the Chair. All application materials should be received by 15 September 1981 and should be sent to:

Dr. Harold Ticho TMT/ENC Chair c/o Lick Observatory University of California Santa Cruz, California 95064

An Equal Opportunity/Affirmative Action Employer (NW836)A

RESEARCH ASSOCIATE and

POSTDOCTORAL FELLOWS

Positions immediately available to investigate monoclonal phagocyte biology as part of an active research group devoted to this area. Emphasis on monoclonal antibodies as probes of function and differentiation. PhD in immunology, biochemistry or pharmacology preferred. Salary commensurate with experience.

Send curriculum vitae and three references to: Dr Stephen W Russell, Director, Comparative and Experimental Pathology, Box J-145, JHMHC, University of Florida, Gainesville, FL 32610. Closing deadline for applications is 15 October, 1981. The University of Florida is an equal opportunity/affirmative action employer. (NW838)A

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts AL5 2JQ PLANT PROTOPLASTS

Applications are invited from graduate biologists to join a team working in the Biochemistry Department on the genetic manipulation of crop plants. The successful applicant will be expected to develop techniques for the regeneration of crop plants from isolated protoplasts. Applicants must have a proven record of success in plant regeneration and protoplast technology and preference will be given to those with a PhD and some post-doctoral experience.

Qualifications: 1st or Upper 2nd class honours degree.

The successful applicant will be appointed in one of the following grades according to qualifications and experience: Higher Scientific Officer (£6,075 — £7,999); Senior Scientific Officer (£7,644 — £9,619); Principal Scientific Officer (£9,690 — £12,540).

At least 2 years relevant experience is required for HSO grading, and 4 years for SSO. Grading at PSO level will only be considered for exceptional candidates. Pay award pending. Non-contributory superannuation.

Apply in writing to the Secretary naming two referees and quoting Ref. 459 by 19th September 1981. Further details on request.

(X9326)A

DURHAM UNIVERSITY CHEMISTRY DEPARTMENT Reactivity of Nitrous Acid Scavengers

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

for one year to study the kinetics of nitrosation using fast reaction techniques under the direction of Dr D L H Williams.

Initial salary in the range £6,070 to £6,880 on Range IA plus superannuation.

PHYCHOLOGY DEPARTMENT

Applications are invited from graduates in psychology or other biological subjects for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANT IN PSYCHOLOGY

for one year from 1 October or as soon as possible thereafter to work on reproductive behaviour with Dr R F Drewett.

Salary up to £7,700 on Range IA plus superannuation.

For both posts, applications (3 copies) naming three referees should be sent to the Registrar, Science Laboratories, South Road, Durham DH1 3LE by 21 September 1981, from whom further particulars may be obtained. (9401)A



INVERESK RESEARCH INTERNATIONAL

SENIOR APPOINTMENTS IN BIOTECHNOLOGY

IRI, a leading company in the contract biomedical research field, is expanding into the area of application of monoclonal antibody technology and genetic manipulation to problems of industrial and medical interest. Applications are therefore invited from scientists interested in applied contract research, both positions are at senior level and will provide ideal opportunities for imaginative scientists to become involved in the scientific and policy developments in the following areas:

MONOCLONAL ANTIBODY TECHNOLOGY

Applicants should have post-graduate experience in immunology and/or tissue culture, preferably with direct experience of hybridoma technology. (Ref. 1701).

GENETIC MANIPULATION

Applicants should have post-doctoral experience in plasmid manipulation and/or expression of foreign genes. (Ref. 1702).

Salaries reflecting the seniority of the posts will be on an incremental scale system, with placement according to relevant experience. Benefits include an excellent pension and life assurance plan, BUPA and relocation assistance if required. IRI is situated in a pleasant rural area within easy reach of Edinburgh and the surrounding country.

Application forms, quoting the appropriate reference, are available from the Personnel Department, Inveresk Research International Limited, Musselburgh EH217UB. (9406)A

UNIVERSITY COLLEGE DUBLIN

DEPARTMENT OF PHARMACOLOGY

A temporary one-year vacancy exists for the 1981/82 session in the Department of Pharmacology at

ASSISTANT LECTURER OR COLLEGE LECTURER LEVEL

Applications are invited from candidates with:

didates with:

(a) an appropriate honours primary degree and

(b) PhD, (except in the case of medically qualified candidates).

The current salary scales are: Assistant Lecturer: Ir£6,839 — £10,933. College Lecturer: Ir£10,572 —£14,556.

Entry point on the relevant scale will be in accordance with qualifications and experience.

Prior to application details of application procedure should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244 ext 431.

The latest date for receipt of completed applications is Thursday, 17th September 1981. (9425)A

ST THOMAS' HOSPITAL MEDICAL SCHOOL

DEPARTMENT OF HISTOPATHOLOGY

London SE1 7EH

BIOCHEMIST

With a good honours degree in Biochemistry or a related subject, to work on a project involving the immunocytochemical localisation of lung tumour markers.

The appointment will be for One year in the first instance.

The work will include protein purification techniques; affinity and gel chromatography. ELISA and radioimmunoassay of a variety of antigens and antibodies and quantitation of immunocytochemical results. This appointment would suit someone wishing to write a PhD thesis or a post doctoral scientist, and will be carried out in the department of Histopathology. The emphasis of the work will be on the clinical application of the techniques.

Salary up to £7,110+Londen Weighting depending on age and experience.

Apply in writing with the names of 2 referees to Dr E Heyderman, REF BC, Department of Histopathology, St Thomas' Hospital Medical School SE1 7EH by Sept 11th 1981.

(9407)A

Assistant Professor of Pharmacology/ Division of Neuropsychopharmacology

The Department of Pharmacology at The Medical College of Pennsylvania invites applications for a full time appointment within the Division of Neuropsychopharmacology as a Behavioral Physiologist or Psychopharmacologist. Applicants should have a PhD or equivalent degree with at least 2 years post doctoral experience. This position requires an individual to set up his/her own research program and also collaborate with other individuals in basic neurochemistry and clinical psychopharmacology. Submit curriculum vitae and references to:

Benjamin Weiss, PhD Director, Division Of Neuropsychopharmacology

MEDICAL COLLEGE OF PENNSYLVANIA

Department of Pharmacology 3300 Henry Avenue Philadelphia, Pa. 19129

An Equal Opportunity Employer, M/F

(NW835)A

MEDICAL LABORATORY SCIENTIFIC OFFICER

Required to work on projects involving radioimmunoassay to determine the antibody responses in patients allergic to food. Only one year left on the grant. Salary on Whitley Council Scale according to qualifications and experience.

Applications in writing, enclosing ev and the names of two referees to: Dr J Brostoff, Department of Immunology, The Middlesex Hospital Medical School, Arthur Stanley House, 40-50 Tottenham Street, London W1P9PG. (9418)A

MEDICAL LABORATORY SCIENTIFIC OFFICER

(Locum for seven months)

to do assays for parathyroid hormone for Regional Assay Service. Present encumbent on Maternity Leave. Interesting work in modern laboratories. Previous radio-immunoassay experience desirable.

Applications in writing to Dr J L H O'Riordan, Department of Medicine, The Middlesex Hospital Medical School, London W1P 7PN. (9417)A

Research Associate in Biochemistry

To collaborate in the Department of Biochemistry of the Kirksville College of Osteopathic Medicine on research into the biochemical basis of experimental epilepsy by the measurement of structural and functional changes in brain synaptosomal membranes. The work will involve measuring uptake of biogenic amines and cations by brain synaptosomes; assessing synaptosomal membrane structure using fluorescence probes and assessing changes in lipid domians of key synaptosomal enzymes. Applicant must have a PhD in biochemistry, 2 or more years postdoctoral research experience, some background in membrane biochemistry and familiarity with methods in lipid biochemistry, electrophoresis, chromatography, enzymology and spectrophotometry. Excellent working conditions with air-conditioned research laboratory, 40 hours per week. Salary \$17,000 per year.

Please send résumé (order 549641) to: Kirksville Job Service, 310 N. Elson, Kirksville, MO 63501.

Applicant must be legally qualified to accept U.S. employment. Equal Opportunity/Affirmative Action Employer M/F/H. (NW839)A

UNIVERSITY OF CAMBRIDGE PHYSIOLOGICAL LABORATORY

Applications are invited for a

GRADUATE RESEARCH ASSISTANT

to work on the molecular mechanism of voltage gating in the sodium channel. Applicants should have a First Class Honours Degree in physics or biophysics, and some experience of computer programming. Salary on range 1A scale, starting point up to a maximum of £6,880 per annum, with placing according to age.

The post will be tenable for 3 years from 1 October 1981. Further particulars may be obtained from Professor R D Keynes FRS with whom applications should be lodged by 15 September 1981. (9421)A

LABORATORY Scientific Officer required for two years to join a team using new techniques to study mammalian organogenesis/gametogenesis. Histological proficiency essential. Apply is writing to the Administrative Assistant, Paediatric Research Unit, Guy's Hospital Medical School, Guy's Tower, London Bridge SE1 9RT. (9395)A

MRC MEDICAL RESEARCH

COUNCIL

Clinical Oncology and Radiotherapeutics Unit

Applications are invited for this NON-CLINICAL SCIENTIFIC

post which may be filled by either a Short-Term appointment of 3(to 5) years or a Career appointment according to the age and experience of the successful candidate. A Career appointment will be tenable at this unit in the first instance. The person appointed will join a multidisciplinary research team directed towards the improvement of clinical cancer therapy. This appointment is for a scientist with experience in some aspect of mammalian cell biology who will develop his/her own research interest within the context of the unit's work. It is anticipated that the successful candidate will also participate in ongoing collaborative studies with experimental human tumour systems. Remuneration will be at an appropriate point on the scales for university non-clinical academic staff.

Further information and an application form may be obtained from The Administrator, MRC Centre, University Medical School, Hills Road, Cambridge CB2 2QH with whom applications — including a full CV and the names of 2 professional referees — should be lodged within two weeks quoting reference number CO/5. (9400)A

RESEARCH ASSOCIATE (PROTOZOOLOGY/TISSUE CULTURE)

CENTRE FOR TROPICAL VETERINARY MEDICINE University of Edinburgh

Applications are invited from honours graduates in biological sciences with an interest and research experience in parasitology for the position of Research Associate in the Protozoology Section of the Centre for Tropical Veterinary Medicine.

The person appointed will be expected to work within a group studying the characteristics of African pathogenic trypanosomes and would be primarily concerned with the growth of these parasites in arthropod and mammalian tissue culture.

The appointment will be on contract, starting as soon as possible. The length of contract will be subject to the continuing provision of funds by the Overseas Development Administration. Salary, according to experience, will be in the range £6,070 to £8,925.

Application by letter giving a full curriculum vitae, stressing post graduate research experience and interests and including the names of two referees, should be addressed to the Director, CTVM, Veterinary Field Station, Easter Bush, Roslin EH25 9RG, Scotland. (9411)A

nature

LONDON OFFICE— Jean Neville 4 Little Essex Street London WC2R 3LF Tel: 01 240 1101 Telex: 262024

NEW YORK OFFICE Cathy Moore 15 East 26 Street New York, NY 10010 Tel: (212) 689 5900

TORONTO OFFICE
Peter Drake Associates
32 Front Street West
32 Toronto, Ontario
M5J 1C5
Tel: (416) 364 1623

THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN CHEMISTRY

postdoctoral research position is vailable for one year initially, on a ooperative Research Grant. The prointee will work on the synthesis f compounds of potential interest as hibitors of the enzymes of the ikimate pathway, in collaboration ith Professor J K Sutherland, of the epartment of Chemistry (to whom oplications, giving the names of two ferees, should be addressed at the lepartment of Chemistry, The niversity, Manchester M13 9PL) nd Dr Gareth Davies, ICI Limited Pharmaceuticals Division). Salary inge pa £6,070 - £6,880. (9420)A

ST THOMAS' HOSPITAL MEDICAL SCHOOL (University of London) London SE1 7EH RESEARCH ASSISTANT

Postgraduate biochemist/phyologist/pharmacologist is required) join a research team investigating arious aspects of cardiac biochenistry and pharmacology. Experience 1 isolated heart perfusion, tissue netabolite analysis, spectrophoto-netry or radiochemical techniques rould be advantageous.

The Research Unit is located in the lew Rayne Institute and appoint-nents will be made through St 'homas' Hospital Medical School.

Applications, together with a urriculum vitae should be sent to Dr S Manning, Myocardial Metaolism Research Laboratories, The tayne Institute, St Thomas Iospital, London SE1 (9409)A



ACULTY Position — Somatic Cell Jeneticist - Molecular Cell Bioogist with interest in human iereditary diseases is being sought to oin the Department of Microbiology it the University of Alabama in 3irmingham. Recruitment is possible is an Assistant, Associate, or full Professor. Preference will be given to in individual with the potential for ind commitment to developing an ndependent research program or to hose established investigators who rave already demonstrated success in stablishing an independent research program. Interest in and aptitude for eaching graduate, medical and lental students is also important. Position available during 1981-82 icademic year. Salary commensurate vith experience but not less than 326,000 for a twelve-month appointnent at the Assistant Professor level. Applicants should submit a letter outlining current and future research nterests, curriculum vitae and bibliography and arrange to have three etters of reference sent to: Dr Roy Curtiss III, Department of Micro-piology, Box 11 SDB, The University of Alabama in Birmingham, Birmingiam, Alabama 35294. An Equal Opportunity/Affirmative Action Employer. (NW837)A Employer.

UNIVERSITY OF FLORIDA DEPARTMENT OF MEDICINE

University of Florida seeks Scientist for appointment as ASSISTANT PROFESSOR OF MEDICINE

to join Medical Oncology Research team primarily involved in immunology and experimental Hema-Candidates must have a tology. Candidates must have a doctoral degree (PhD or equivalent), at least 2 years experience, and demonstrated research productivity in human cellular immunology. Responsibility will include teaching at all Salary \$23,000 levels. negotiable.

Application recruiting deadline 12-31-81, anticipated starting date 1982. Contact Roy S Weiner, MD, Division of Oncology, Box J-277, J Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610. An Equal Employment Opportunity/ Affirmative Action Employer. (NW820)A

UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSIOLOGY GRADUATE RESEARCH

ASSISTANT NERVE CELL PHYSIOLOGY

To work on a 3 year MRC supported project involving voltage-clamp studies of calcium conductance and calcium-dependent potassium conductance in mollusc neurones.

Salary on scale £5,285 - £7,700. The successful applicant will be encouraged to register for a PhD degree.

Applications, including the names of two referees, to, or further information from: Dr N B Standen, Department of Physiology, University of Leicester, Leicester LE1 7RH. Telephone 0533-554455 ext 331.

(9394)A

SOUTH HAMMERSMITH HEALTH DISTRICT West London Hospital JUNIOR 'B' MEDICAL LABORATORY SCIENTIFIC **OFFICER**

We wish to appoint an Immunology/ Biochemistry graduate to work in Obstetrics and Gynaecology at West London Hospital, Hammersmith Road, London W6.

This post is of a fixed term nature for one year only in the first instance, from 1st October, 1981 and will involve assisting with a project on "Development of monoclonal antibodies for immunoassay of human placental alkaline phosphatase in normal and abnormal pregnancy".

Inclusive salary scale: £3,470 -£4,631 (under review).

Applicants wishing to discuss the post informally should contact Dr SF Contractor, 01-748 3441 ext 127, after 1st September, 1981.

For an application form and job description, please contact the District Personnel Department, on 01-748 2040 ext 2992. (9399)A

THE EUROPEAN MOLECULAR **BIOLOGY LABORATORY**

in Heidelberg, Germany, invites applications for a post in the Biological Structures Division as

RESEARCH TECNICIAN

The successful applicant will work on the purification and crystallisation of proteins in a group headed by Professor Demetrius Tsernoglou. Experience in the fields of protein chemistry or crystallography would be advantageous, but a good general background with possibly a technical or scientific qualification, is more

Net salary after tax will be between DM 2,600 and DM 2,900 monthly, in addition to which certain allowances are payable, depending on personal circumstances. Initial contract of 3 years' duration and financial assistance with relocation.

Please contact EMBL, Personnel Section, Postfach 10.2209, D - 6900 Heidelberg, Germany, quoting reference 81/15, for (W406)A an application form.

IMPERIAL CANCER RESEARCH FUND TECHNICIAN/RESEARCH **OFFICER**

Required in our Cytogenetics Laboratory to analyse and identify the human chromosomes in human/ mouse somatic cell hybrids and analyse some human tumour material. Experience in human cytogenetics essential.

Degree/HNC Salary range £5,637 to £7,881. For further information and application form write or tele-phone to Miss S. M. Hurley, Imperial Cancer Reseach Fund, Lincoln's Inn Fields WC2 on 242 0200 ext 305, quoting ref 137/81. (9424)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

PATHOGENIC MICROBES RESEARCH LABORATORY

A graduate, or well-qualified MLSO or Junior 'B' MLSO is required to assist in a study of the growth and metabolism of bacterial populations implicated in the pathogenesis of dental caries. The project will involve the use of continuous culture techniques. The appointment, sponsored by

the Medical Research Council, will be for up to two years and in the salary range £2,943 - £6,597.

Further details of the post can be obtained from Dr P D Marsh, Tel: 0980-610391

The appointment will be either at MLSO or Junior 'B' MLSO, depending on age, experience and qualifications.

Applications, including curriculum vitae and the names and addresses of two referees, should be sent to Mrs M Bushby, Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts.

Closing date for applications 18th September 1981. (9396)A

DEVON AREA HEALTH AUTHORITY **Exeter Health Care District**

SENIOR PHYSICIST (£7,574 - £9,921 pa) (pay award pending)

The Department of Urodymanics/ Sphincter Unit (Physics Service) based at the 7 year old Royal Devon and Exeter Hospital (Wonford), provides an investigative and diagnostic service to referring Consultants. A Senior Physicist is required to assist the Scientific Director in this work, which deals with urological disorders, and to expand the service of other fields including oesophageal investiga-

Candidates must have considerable interest in this type of applied clinical science and the ability not only to integrate well with staff of various disciplines but also to communicate effectively with patients Opportunities will exist for introducing new techniques and for developing instrumentation for diagnostic and therapeutic purposes. Training in the techniques of conducting urodynamic investigations and interpreting relevant data will be provided.

The Unit, which has close links with the Medical Physics Group at the University of Exeter, is housed in purpose-built, well-equipped

accommodation.

Candidates with a degree or equivalent qualifications, appropriate experience with a good background in electronics and a genuine interest in patient care are invited to obtain the application form and job description of this new and challenging appointment from the Personnel Officer, Royal Devon and Exeter Hospital (Wonford), and Exerer Hospital (Wontord), Barrack Road, Exeter EX2 5DW, tel Exeter (0392) 77833 ext 2188, or contact Dr E D James, Scientific Director ext 2278 for further details. (9398)A

ANNOUNCEMENTS

BAYLISS & STARLING SOCIETY NATIONAL SCIENTIFIC MEETING 18th — 19th DECEMBER 1981 **ROYAL POSTGRADUATE MEDICAL SCHOOL,** LONDON

A two-day programme of submitted platform presentations and posters on the topic of

Regulatory Peptides and **Gut Hormones**

To bring together all the workers in this currently disparate field, Anatomists, Biochemists, Biologists, Physicians, Physiologists, Veterinarians, etc - All Welcome

Invited Address by

Prof. Viktor Mutt

Abstract Deadline: Receipt before 27th October

(Abstracts to be published in Regulatory Peptides)

For Abstract forms and further information on low cost accommodation etc write to: Dr. S. R. Bloom/Dr. J. M. Polak Hammersmith Hospital Du Cane Road London W12 0HS

The Bayliss & Starling Society is a Registered Charity (No. 279143)

> Council Members: S. R. Bloom, J. M. Polak, Sir F. Avery Jones, J. H. Baron, J. C. D. Hickson,

G. J. Dockray, K. D. Buchanan.

R. A. Gregory.

(9423)G

STUDENTSHIPS

UNIVERSITY OF READING

DEPARTMENT OF AGRICULTURE AND HORTICULTURE

POSTGRADUATE STUDENTSHIPS RABBIT NUTRITION

Applications are invited for two studentships available in the Department of Agriculture and Horticulture for research leading to a PhD degree. The studentships are provided by the Tropical Products Institute under conditions comparable with the Science and Engineering Research Council scholarships and are for work on (1) the protein and amino acid requirements of rabbits and (2) determination of the metabolisable energy content of feedstuffs and development of methods of estimating ME for rabbit feed from chemical composition.

Applications from graduates with First Class or Upper Second Class Honours degrees in Biological Sciences or Agriculture giving details f academic training and experience and names and addresses of two cademic referees should be sent to or T R Morris, Department of Agriculture and Horticulture, University of Reading, Earley Gate, Reading RG6 2AH. A selected list of candidates will be invited to attend for interview on 29 September 1981. (9405)F

FELLOWSHIPS

THE OPEN UNIVERSITY Faculty of **Mathematics**



Research Fellowship in Applied **Mathematics**

Applications are invited for a research fellow post (funded by the Science Research Council) to work on semi-classical theories of molecular scattering. Can-didates should have a PhD in mathematics or physics and should have some knowledge of classical dynamics and semiclassical theories.

Appointment will be made at an appropriate point on the Research Fellow scale (£6,070 £10,575), but not exceeding £6.880 at the highest point. The post is for two years and is available from 1st October 1981. Application forms and further particulars may be obtained from Ms R L Johnson, (4236/2), Assistant Secretary (Maths), The Open University, Milton Keynes MK7 6AA or telephone Milton Keynes 653784: there is a 24 hour answering service on 653868.

Closing date for applications: (9416)E 10th September.

Training Fellowships in Human Nutrition

The Medical Research Council and the Agricultural Research Council wish to increase the cadre of research workers with training in subjects relevant to human nutrition, particularly in areas suitable for collaboration between the Councils.

Applications are invited from clinicians or postdoctoral non-clinical scientists for MRC training fellowships. Details of the areas of work being encouraged are available, but applications will be considered in any modern aspects of human nutrition and the underlying sciences. The followships will be available for a programme of research training divided between an MRC supported establishment or university department and a similar ARC supported group. Tenure will be for any period from six months to three years.



Application forms, closing dates and further details may be obtained from Training Awards Group, Medical Research Council, 20 Park Crescent, London W1N 4AL. (Telephone 01-636 5422 ext 448). Medical Research Council

ASSISTANTSHIPS

UMIST DEPARTMENT OF **BIOCHEMISTRY Agricultural Research Council Group in Lignin** Biodegradation Ref: BIO/117/AI

Applications are invited for two RESEARCH ASSISTANTSHIPS

The team (7 members) is supported by the ARC and by British Petroleum's Venture Research Unit, and is in completely new and well equipped laboratories. Applicants should have at least a good Honours degree and postgraduate experience or a PhD in microbial physiology, biochemistry, mole-cular biology or a related subject. Appointments are for up to 3 years in the first instance, with a possibility of extension, and will commence as soon as possible. Further information can be obtained by contacting Professor P Broda (tel: 061-236 3311 ext 2119). Salaries will be within a range up to £9,335 per annum.

Requests for application forms, quoting the above reference, should be addressed to the Registrar, Room B6, UMIST, PO Box 88, Manchester M60 1QD. The closing date is 18 September 1981. (9410)P

UNIVERSITY OF OXFORD **Dyson Perrins** (Organic Chemistry) Laboratory

Applications are invited from suitably qualified

ORGANIC CHEMISTS for a postdoctoral research assistantship to work on the synthesis of reversible oxygen carriers. The appointment, in Grade 1A, supported by BP would be for one year from October 1981, at a salary within the range £6,070 to £6,880 p.a. (according to age and experience) plus USS.

Applications, quoting Reference R81/3, with curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Professor J E Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. (9413)P

UNIVERSITY OF LEICESTER

DEPARTMENT OF ZOOLOGY

RESEARCH **ASSISTANTSHIP**

Applications are invited for the post of Research Assistant at post-doctoral or graduate level to join a group working on the arrangement and expression of DNA sequences in the chromosomes of amphibians.
Applicants should have some experience with recombinant DNA and related technologies. The appointment will be for one year in the first instance, commencing 1st October, 1981, but is likely to be renewable for a further 2 or 3 years. Salary scale £5,285 to £7,700.

Applications, with the names of 2 academic referees, to Professor H C Macgregor, Zoology Department, University of Beicester, Leicester LE1 7RH. Enquiries by telephone welcome (0533-55465 ext 113).

DURHAM UNIVERSITY BOTANY DEPARTMENT

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANTSHIP

from suitably qualified cell biologists to work on aspects of crop improvement using recombinant DNA methods. The post is tenable for up to 3 years from 1 October 1981 or as soon as possible thereafter. Experience in plant cell, protoplast or tissue culturing methods preferred.

Salary on 1A Scale plus superannuation.

Applications (3 copies) naming three referees to be sent by 11 September 1981 to the Registrar, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

(9403)P

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Definitive, Complete, Authoritative

NATURE announces the publication in 1981 of the Annual Directory of Biologicals and Disposable Chemicals. The directory will be available for distribution to all NATURE subscribers in the Autumn of 1981. It will also be marketed separately, offering an even larger distribution.

A New Buyers guide

The basic thrust of the directory will be to provide NATURE subscribers with a definitive reference catalogue from which they may purchase the chemicals and disposables listed.

From research conducted around the world the NATURE marketing staff has determined the need for a directory of biologicals, disposable chemicals and gases. Probably more than any other scientist, the NATURE reader represents the largest potential market for the sale of these chemicals.

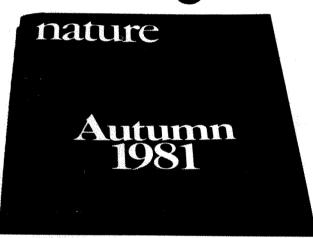
very attémpt will be

made to produce a catalogue that will reference the worldwide market of chemical manufacturers, distributors, suppliers, and exporters.

No instrumentation or apparatus listings will be included in this directory; its sole purpose is to provide detailed information for our readers as to where they may secure the biologicals, chemicals and gases for use in their

laboratories.
The directory will be cross-referenced and include chemical classifications, product classifications, geographical location of suppliers and other referenced information as needed.

NATURE Directory of Biologicals



What's in It?

The directory will include (but not be limited to) these biological reseach chemicals

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